Cryptogamie, Mycologie, 2013, 34 (3): 259-279

Type studies in *Russula* subsection *Lactarioideae* from North America and a tentative key to North American species

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Abstract – Detailed microscopical observations on four type specimens of *Russula* subsect. *Lactarioideae* from North America are presented and illustrated. As the result of our analyses, all four species are accepted as good species in *Lactarioideae*, although sometimes with slightly modified concept. *R. brevipes* is recognized by the rather short and often inflated cells in the pileipellis, large spores with spiny, subreticulate ornamentation and the distinctly amyloid suprahilar spot. Whether or not the European *R. chloroides* is a later synonym remains to be examined. *R. inopina* is characterized by filiform, flexuous-undulate terminal cells in the pileipellis, as well as on the gill edge, and it lacks shorter, mucronate pileocystidia. *R. romagnesiana*, originally described to replace the invalidly published, European *R. chloroides* var. *parvispora*, is here considered to be quite different from this European taxon and is accepted as distinct, native American species characterized by having equally small spores, frequently bicapitate pileocystidia near the pileus center as well as hyphal terminations with a glutinous sheath, but lacking distinct incrustations. *R. vesicatoria* is defined as a species of sandy *Pinus* stands with crowded gills, large and clavate, obtuse to capitate, often thick-walled pileocystidia, very long and slender hyphal extremities and spores with an inamyloid suprahilar spot. A tentative key to all North American *Lactarioideae* introduces objective microscopic features for the identification of all recognized species in this group.

Identification key / morphology / *Russula brevipes* / *R. inopina* / *R. romagnesiana* / *R. vesicatoria*

INTRODUCTION

The objective of this paper is to discuss some of the American species in one of the more difficult groups of the genus *Russula* Pers., starting from the careful examination of type-specimens.

Russula subsect. *Lactarioideae* Maire groups the white russulas that are better known as the "*R. delica*-group". Once a possible confusion with the very similar, white milk caps of genus *Lactifluus* (Pers.) Roussel has been eliminated, the recognition of a *Russula* as member of the *R. delica*-group is quite straightforward, mainly because of the combination of the fleshy, whitish cap and stipe (which may stain yellowish brown or ferrugineous with age) and the regularly unequal, whitish to greenish white or pale yellowish gills. The precise

doi/10.7872/crym.v34.iss2.2013.259

identification of the species, however, is quite another matter. Microscopic features have rarely been used for identification and characterization of individual species and their different varieties. Field characters such as taste, general stature, gill density etc… are the more commonly used key features.

In North America, *Lactarioideae* are quite diverse, but the group has only received little attention from taxonomists. North American *Lactarioideae,* as a group, have been the subject of a single taxonomic revision (Shaffer 1964), which fortunately included also the examination of material for the then known European species in this subsection. Shaffer (l.c.) correctly observed that a precise interpretation of the native American species had suffered to a large extent from the blurry species concepts of the European *Lactarioideae*. Although Shaffer blames the absence of type material as one of the main causes for this situation, we think that the difficulty of delimiting species comes above all from the lack of good characters to distinguish between them due to the high variability of many features, including color of spore print, size as well as ornamentation height of spores, spacing of gills, etc… (Romagnesi 1967). This resulted in very wide species concepts with several varieties within each individual species and a lot of controversial interpretations between authors. This was particularly the case for *R. delica* Fr. itself, the type-species of *Lactarioideae*, which has no less than 11 different varieties (Adhikari & Durrieu 1999, Bataille 1908, Blum 1963, Kauffman 1918, Melzer 1944, Melzer & Zvára 1927, Quélet 1902, Romagnesi 1967, Singer 1938, 1989). Because of the characters mentioned in the original description of *R. delica* (Fries 1838), Shaffer (1964) even considered the use of this name by European authors a misinterpretation of Fries' original concept, which, according to Shaffer, indicated a species that not even belonged in *Lactarioideae*. The European *R. delica* was first reported from the United States by Peck (1880), ten years before Peck (1890) described his *R. brevipes* Peck in *Lactarioideae*, a species that would only become accepted by the mycological community some 80 years later thanks to Shaffer (1964). Burlingham (1913) had synonymized Peck's *R. brevipes* with *R. delica*, Kauffman (1918) combined it as *R. delica* var. *brevipes* (Peck) Kauffman, whereas Singer (e.g. 1975) was for most of his career also of the opinion that *R. brevipes* was nothing else but a mere variety or form of *R. delica* and he listed both for the first time as two separate species only in the latest edition of his Agaricales (Singer 1986). Principally motivated by the fact that many of the European *Lactarioideae* were in need of typification in order to stabilize nomenclature, Shaffer (1964) dismissed all American collections identified as *R. delica* as misidentifications, and he suggested to use Peck's *R. brevipes* for them. Shaffer suggested equally that the European *R. chloroides* (Krombh.) Bres. was another misinterpretation of the original description, and he considered this name, as used in Europe and America, equally a synonym of the American *R. brevipes*, which would then have priority, but was never followed in this.

In addition to *R. brevipes*, Shaffer (1964) recognized in the United States five more species, principally distinguished by differences in smell and taste, as well as in height of spore ornamentation:

- *R. vesicatoria* Burl., is a species with low spore ornamentation, a bitter to astringent taste becoming rapidly very acrid, and a strong odor of *Lactarius camphoratus.*
- *R. cascadensis* Shaffer differs from *R. vesicatoria* by its indistinctive smell and it lacks the bitter component in the acrid taste.

- *R. inopina* Shaffer differs from *R. vesicatoria* in its mild taste and less reticulated spores.
- Shaffer accepted the European *R. delicula* Romagn. and reported its presence in the United States on the basis of the very prominent spore ornamentation, mild taste and small size. Later, however, Romagnesi himself (1967) synonymized his *R. delicula* with *R. chloroides.*
- Shaffer introduced *R. romagnesiana* Shaffer as new species for the then invalidly published, European *R. chloroides* var. *parvispora* Romagn., because of the very small spores with prominent ornamentation and mild taste. He reported a single collection from North America (Michigan), which he chose as type.

Since Shaffer's revision, two more *Lactarioideae* have been described from the United States:

- *R. angustispora* Bills, described from Virginia (USA), was said to differ from *R. vesicatoria* by its much narrower and less reticulate spores and is an exclusive associate of *Pinus virginiana* (Bills 1986).
- *R. glutinosa* Fatto (1999), described from beech-oak woods in New Jersey, belongs clearly in sect. *Archaeinae* Buyck & Sarnari (Buyck unpubl.).

Some other species have been suggested as members of this subsection in the past, but are now generally admitted as unrelated to *Lactarioideae*:

- *R. lilacipes* Shear is now generally accepted as member of sect. *Ingratae* Quél. (Shaffer 1972).
- *R. morgani* Sacc. is now considered a synonym of *R. eccentrica* Peck (Bills 1985).
- *R. polyphylla* Peck has been transferred (Singer 1951) to its own subsection (*Polyphyllinae* Singer).

Several other *Lactarioideae* described from Central and South America seem quite different and rather well characterized by a unique combination of characters:

- *R. fuegiana* Singer is a *Nothofagus*-associated, moderately acrid species from Argentina, with a nice smell of apples. It was said to be similar to *R. vesicatoria* but smaller and with a viscid cap (Singer 1950).
- *R. austrodelica* Singer, another *Nothofagus*–associated *Russula,* but described from Chile, is characterized by acrid taste, fishy odor and IIc spore print according to Romagnesi (1967) (Singer 1969).
- *R. idroboi* Singer, associated with *Quercus humboldtii* in the highlands of Colombia, with moderately acrid taste, fishy smell and spiny, large spores, is said to differ from *R. fuegiana* by its larger size and habitat (Singer 1963).
- *R. metachromatica* Singer, described from the mountains in Venezuela, was suggested as possible *Lactarioideae* (Singer 1952) but became type-species of subsect. *Metachromaticae* Singer (Singer *et al.*, 1983).
- *R. aucarum* Singer, described from lowland rain forest in Equador (Singer 1975), is now the type species of subsect. *Delicoarchaeae* Singer (Singer *et al.*, 1983).
- *R. littoralis* Pegler *nom. illeg*. was also placed in *Lactarioideae* by its author (Pegler & Singer 1980). Because of an earlier, validly published homonym (*R. littoralis* Romagnesi, non McNabb), it was renamed as *R. cremeolilacina* Pegler var. *coccolobicola* Singer (Singer *et al.*, 1983), and moved to subsect. *Cyanoxanthinae* Singer, whereas Buyck (1992, 2003) suggested subsect. *Ilicinae* Buyck as correct placement.

MATERIAL 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 element (in some cases 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 Cresyl blue to verify presence of ortho- or metachromatic reactions as explained in Buyck (1989). Trama and cystidia were examined in sulfovanillin solution, acidoresistant incrustation of primordial hyphae was colored in karbolfuchsin and observed in distilled water after staining for a few seconds in a 10% solution of HCl (cf. Romagnesi 1967).

Spores were scanned with an Artray Artcam 300MI camera and measured by 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 our descriptions is given following Adamčík & Marhold (2000). Herbarium abbreviations follow Holmgren *et al.* (1990). Years of Peck's publications in Annual Reports of the State Botanist follows Petersen (1980).

TAXONOMY

Russula brevipes Peck, Ann. Rep. N.Y. St. Mus. Nat. Hist. 43: 20. 1890 **Figs 1-8**

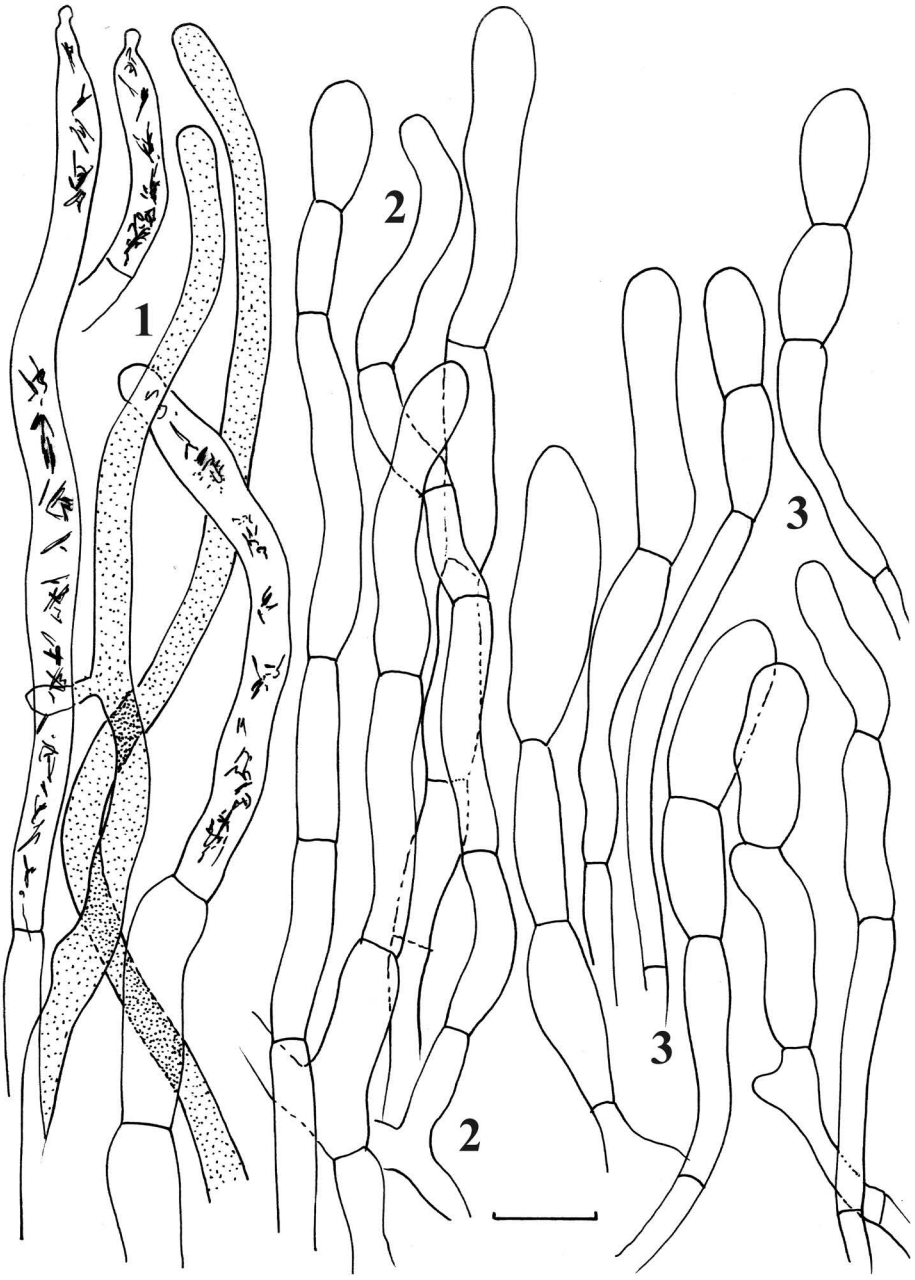
Original description:

Pileus at first convex and umbilicate, then infundibuliform, dry, glabrous or slightly villose on the margin, white, sometimes varied with reddish-brown strains, flesh whitish, taste mild, slowly becoming slightly acrid; lamellae thin, close, adnate or slightly rounded behind, white; stem very short, solid, white; spores globose, verruculose, .0004 to .0005 in. in diameter.

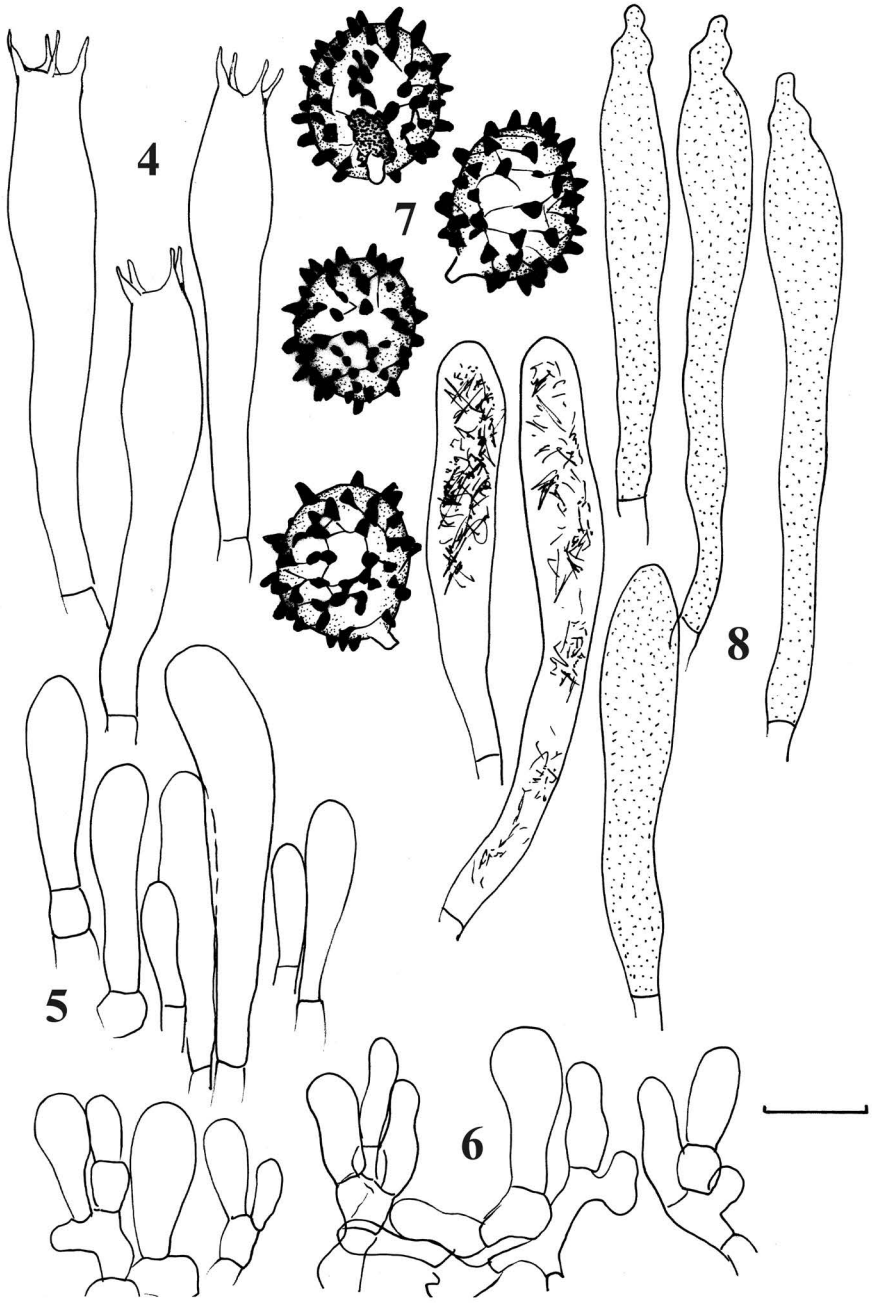
Pileus 3 to 5 in. broad, stem 6 to 10 lines long, 6 to 10 lines thick.

Sandy soil in pine woods. Quogue. September.

This species is related to *Russula delica*, but is easily distinguished by its short stem and crowded lamellae. The pileus also is not shining and the taste is tardily somewhat acrid. From *Lactarius exsuccus* it is separated by the character of the lamellae and the very short stem which is about as broad as it is long. The spores also are larger than in that species. The lamellae in the young plant are sometimes studded with drops of water. They are not clearly decurrent. Some of them are forked at the base. The pileus is but slightly raised above the surface of the ground and is generally soiled by adhering dirt and often marked by rusty or fuscous stains. The plant grew in old roads in the woods where the soil had been trodden and compacted.



Figs 1-3. *Russula brevipes* (holotype). **1.** Pileocystidia of sub- and suprapellis. **2.** Hyphal terminations near the pileus margin. **3.** Hyphal terminations near the pileus center. Cystidial contents as observed in Congo Red, but most elements with contents indicated schematically. Scale bar = 10 µm.



Figs 4-8. *Russula brevipes* (holotype). **4.** Basidia. **5.** Basidiola. **6.** Marginal cells of the gill edge. **7.** Spores as seen in Melzer's reagent. **8.** Hymenial cystidia with contents as observed in Congo Red, but for some elements with contents indicated schematically. Scale bar = 10 µm, but only 5 µm for spores.

Spores shortly ellipsoid, (8.5-)8.7-9.1-9.5(-9.9) × (7.2-)7.5-7.9-8.2(-8.6) µm, Q=(1.09-)1.11-1.16-1.21(-1.29), with a subreticulate ornamentation of conical, strongly amyloid, distant spines [(2-)3-5 in a 3 µm diam. circle], 0.8-1.3 µm high, connected by fine line connections [2-3(-4) in the circle] or occasionally fused in pairs or short ridges [0-2 fusions in the circle], with a relatively small, but distinctly amyloid suprahilar plage. **Basidia** (45-)55.5-60.7-68 × 9.5-11.4-14 µm, 4-spored, pedicellate-clavate. Basidiola first cylindrical, then narrowly to broadly clavate, 4-10(-13) µm wide. **Subhymenium** pseudoparenchymatic. **Lamellar trama** mainly composed of large sphaerocytes. **Hymenial cystidia** on sides of gills numerous, ca. 1500-2500/mm^2, some originating deep in the gill trama, narrowly fusiform to pedicellate-clavate, thin-walled, mostly mucronate or appendiculate, measuring (49-)62-73.6-93 × 7.5-9.7-11 µm, with contents weakly sulfovanilin-positive, refringent, crystalline-granular and weakly metachromatic walls in Cresyl blue, near the gill edge dispersed, mostly not pedicellate and shorter, with simply obtuse-rounded apices. **Marginal cells** similar to basidiola on sides of the gills but shorter, clavate or subcylindrical, obtuse, measuring 9-15.4-19.5(-26) × 3.5-5.7-9 µm. **Pileipellis** weakly and indistinctly metachromatic in Cresyl blue, ca. 100 µm deep, composed of strongly gelatinized hyphae, becoming less gelatinized and more anastomosed before passing abruptly to the inflated, short cells and sphaerocytes of the trama, without zebroid incrustations. Suprapellis near the cap margin composed of long, thin-walled, repent to fasciculate hyphal terminations with terminal cells measuring (15-)24.5-30.8-43 × 4.5-5.9-7.5 µm, cylindrical, clavate or sometimes almost fusiform or subapically slightly constricted, obtuse-rounded; originating from short chains of 3-6 unbranched, mostly subcylindrical cells. Towards the pileus center with more anastomoses between subterminal cells and more frequently inflated, often shorter terminal cells, 14-22.3-28.5(-38) × 5-7.3-10 µm. Pileocystidia long and slender, mostly non-septate, towards the surface dispersed and occasionally 1-2-septate and minutely capitate, continuing in the trama as "cystidioid hyphae" (or endocystidia), 5-7.5 µm wide, with scattered to abundant crystalline-granular contents that are weakly graying in sulfovanilin. **Clamp connections** absent in all parts.

Examined material: UNITED STATES. **New York State**. Long Island, Suffolk Co., Quogue, *C.H. Peck* (NYSf529, holotypus).

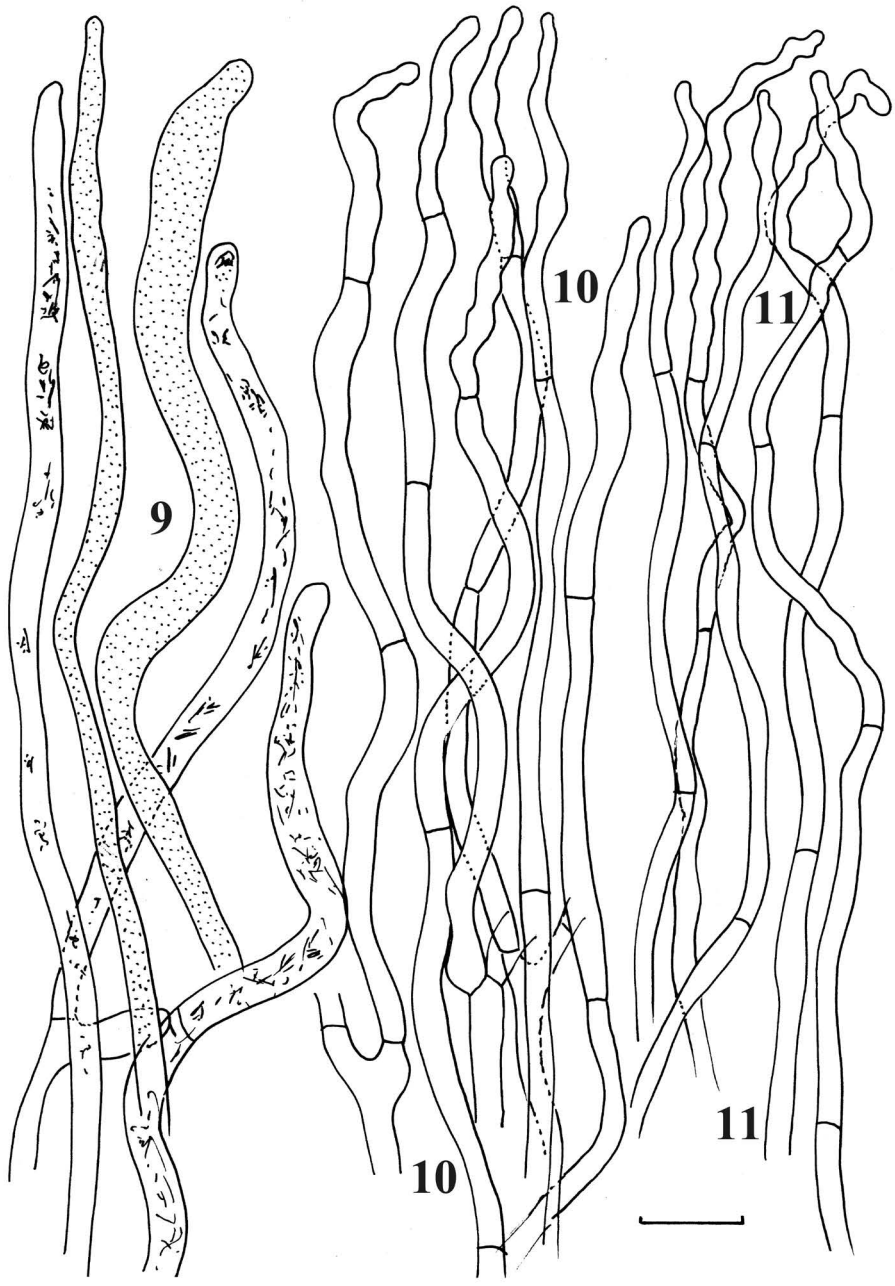
Commentary: The type of *R. brevipes* has near identical features as the European *R. chloroides* (e.g. Romagnesi 1967, Sarnari 1998). Both species share the large spores with a spiny, subreticulate ornamentation and the distinctly amyloid, suprahilar spot, and both have hyphal extremities in the pileipellis that are composed of rather inflated, short cells. *R. delica* differs in its, on average, smaller spores with lower ornamentation and the more distant, broader gills (Romagnesi 1967, Sarnari 1998).

Shaffer (1964) described two additional varieties of *R. brevipes*: one for specimens having larger spores (var. *megaspora* Shaffer) and one for very acrid specimens (var. *acrior* Shaffer), two rather questionable features. As *R. brevipes* has, to our knowledge, not yet been reported from pure broad-leaved forests in North America, it appears to be (principally?) conifer-associated.

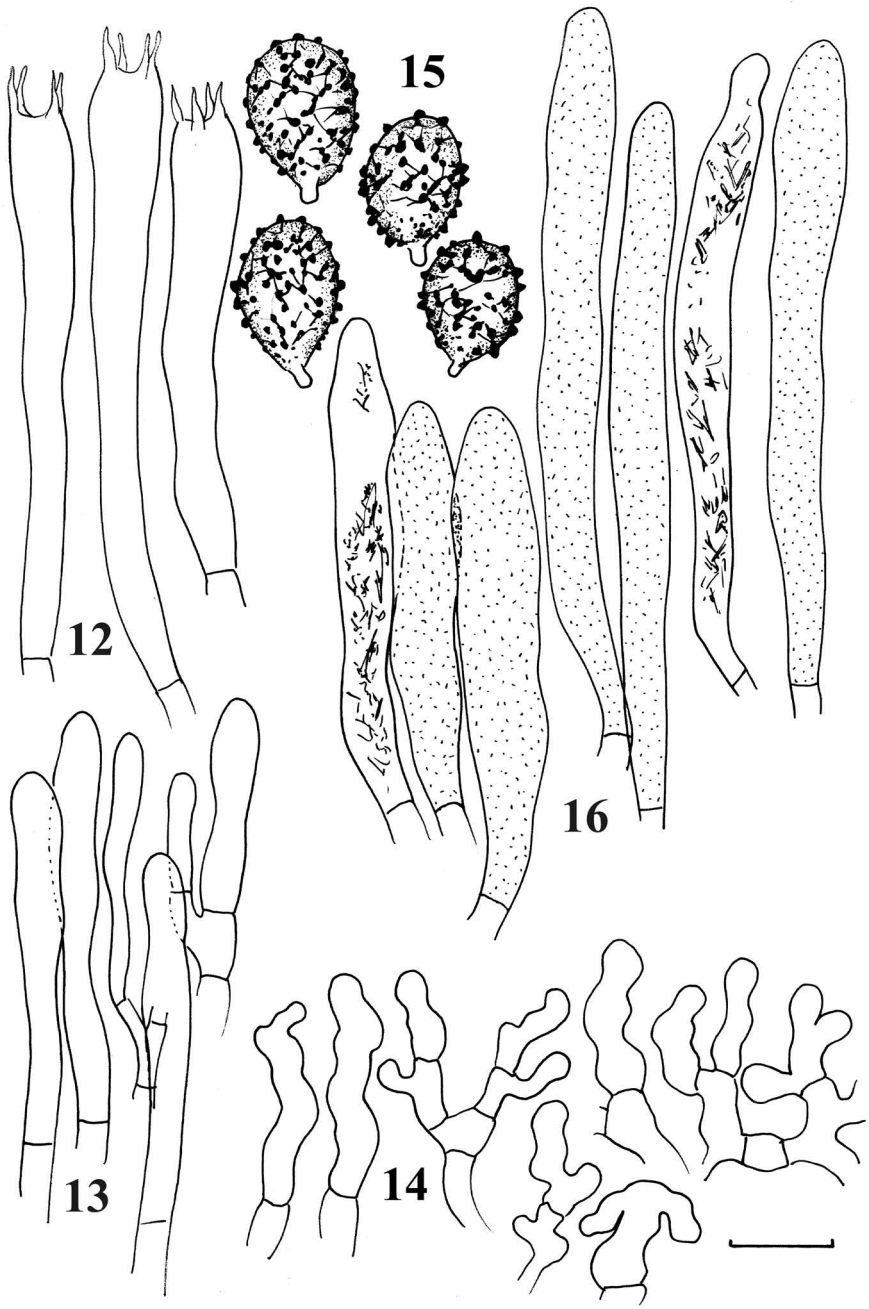
Russula inopina Shaffer, Mycologia 56: 208. 1964 **Figs 9-16**

Original description:

Pileus siccus, impolitus, margine tenuiter coactus, cremeo-albus, umbrino-maculatus; trama rigida, cremeo-alba, sapore miti; lamellae angustae, adnatae vel subdecurrentes, confertae, pallido-luteae; lamellulae numerosae; stipes impolitus, albus,



Figs 9-11. *Russula inopina* (holotype). **9.** Pileocystidia of sub- and suprapellis. **10.** Hyphal terminations near the pileus margin. **11.** Hyphal terminations near the pileus center. Cystidial contents as observed in Congo Red, but most elements with contents indicated schematically. Scale bar = 10 µm.



Figs 12-16. *Russula inopina* (holotype). **12.** Basidia. **13.** Basidiola. **14.** Marginal cells of the gill edge. **15.** Spores as seen in Melzer's reagent. **16.** Hymenial cystidia near the gill edge (on the left) and on gill sides (on the right) with contents as observed in Congo Red, but for some elements with contents indicated schematically. Scale bar = 10 µm, but only 5 µm for spores.

umbrino-maculatus; sporae pallido luteae in cumulo, 6.8–9.3 × 5.3–7.2 µ (ornamentatione exclusa), ornamentatione 0.2–0.7 µ altitudine, plerumque non reticulata; cuticula pilei ex hyphis intertextis 1.0–4.0 µ latitudine etiam hyphis oleiferis 2.7–8 µ latitudine constans.

Holotypus: Colonial Point, Burt Lake, Cheboygan Co. Mich.; 11 Aug. 1961; *Smith 63889* (MICH).

Spores ellipsoid, (6.5-)6.9-7.2-7.5(-7.8) × (5-)5.2-5.5-5.7(-6) µm, Q=(1.22-)1.25-1.31-1.37(-1.44), with a subreticulate ornamentation of rather dense, convex to conical, amyloid warts [(6-)7-10 in 3 µm diam. circle], 0.4-0.7 µm high, connected by numerous, very fine and often indistinctive line connections [1-4(-5) in the circle] or fused in pairs or short ridges [(0-)1-5 fusions in the circle]. Suprahilar spot small and indistinct, inamyloid to partly amyloid or covered by minute, amyloid punctuations. **Basidia** very slender and long, (52-)57-63.5-70(-80) × 8-9.5 µm, 4-spored, narrowly pedicellate-clavate; also basidiola very slender and long. **Subhymenium** pseudoparenchymatic. **Lamellar trama** mainly composed of large sphaerocytes. **Hymenial cystidia** on sides of gills abundant, ca. 3000-4500/mm^2, becoming even more numerous towards gills edge, sometimes originating deep in the gill trama, narrowly fusiform or lanceolate, thin-walled, not mucronate or appendiculate, 71-83.3-93(-110) × 7-8.6-10 µm, shorter and wider on the gill edge, measuring (37-)47-55.6-67 × 8-9.2-11 µm, with heteromorphous contents that are graying in sulfovanilin, with weakly metachromatic walls in Cresyl blue. **Marginal cells** very short and irregularly nodulose-flexuous, 12-17.8-23(-28) × 3-4.6-6.5 µm. **Pileipellis** orthochromatic in Cresyl blue to very weakly metachromatic in deeper layers, not very sharply delimited from the underlying sphaerocytes of the context, ca. 300 µm deep, not gelatinized near cap surface but distinctly gelatinized downwards, composed of irregularly oriented, intricate, branched, narrow hyphae, ca. 2.5-10 µm wide in subpellis, without incrustations. Hyphal extremities near the surface sometimes aggregated in fascicules, locally with some gelatinous, hyaline coating that is resistant to color reagents , composed of very slender, rarely branched, cylindrical cells, 2.5-3.2-4 µm wide (only up to 3 µm wide in cap center), with terminal cells (24-)31-41.3-51.5(-68) µm, long and strongly flexuous-undulate, often narrowed or subcapitate at the tip. Pileocystidia all very long and usually with a basal septum that is difficult to trace, continuing as cystidioid hyphae (or endocystidia) in underlying trama, cylindrical-flexuous, 5-7(-8) µm wide towards pileus margin, only 3.5-5 µm wide in pileus center, with obtuse tips or subcapitate, partly filled with yellowish amorphous to refringent, crystalline contents, with weak grayish SV reaction. **Clamp connections** absent in all parts.

Examined material: UNITED STATES. **Michigan**. Cheboygan Co., Colonial Point Hardwoods, Burt Lake, under pine, 11 Aug 1961, *A.H. Smith 63889* (MICH 12222, holotypus).

Commentary: Shaffer (1964) characterized *R. inopina* as a species with pale cream spore print, mild taste, indistinct smell and less reticulate spores.

Although Shaffer does not discuss this species any further, our type study shows it to have several interesting features that set it apart from the other species that are here studied. It differs notably in the irregular, flexuous-undulate terminal cells present in the pileipellis and on the gill edge, in the very long basidia as well as in the absence of shorter, mucronate pileocystidia. The gelatinous coating of the hyphae in the pileipellis is completely different from the typical zebroid incrustations that can be observed in many other *Russula,* including *R. romagnesiana* (see below), but is comparable to what is observed in some species in sect. *Archaeinae* (Buyck 1998).

Russula romagnesiana Shaffer, Mycologia 56: 217. 1964 **Figs 17-25**

Original description:

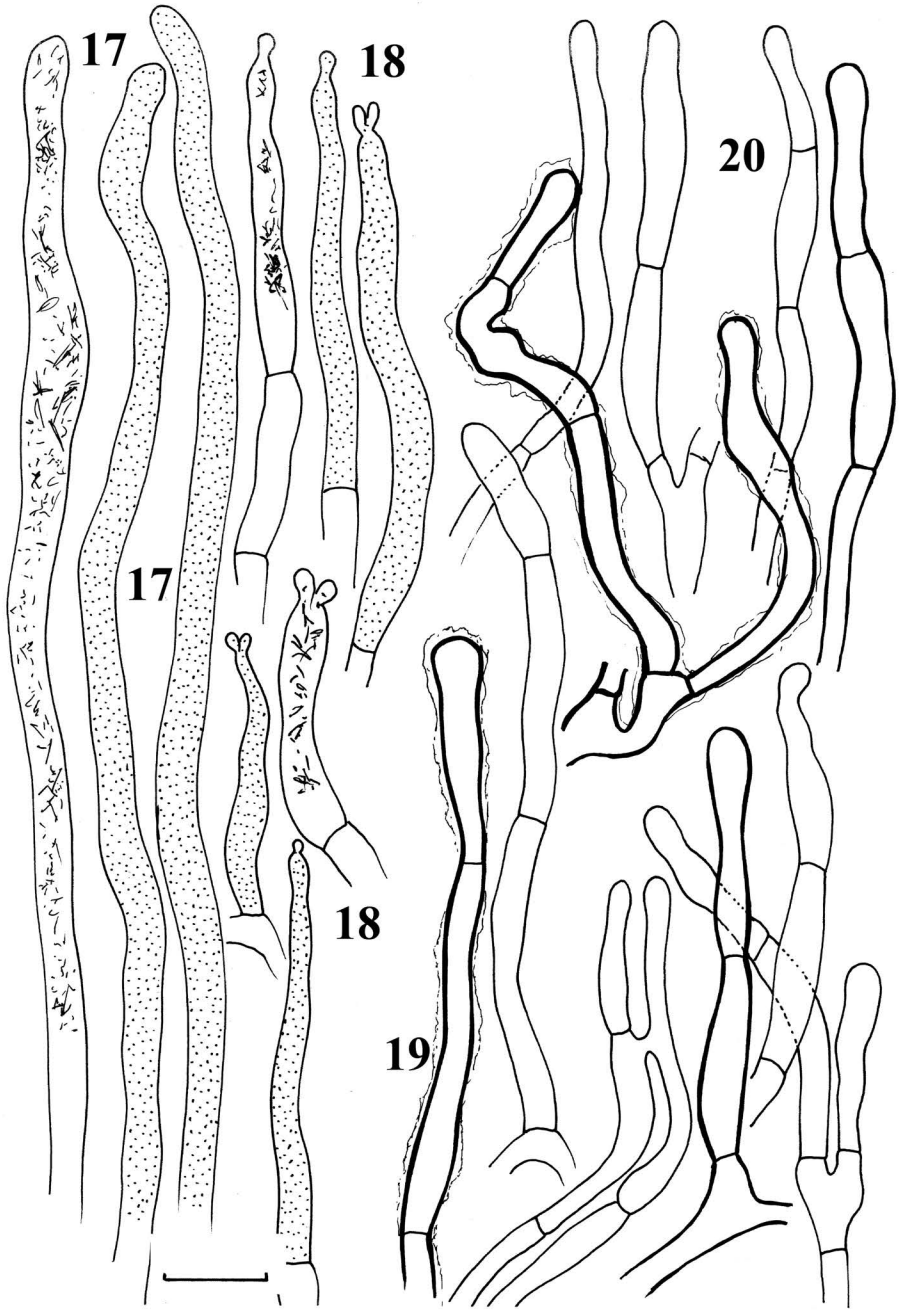
Pileus siccus, glaber, cremeo-albus, isabellino-maculatus; trama rigida, odore parvo, sapore nullo; lamellae angustae, subdecurrentes, confertae, pallido-glaucae; lamellulae numerosae; stipes glaber, cremeo-albus; sporae 6.4–8.0 × 6.1–6.8 µ (ornamentatione exclusa), ornamentatione (0.5–)0.8–1.6 µ altitudine, plerumque non reticulata; cuticula pilei circa 150 µ, crassitudine, ex hyphis intertextis, nonnunquam aspero-tunicatis 3.3–8.0 µ latitudine constans.

Holotypus: Pinckney Recreation Area, Washtenaw Co. Mich.; 19 Jul. 1960; *Shaffer 2421* (MICH).

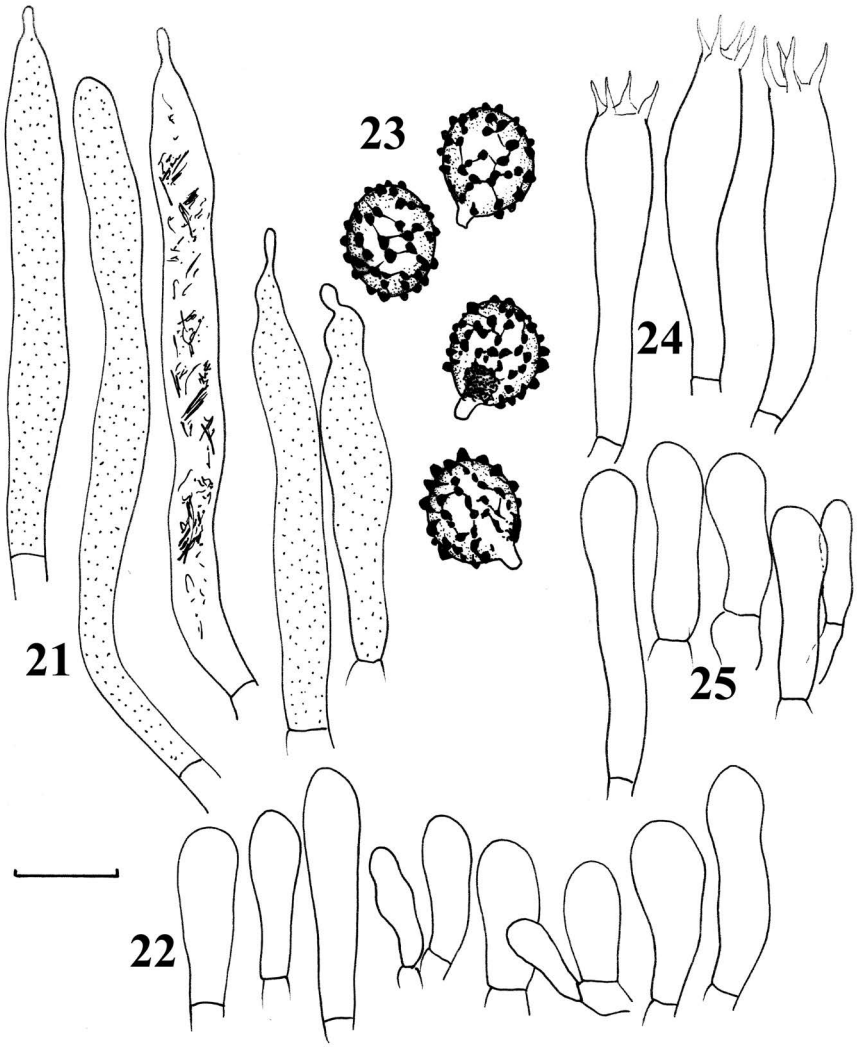
Spores subglobose to shortly ellipsoid and small, (5.8-)6-6.3-6.7(-7.3) × (5.1-)5.2-5.4-5.7(-6.1) µm, Q=(1.09-)1.12-1.16-1.2(-1.25), with a subreticulate ornamentation of convex to conical, moderately distant [(4-)5-7 in a 3 µm diam. circle], amyloid warts measuring 0.6-0.9 µm high, connected by numerous line connections [1-3(-4) line connections in the circle] or frequently fused in pairs or short ridges [(0-)1-3(-4) fusions in the circle]. Suprahilar spot amyloid, but relatively small. **Basidia** 40-44-48 × 8.5-9.7-11 µm, 4-spored, narrowly clavate to fusiform. **Subhymenium** pseudoparenchymatic. **Lamellar trama** mainly composed of large sphaerocytes. **Hymenial cystidia** on gill sides numerous to abundant, ca. 3000-3500/mm^2, narrowly fusiform to subcylindrical, thin-walled, with mostly mucronate to appendiculate tips, (47-)60.5-75.6-91(-103) × 7-7.3-8 µm, some originating deep in the gill trama and then usually obtuse-rounded, with heteromorphous, weakly sulfovanilin-positive contents and weakly metachromatic walls in Cresyl blue. **Marginal cells** hardly differentiated from basidiola, mixed with similar cheilocystidia, but these are filled with yellow, heteromorphous contents, (13-)15.5-22-28.5(-40) × (3-)5-6.6-8(-9.5) µm. **Pileipellis** not sharply delimited from the underlying trama, ca. 100 µm deep, orthochromatic in Cresyl blue but locally with metachromatic granular wall deposits, zebroid wall incrustations distinctly present. Pileipellis near pileus margin poorly developed or vanished, in pileus center composed of hyphal terminations 3.5-4.5-6 µm wide, sometimes more or less thick-walled (0.5-1 µm thick) or covered with a glutinous coating that remains hyaline in Congo red and other color reagents, with frequent anastomoses; the terminal cells (13-)17.5-27.8-38(-55) µm long, subcylindrical, subclavate or slightly subapically constricted. Pileocystidia of two types, near the surface of the pileus center mostly one-celled, (27-)34.5-47-59.5(-80) × 4.5-5.6-6.5 (-7.5) µm, narrowly fusiform to subulate, thin-walled, minutely 1-2-capitate, near the pileus margin and in deeper layers (also continuing in pileus trama) very long and subcylindrical, 4-8 µm wide, obtuse-rounded at the apex. All cystidia with yellowish-refringent, granular to crystalline contents. **Clamp connections** absent in all parts.

Examined material: UNITED STATES. **Michigan**.Washtenaw Co.: Solitary in oak-hickory woods, Pinckney Recreation Area, 19 Jul. 1960, *Shaffer 2421* (Holotype).

Commentary: Shaffer (1964) validated Romagnesi's variety "*parvispora*", at that time a still invalid (art. 37.1 ICBN) variety of *R. chloroides,* as an independent species, *R. romagnesiana*, and indicated for holotype the single American collection. In 1967, Romagnesi's monograph automatically validated his variety by reference. To our knowledge, there are no publications that discuss new collections of either taxon.



Figs 17-20. *Russula romagnesiana* (holotype). **17.** Long pileocystidia of subpellis and pileus margin. **18**. Shorter, minutely capitate pileocystidia at the surface of the pileus center. **19.** Hyphal terminations of the pileus center with slightly thickened walls and embedded in a glutinous sheat. **20.** More or less thin-walled hyphal terminations near the pileus center. Cystidial contents as observed in Congo Red, but most elements with contents indicated schematically. Scale bar = 10 µm.



Figs 21-25. *Russula romagnesiana* (holotype). **21.** Hymenial cystidia near the gill edge (on the right) and on gill sides (on the left) with contents as observed in Congo Red in one element, otherwise with contents indicated schematically. **22.** Marginal cells of the undifferentiated gill edge. **23.** Spores as seen in Melzer's reagent. **24.** Basidia. **25.** Basidiola. Scale bar = 10 µm, but only 5 µm for spores.

Romagnesi (1967) was very skeptical concerning Shaffer's decision and preferred to maintain his var. *parvispora* at varietal level because of the existence of all intermediary forms between this variety and the type variety. Shaffer motivated his new species by the fact that, apart from the smaller spores, this new species is also characterized by the encrusted hyphae in the pileipellis and the presence of large, blunt, non-amyloid warts on much less reticulate spores. In our experience, the latter character is a common malformation and a simple artifact

due to an unfavorable spore development as frequently observed in many other *Russula* species (Buyck unpubl.). When examining the gill surface of the type of *R. chloroides* var. *parvispora* under the microscope, this is clearly the case here: spores are extremely variable, both in size and in ornamentation, going from strongly amyloid and completely smooth spore surfaces, over the presence of a few large, isolated, papillate droplets to the normal, subreticulate ornamentations typical for the group. The spore development of the American holotype, however, appears completely normal. The encrusted hyphae mentioned by Shaffer for his species are different from what is often referred to as incrustations in the pileipellis of *Russula*. In the case of the holotype, there are irregular deposits of some kind of refringent, glutinous matrix on the cell walls. The European type of var. *parvispora*, on the contrary, has very distinct, zebroid incrustations in the pileipellis, particularly on pileocystidia, that take up color reagents in microscopic mounts, not at all like the refringent, glutinous deposits of the *romagnesiana* type that are insensitive to any color reagent. When examining the American holotype, the most striking difference with Shaffer's description mentioning complete lack of pileocystidia, resides in the presence of abundant, distinct pileocystidia in both supra- and subpellis with typical heteromorphous contents, in particular in the cap center (Figs 17-18). These pileocystidia are also present in the European *parvispora*-type, but there they are indeed very inconspicuous, almost optically empty and usually without mucronate apices.

In short, we are strongly inclined to think that Shaffer's microscopical description applies to the European *parvispora*-type and not to the American holotype. In our opinion, both types belong to (very) different species and the main features cited by Shaffer for his *R. romagnesiana* actually do not apply to the American holotype. Our examination of the holotype (and still single known collection) suggests that the small spores and the frequently bicapitate pileocystidia in the pileus center may be a good feature to recognize it. Whether this species differs from the other species that are here discussed in its association with broad-leaved trees, as suggested by the habitat of the type specimen, remains to be verified. Although we accept *R. romagnesiana* provisionally in *Lactarioideae*, it is certainly the most atypical one among the here studied species and possesses characters that are reminiscent of other groups in North America, i.e. two-knobbed dermatocystidia (*R. dissimulans*-group), smaller spores and hyphae with a glutinous sheath (*R. earlei*-group).

Russula vesicatoria Burl., Mycologia 36 (1): 118. 1944 **Figs 26-34**

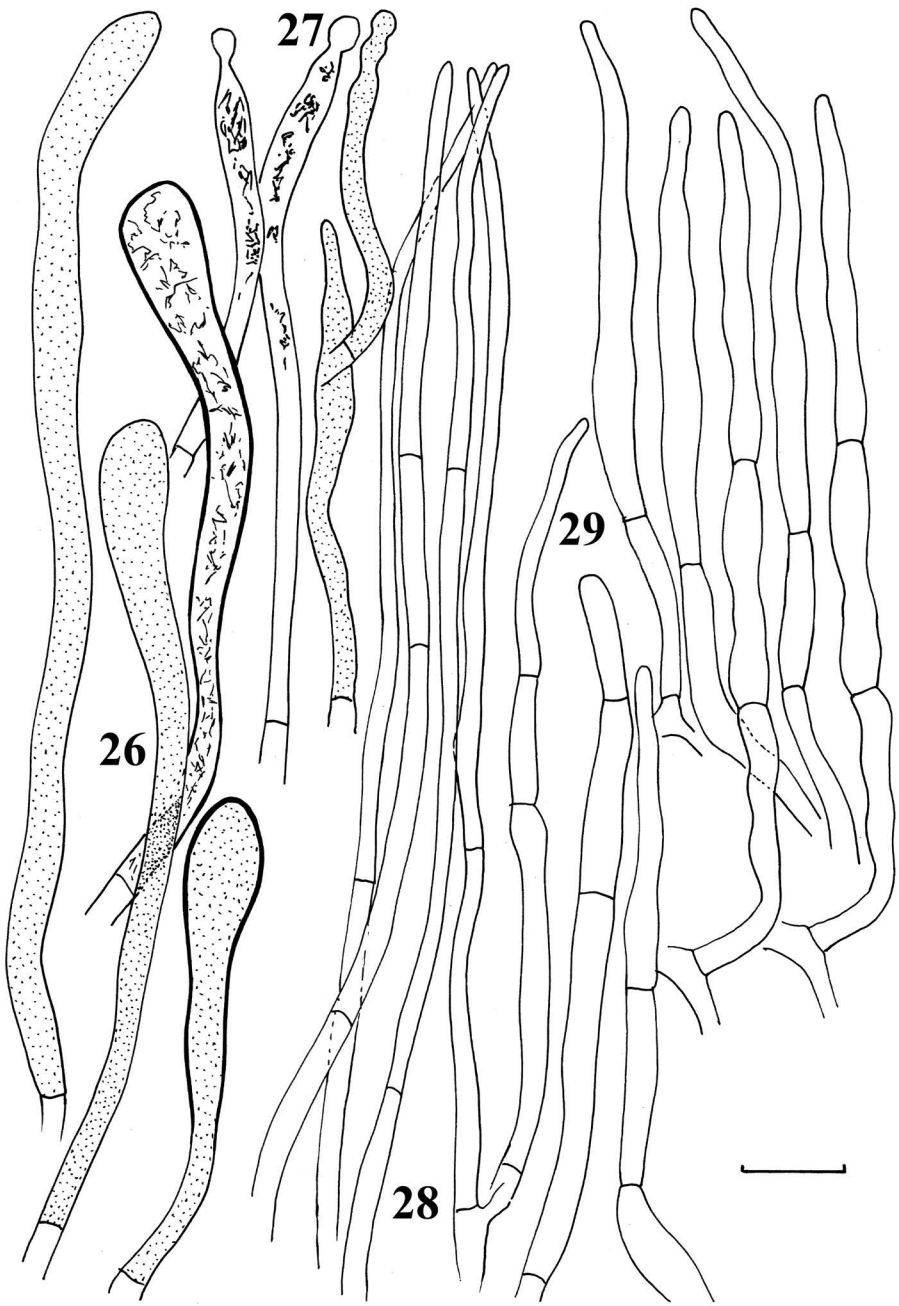
Original description:

Pileo carnoso, firmo, umbilicato, margine incurvato, pruinoso-puberulo sub lente, postea expanso et centro depresso, albo, postea disco albidulo aut pallido-luteolo, margine cum udus est viscidulo, mox sicco, exstrio, centro cum exoletum est saepe areolato, 6–11 cm. lato; carne alba, astricta, amara, turn tarde acri, postremum et diu acerrima, subolida cum fracta est; lamellis albidulis, inaequalibus, furcatis, angustis, confertis; stipite albo, solido, ad apicem minute pruinoso-puberulo, constricto deorsum; sporis albidulis (9-t4 aut 67 t1), echinulatis, et lineis delicatis reticulatis, 6.87–7.5 μ × 7.5–8.37 μ.

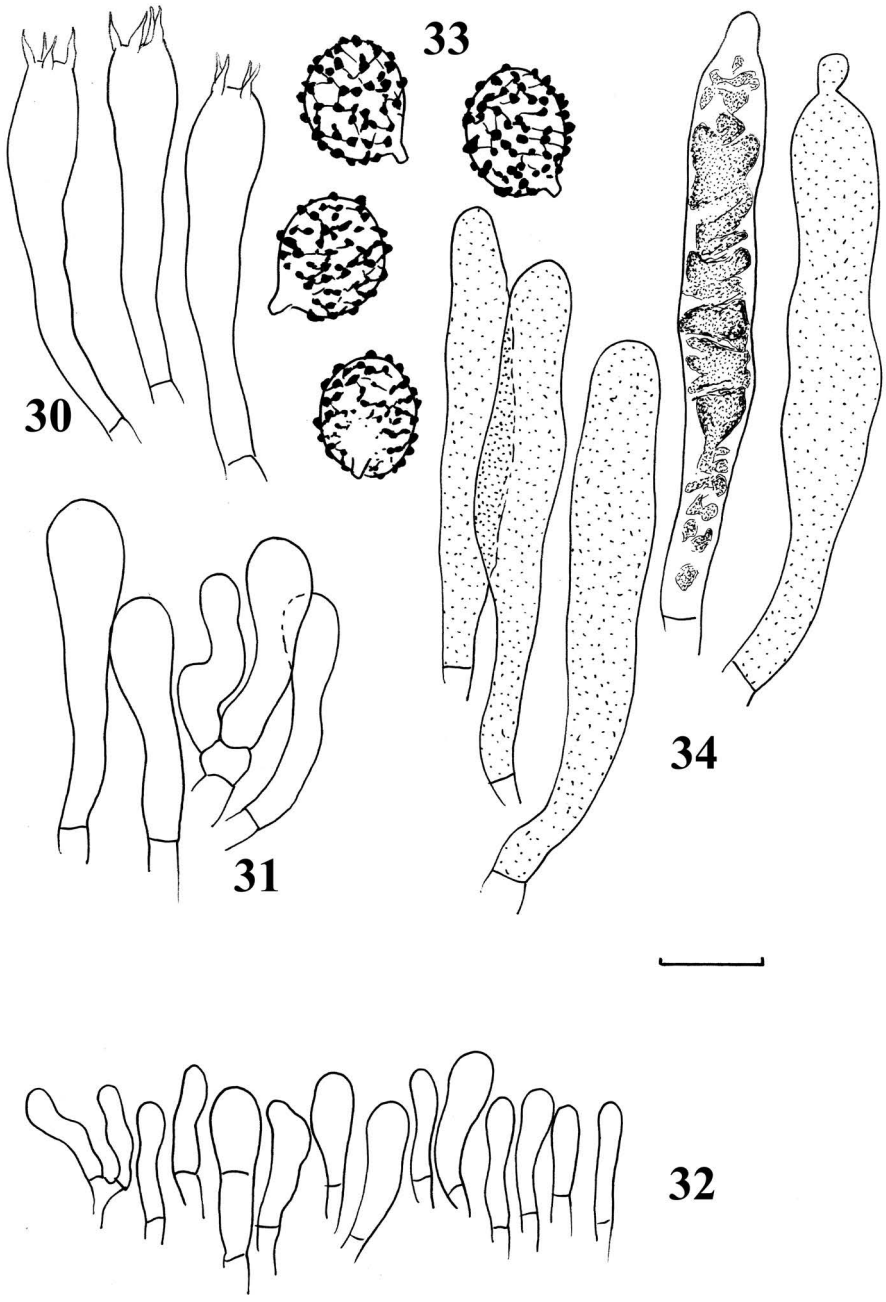
Type locality: Near Lake Wildmere, Longwood, Florida. Type 1-Oct. 23-1941.

Habitat: In black humus of lawn under scattered pines.

Spores shortly ellipsoid, (7-)7.3-7.6-7.9(-8.2) × (5.8-)6-6.3-6.7(-7) μm, Q=(1.12-)1.15-1.19-1.23(-1.31), with a subreticulate to reticulate ornamentation of moderately numerous, convex, rather large, amyloid warts [5-7(-8) in a 3 μm diam. circle], 0.4-0.7 μm high, connected by numerous line connections [1-4(-5) line connections in the circle] or fused in short to longer chains [(0-)1-3(-5) fusions in



Figs 26-29. *Russula vesicatoria* (holotype). **26.** Large, mostly clavate pileocystidia arising from or restricted to the subpellis and underlying trama. **27.** Smaller and slender capitate pileocystidia near the pileus surface. **28.** Tips of the very long and slender hyphal terminations near the pileus surface. **29.** Shorter, "normal" hyphal terminations of the suprapellis. Cystidial contents as observed in Congo Red, but most elements with contents indicated schematically. Scale bar = 10 μm.



Figs 30-34. *Russula vesicatoria* (holotype). **30.** Basidia. **31.** Basidiola. **32.** Marginal cells of the gill edge. **33.** Spores as seen in Melzer's reagent. **34.** Hymenial cystidia near the gill edge (on the left) and on gill sides (on the right) with contents as observed in Congo Red for one element, otherwise with contents indicated schematically. Scale bar = 10 µm, but only 5 µm for spores.

the circle]. Suprahilar spot small and indistinct, not amyloid. **Basidia** narrowly clavate, 41-46-50 × 8-9.5-10.5 µm, 4-spored. **Subhymenium** pseudoparenchymatic and distinctly metachromatic, as is the lower part of the hymenium. **Lamellar trama** mainly composed of large sphaerocytes. **Hymenial cystidia** on gill sides moderately numerous, ca. 1000-1200/mm^2, cylindrical, clavate or narrowly fusiform, thin-walled, obtuse-rounded or minutely capitate, (53-)63-74.5-91 × 8-10-11.5 µm, with abundant, refringent-oily and yellow contents, insensitive to sulfovanilin, near the gill edge mostly fusiform-subclavate, 42-55.5-71 × 8-9.6-12 µm, sometimes with slightly thickened walls. **Marginal cells** poorly differentiated, similar but smaller than basidioles, (7-)11-14.2-20 × 3-4.5-5.5(-7) µm, densely packed over the whole gill edge surface. **Pileipellis** orthochromatic in Cresyl blue, not gelatinized, ca. 150-250 µm deep, in subpellis with 2-8 µm wide hyphae, not sharply delimited from the underlying trama. Pileipellis near the pileus surface with sometimes > 100 µm high, erect fascicules of long, unbranched, parallel, filiform hyphal terminations, 2-3 µm wide, with terminal cells (29-)37-52-67(-91) long, other hyphal extremities with cells (2.5-)3.5-4-5 µm wide, mostly cylindrical to subulate terminal cells, (15-)19-29.3-40(-68) long, and subapical cells often distinctly shorter, rarely branched, some hyphal walls locally with a gelatinous hyaline coating that is insensitive to color reagents. Pileocystidia of two types, the first type long cylindrical to distinctly clavate, one-celled, frequently with slightly thickened walls, near margin of the cap measuring (40-)48-75.1-102(-143) × (5.5-)6.5-8.5-10.5(-12) µm, continuing also deeper in trama as "cystidioid hyphae" (or endocystidia), obtuse-rounded, filled with yellowish, amorphous and abundant contents insensitive to sulfovanilin. Near the surface in the pileus center also with smaller, narrower, mucronate-capitulate pileocystidia, (27-)45-64.7-84(-97) × 3.5-5.1-7(-10) µm, with less abundant, yellowish, amorphous contents. **Clamp connections** absent in all parts.

Examined material: UNITED STATES. **Florida**. 23 Oct 1941, *G.S. Burlingham* (NY 00761004, holotypus).

Commentary: In his revision of *Lactarioideae*, Shaffer (1964) pointed out that the holotype of *R. vesicatoria* was apparently missing (perhaps unaware that Singer had published on it in 1958). Shaffer therefore designated a lectotype based on a specimen annotated by Burlingham as "co-type". The holotype was later relocated and rapidly back on file at NY. It was cited under "examined material" by Bills (1986) and was here critically re-examined.

Originally described as fleshy white (Burlingham 1944), the spore print color has later been specified on Romagnesi's scale (1967) as IIb-IIc (Singer 1958) and even IIIa (Bills 1986). *R. vesicatoria* is a species with a narrow ecological habitat (Bills 1986), growing in sand or sandy soil under 2-3 needle pines (*Pinus taeda, P. virginiana, P. australis, P. palustris*, etc…), where it can be extremely abundant. The crowded gills (often compared to *Lactifluus piperatus*), together with the completely inamyloid suprahilar spot and conspicuous, sometimes thick-walled, often clavate pileocystidia together with the very long and filamentous hyphal terminations in the pileipellis allow to distinguish it from other species.

Singer (1958) reported very similar, equally acrid, northwestern collections for the southeastern *R. vesicatoria*, differing however in the absence of a bitter component accompanying the acrid taste. Shaffer (1964) introduced *R. cascadensis* Shaffer (not studied here) as new species for such collections without any other argument than their less distinct smell and lack of bitter taste as compared to *R. vesicatoria*. The latter was later re-examined on the basis of abundant material from the southeastern and eastern United States (Bills 1986), allowing for a better appreciation of its variability. Bills pointed out that the

particular fenugreek smell and bitter-acrid taste (used as main diagnostic features for *R. vesicatoria* in the past) are in fact age-dependent and therefore inconstant characters. *R. vesicatoria, R. inopina* and *R. cascadensis* are three conifer-associated species, mostly with *Pinus*, and all three possess nearly identical spores, contrary to Shaffer's opinion that *R. vesicatoria* has more reticulated spores. However, we observed quite important differences in the pileipellis of the three species: whereas the mild *R. inopina* has the irregularly undulate-flexuous hyphal tips, both acrid species differ by absent to very rare pileocystidia in the western *R. cascadensis* (Shaffer's 1964, as "oleiferous hyphae"), and abundant and very distinctive pileocystidia in the eastern *R. vesicatoria.*

Very similar collections, but possessing thick-walled hyphal terminations were reported from Costa Rica (http://www.nybg.org/bsci/res/hall/vesicatoria.html), Japan (as *R. japonica* Hongo) and Chile (as *R. austrodelica* Singer). Molecular data are needed to shed more light on this species complex.

DISCUSSION

All examined type-specimens belong without a doubt in subsect. *Lactarioideae* as interpreted by most authors. However, also Shaffer observed that the species in *Lactarioideae* can easily be divided in two groups: one group for species with darker spore prints, lower spore ornamentation, hardly amyloid suprahilar spots and more slender hyphal extremities in the pileipellis, and a second group for species with paler spore prints, larger spores having higher, conical warts, distinctly amyloid suprahilar spot, as well as sometimes more inflated hyphal extremities. Bon (1988) introduced subsect. *Pallidosporinae* Bon to place species of the former group (European *R. pallidospora* Romagn., *R. flavispora* Romagn., *R. littoralis* Romagn.). Following this concept, *R. vesicatoria* and *R. inopina* should be placed in *Pallidosporinae* (as well as *R. cascadensis* and *R. angustispora*), whereas *R. brevipes* and *R. romagnesiana*, notwithstanding the somewhat atypical features of the latter, are closer to the *R. delica*-group. In addition, our type study seems to confirm previous observations (Shaffer 1964) that *Pallidosporinae* have a distinctly thicker pileipellis.

Broad species concepts, accepting a single species to be present on several continents and in very different climates, were shared by most of Shaffer's contemporary mycologists (e.g. Hesler 1961). In recent years, however, the use of molecular markers now often refutes transatlantic distributions for a single ectomycorrhizal species (e.g. Buyck & Hofstetter 2011). The *R. chloroides* – *R. brevipes* distinction still needs molecular proof, but as far as the other European species are concerned, even morphological features seem to indicate that they are different (e.g. *R. romagnesiana* versus *R. chloroides* var. *parvispora*).

The *Russula delica* group sensu stricto (excl. *Pallidosporinae*) is known for its association with mycoheterotrophic orchids: *Limodorum* in Europe (Girlanda *et al.*, 2006) and *Monotropa* in the United States (Yang & Phister 2006). *R. brevipes* seems also the preferred host for the obligate parasite *Hypomyces lactifluorum* (Schwein.) Tul. & C. Tul. (Rogerson & Samuels 1994), turning it into one of the more appreciated edibles known as the "lobster mushroom".

In view of providing more reliable features for the identification of American *Lactarioideae*, we provide below a tentative identication key for the North American species largely based on microscopic features.

TENTATIVE KEY TO THE SPECIES OF NORTH AMERICAN *LACTARIOIDEAE*

1. Taste mild or bitter, but not acrid **2**
1. Taste bitter or not, but also at least moderately to extremely acrid, sometimes tardily so **4**

2. Spores (sub)globose, < 7 µm (on average 6.3 × 5.4 µm, Q=1.1-1.2), small and with a distinctly amyloid plage, pileipellis with often two-capitate dermatocystidia in the center and hyphal extremities that are relatively wide and short-celled, pileipellis ca. 100 µm deep. Off-white to pale cream spore print. Under broad-leaved trees (only?). . . . . ***R. romagnesiana***
2. Spores ellipsoid to elongate, on average slightly > 7 µm long, with an inamyloid to poorly amyloid suprahilar spot (if spores > 9 µm long and with amyloid suprahilar spot: milder forms of *R. brevipes*), with long and slender hyphal extremities, pileipellis up to 300 µm deep. Spore print pale to dark cream **3**

3. Spores ellipsoid, 6.5-7.8 × 5-6 µm, Q=1.2-1.4. Northeastern, conifer associated species with irregular, flexuous-undulate terminal cells in pileipellis and on gill edge, and pale cream spore print. . . . . ***R. inopina***
3. Spores elongate, 6.5-8.5 × 4.5-5 µm, Q=1.5-1.7 Southeastern species, apparently strictly associated with *Pinus virginiana*, having remarkably elongate spores and dark cream spore print (IIb-c, Romagnesi) . . . . . ***R. angustispora***

4. Spores on average 9 × 8 µm, with large conical warts ca 1 µm high and distinct amyloid spot. Pileipellis with broad, short-celled extremities. Variable acridity and mostly dense, but not crowded gills. Conifers (exlusively?). . . . ***R. brevipes***
4. Spores on average 7-8 × 5.5-6.5 µm, with convex warts ca. 0.5 µm high and inamyloid suprahilar spot. Gills crowded (as in *Lactifluus piperatus*) and usually very acrid. **5**

5. Eastern species in sandy soil under 2-3-needle pines, with distinct and numerous pileocystidia. Spore print ochre IIIa. . . . . ***R. vesicatoria***
5. Western conifer-associated species, without or with rare distinct pileocystidia (fide Shaffer 1964). . . . . ***R. cascadensis***

Acknowledgements. Mss Lorinda Leonardi, collection manager at NYS, is acknowledged for assistance with type material during our visit. Curators of herbaria MICH and NY are acknowledged for loans of studied material. A study visit to the Paris' Herbarium by Adamčík was funded by a Slovak national grant (Vega 2/0028/11). Travel to US herbaria was funded by an EDIT Research Integration Grant (JPA 5 – IRG, PI Buyck).

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