

Two new *Quercus*-associated *Russulas* from Costa Rica and their relation to some very rare North American species

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Abstract – Detailed descriptions are given for *Russula cartaginis* and *R. quercophila* associated with endemic *Quercus* in Costa Rica. Both species are compared with the closely resembling and very rare *R. eccentrica* from southeastern North America and *R. imitatrix* from northeastern North America. Microscopic features for all four species are here illustrated in more detail for the first time.

Résumé – Des descriptions détaillées sont fournies pour *Russula cartaginis* et *R. quercophila* découvert sous les chênes endémiques du Costa Rica. Les deux nouvelles espèces sont comparées avec deux russules fortement ressemblantes : *R. eccentrica*, du sud-est des Etats-Unis, et *R. imitatrix*, du nord-est des Etats-Unis. Les caractères microscopiques de ces quatre espèces sont illustrés ici en plus grand détail pour la première fois.

INTRODUCTION

This is the first paper in a series on the genus *Russula* (Russulaceae – Basidiomycotina) in Costa Rica. Prior work on the agaric diversity of Neotropical oak forests in Costa Rica during the last three decades was summarized by Halling and Franco-M. (1996) and Halling and Mueller (1999). Recently, Gómez and Alfaro (1996) provided a synopsis of the 44 known species of *Russula* including the description of seven new species. We report here the new *Russula cartaginis* sp. nov. and *Russula quercophila* sp. nov., both apparently very rare species known from single sites in Costa Rica. We provide modern morphological descriptions and illustrations for both new species, as well as detailed illustrations for easier comparison with *R. eccentrica* and *R. imitatrix*, two very rare *Russulas* from the southeastern and northeastern United States, with which these new *Russulas* might be easily confused.

MATERIALS AND METHODS

Microscopic features were examined and sketched by B. Buyck. All microscopic observations and measurements – except for basidiospores – were made in ammoniacal Congo red, after a short aqueous KOH pretreatment to improve tissue dissociation and matrix dissolution. Original drawings for all elements of the hymenium or pellis were made at $\times 2400$. Contents of hymenial and dermatocystidia in the illustrations are indicated schematically, except for a single element where contents are indicated as observed in Congo Red preparations from exsiccata. All elements of the basidiomes were also examined for the presence of ortho – or metachromatic contents or incrustations in cresyl blue as explained in Buyck (1989). Observations and measurements of basidiospores were made in Melzer's reagent. The measurements in italics represent the mean value or – in case of several measured collections – the low and high means among these collections. The mean length/width ratio (Q) gives minimum, mean, and maximum values. We refer the reader to Buyck (1991) for explanation of cystidial terminology.

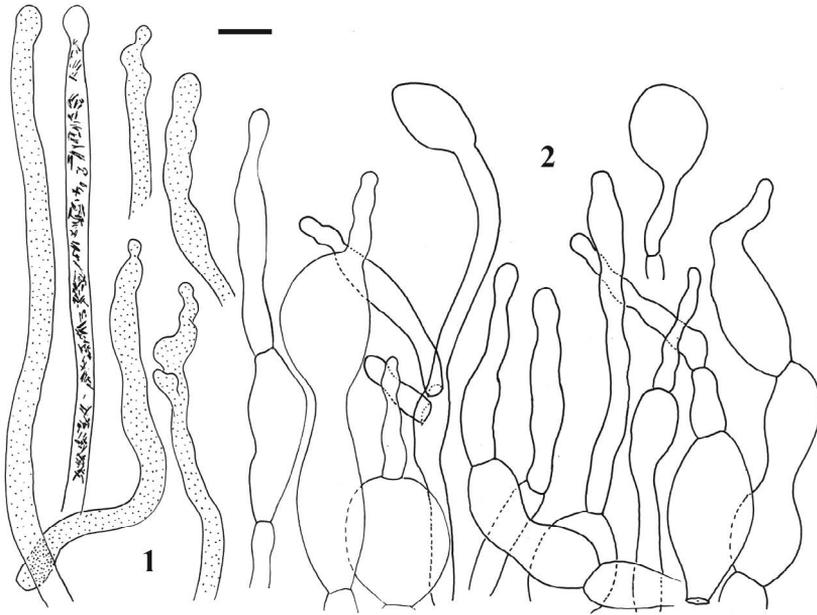
DESCRIPTIONS

1. *Russula cartaginis* Buyck & Halling sp. nov.

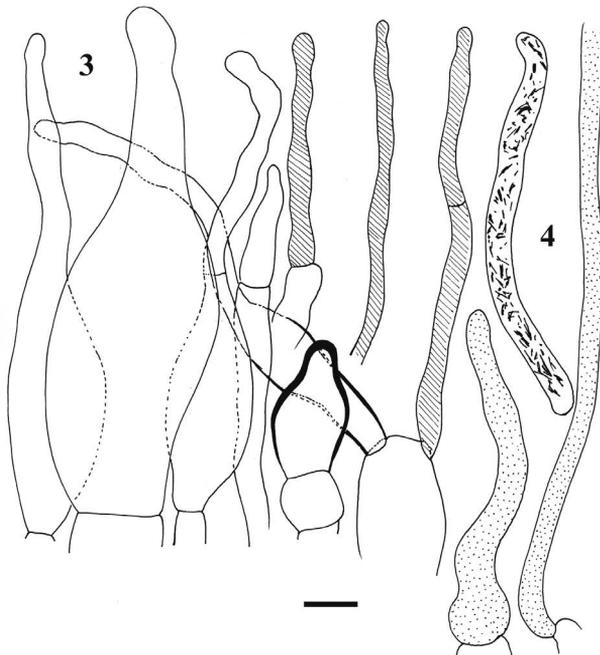
Figs 1-2, 5-8

A R. eccentrica praesertim differt lamellis albidis dein flavescentibus haud rubescentibus lamellarum cellulis marginalibus voluminosis ramosisque. Holotypus: Buyck 01.253 (PC)

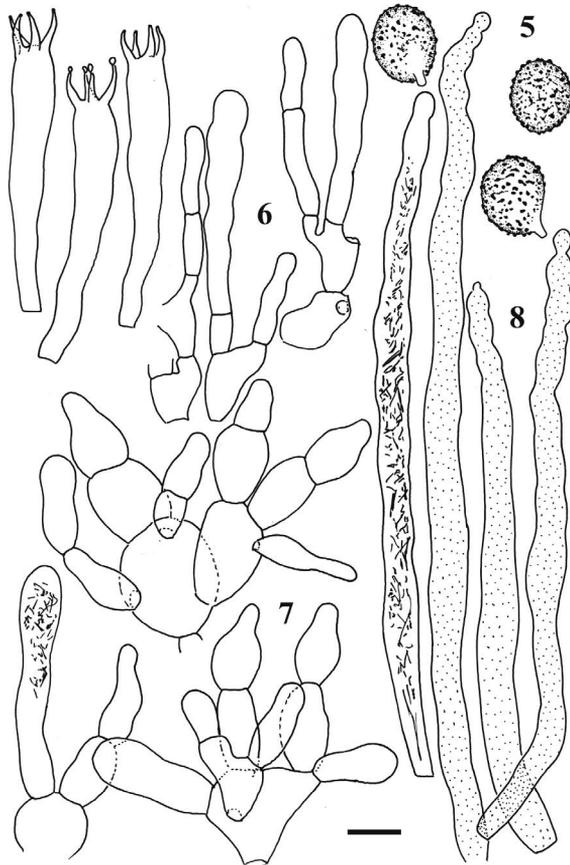
Sporophores occurring in small groups of a few dispersed individuals. **Cap** (25) 40-95 mm diam., broadly depressed, often irregular, near the margin becoming broadly sulcate in age; pellis not viscid when humid, not separable, not pruinose but rather felty-greasy at the touch, first breaking up concentrically, then becoming gradually completely areolate from the margin inwards, a warm dark brown. **Gills** unequal, mostly alternating with 2 much shorter lamellulae, subsistant to distant, not anastomosing in between, not forking near stipe, rarely so closer to the margin, pale when young, a dirty cream, becoming much darker, dirty ochre to pinkish pale brown when old, bruising pinkish brown; edge entire, faintly brown over whole length, less so when aging. **Stipe** (15)20-45(90) \times 9-20(40) mm, more or less cylindrical but often widening near the base, there also often irregularly and strongly furrowed, having a cottony, white to concolorous with the cap but bearing young a pure white pubescence which disappears quickly on handling, becoming glabrous and dirty cream or grayish, turning pink when injured, massive inside but quickly very brittle and irregularly hollowing under a thick cortex. **Context** up to 10 mm thick, extremely brittle, breaking when touched, presenting a faint pinkish tinge when cut, especially the stipe interior, nearly insensitive to FeSO₄. **Taste** mild but nauseous, strongly disgusting. **Smell** very faint, disagreeable, on drying releasing sweeter, almond-like components. **Spore print** not obtained, probably white.



Figs 1-2. *Russula cartaginis* (HOLOTYPE). 1. Extremities of the pileocystidia. 2. Terminal elements of the pileipellis. Scale bar = 10 μ m.

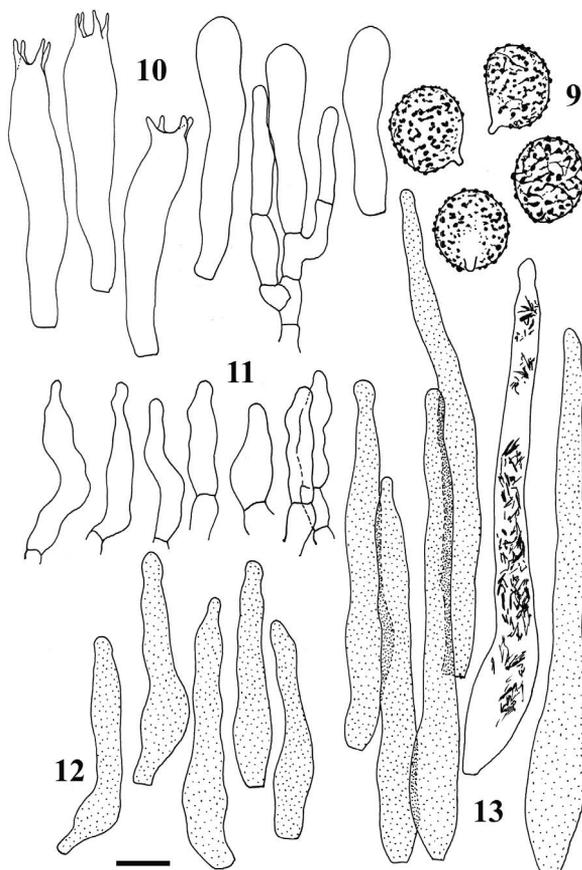


Figs 3-4. *Russula eccentrica* (D. Lewis 4498). 3. Terminal elements of the pileipellis. 4. Extremities of the pileocystidia. Scale bar = 10 μ m.



Figs 5-8. *Russula cartaginis* (HOLOTYPE). 5. Basidiospores. 6. Basidia and basidiola. 7. Marginal cells of the gill edge with one cheilocystidium. 8 Hymenial pleurocystidia. (HOLOTYPE). Scale bar = 5 μ m for spores, 10 μ m for other elements.

Spores (7.0)7.4-7.87-8.21-8.6(8.8) \times (6.0)6.1-6.38-6.76-7.0(7.1) μ m (Q=(1.13)1.16-1.21-1.23-1.27(1.32)), shortly ellipsoid, ornamentation of low obtuse warts of different diam. up to 0.5 μ m high, distinctly amyloid and interconnected or prolonged by very fine lines in varying degrees, mostly very incompletely reticulate, some spores with almost isolated warts; suprahilar spot large but not amyloid. **Basidia** 48-58 \times 8 μ m, clavate-pedicellate, 4-spored; sterigmata stout, 6-8 \times 1-2 μ m. **Cystidia** probably quite numerous, 105 - 240 \times 5 - 8(11) μ m, not emergent, thin-walled, usually very long and slender, cylindrical or slightly tapering towards the top, locally somewhat inflated, mostly in lower part or at the base, contents not greying in sulfovanillin (SV-), although filled with abundant, minutely crystalline contents, near the gill edge dispersed and much smaller, e.g. 57 \times 6 μ m, sometimes with slightly thicker wall. **Marginal cells** occupying the whole edge, the latter completely sterile and covered by a well-developed layer of small but mostly strongly inflated cells composing highly branched tufts with virescens-like structure; cells mostly sphaerical, ampullaceous, ellipsoid to utriformous, some filled with a brown diffuse pigment.



Figs 9-13. *Russula eccentrica* (D. Lewis 4498). 9. Basidiospores. 10. Basidia and basidiola. 11. Marginal cells of the gill edge. 12. Hymenial cheilocystidia. 13. Hymenial pleurocystidia. Scale bar = 5 μ m for spores, 10 μ m for other elements.

Subhymenium except for the 1-3 narrow cylindrical cells immediately under the basidium, composed of rapidly inflating cells towards the trama, forming a well-inflated and relatively loose tissue, which seems like “perforated” by many bases of cystidia that originate in the trama. **Lamellar trama** mainly composed of loose sphaerocytes and relatively short, narrow and slender, often more sinuous cystidioid elements, measuring for ex. $150 \times 7 \mu\text{m}$ diam.. **Pileipellis** orthochromatic in cresyl blue, an ill-structured layer of irregularly inflated, thin-walled elements, mostly containing a brown diffuse pigment; terminal cells very variable, cylindrical, inflated-appendiculate, ampullaceous, clavate etc. Pileocystidia dispersed but distinct, arising from deeper layers, cylindrical, 3-7 μm diam., often capitulate or irregularly constricted to somewhat inflated near the top, filled with abundant crystalline contents, not reacting in sulfovanillin. **Stipitipellis** surface is a loosely interwoven tissue of thin-walled hyphae with abundant caulocystidia reminiscent of the hymenial cystidia with distinct and abundant contents, towards the base of the stipe also with more inflated elements as on the cap. **Clamps** absent.

Specimens examined

COSTA RICA: Cartago, Estrella, 5 km E. of Interamerican Highway at km 31, near town of Estrella, , 9° 46' 04" N, 83° 57' 19" W, 1717 m alt., under *Quercus oocarpa* and *Quercus* sp., Buyck 01.253 (holotypus PC, isotypi F, USJ); *ibid.*, 6 June 1997, Halling 7697; *ibid.*, 24 June 2000, Halling 7917 (paratypim NYBG, USJ)

Comments

A coloured illustration of this species is provided on the New York Botanical Garden website for Costa Rican macrofungi (<http://www.nybg.org/bsci/res/hall/>)

In the field, *R. cartaginis* immediately reminds one of the rare North American *R. eccentrica* with which it shares identical habit and overall colour, except for the gills which do not turn pink with age. Although *R. eccentrica* has been listed as present in Costa Rica in the past (<http://www.nybg.org/bsci/res/hall/>), there is – as far as we are aware – no confirmed report yet of its presence in this country. Nor has this species been recorded from any neighbouring or South American country, but the senior author has been able to examine and confirm the identity of *R. eccentrica*-collections associated with indigenous oak in Mexico. Although a modern description for *R. eccentrica* was given by Bills (1985), more precise illustrations for microscopic features are here provided (fig. 3-4, 9-13) for easier comparison with *R. cartaginis*. Our illustrations show clearly that both species are indeed very close, both possess very similar spores (perhaps a little less ornamented in *R. cartaginis* although more collections are needed to verify) and similar elements in pileipellis and hymenium. It is indeed easier to separate both species in the field rather than under the microscope.

The mention of abundant lactifers for the lamellar trama (Bills, 1985) is not correct according to our interpretation of these structures: lactifers – such as these can be observed in *Lactarius* – are long and repeatedly branching hyphae filled with the typical “milk” content, not the kind of short, cystidoid elements that are observed in the lamellar trama of the *R. eccentrica*-group. Other important features for both species are – in our opinion – the often swollen portion near the base of hymenial cystidia, as well as the sterile cells occupying the gill edge, both unnoticed in previous studies.

In the herbarium of the first author, at least one collection of *R. eccentrica* developed a typical, strong and repelling smell, which has not developed in our collections of *R. cartaginis*, but which was observed in exsiccata of several African *Russula* of sect. *Compactae* s.l., such as *R. pseudolateriticola* Buyck (Buyck, 1999).

Neither *R. cartaginis*, nor *R. eccentrica* possess the typical anastomoses that can be observed in between the gills near the margin cap of *R. quercophila* (see below).

2. *Russula quercophila* Buyck & Halling sp. nov.

Figs 14-19

a R. imitatrice praesertim differt pileocystidiorum absentia atque consociatione quercibus. Holotypus: Buyck 01.219 (PC)

Cap (60)75-100 mm diam., convex, firm, irregularly waving-sinuuous near the margin, the latter smooth to very shortly subsulcate; pellis not separable, entirely cottony or finely woolly when young, later only so near the extreme margin, dry to somewhat greasy, not viscid when humid, smooth or with minute veins or ridges, continuous, very pale cream when young because of the cottony hairy covering, becoming later pale brown to coffee with milk or greyish brown, finally

dark reddish brown to almost black when old. **Gills** 8-12 mm high, decurrent, unequal with numerous lamellulae, brittle, widely spaced, somewhat attenuate and strongly anastomosing towards the cap margin, not forked or only close to the stipe; edge looking paler when young, entire. **Stipe** (30)50-70 × (14)28-38 mm, slightly eccentric, subcylindrical or narrowing to the base or sometimes to the top, very hard and firm, compact, whitish to cream or slightly brownish, paler than cap, more or less irregularly veined – wrinkled and showing some white mycelium at the base. **Veils** absent. **Context** 6-8 mm thick above gill attachment, dirty cream to pale brown, turning browner with time, locally with distinct orange tinge. Mild. Odour not strong at first, but strongly disagreeable to nauseous when old. FeSO₄ very slow and ambiguous reaction, developing greenish as well as a reddish orange tint. **Spore print** not obtained.

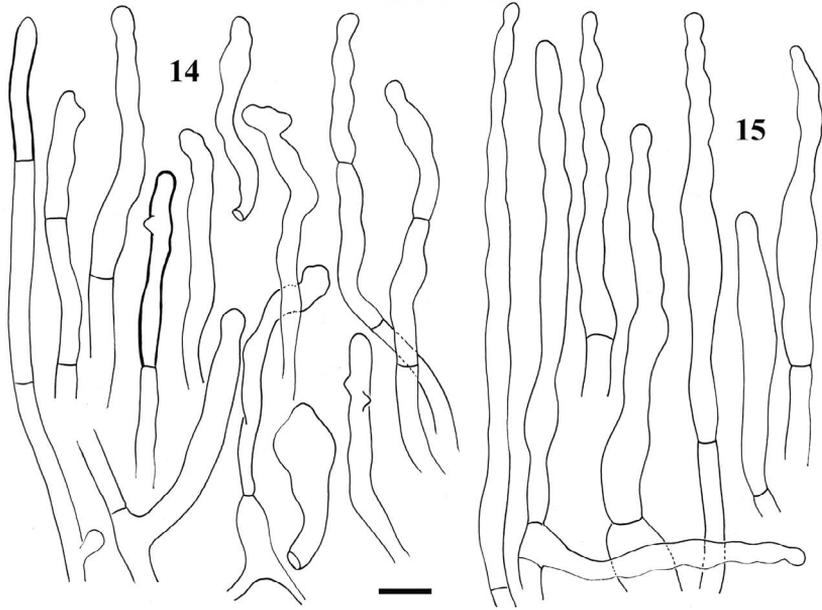
Spores (8.2)8.3-8.64-8.9(9.1) × (7.6)7.7-7.89-8.0(8.2) μm (Q= (1.03)1.10 (1.13)), subglobose, subreticulate with irregular, often large meshes; ornamentation of interconnected, low and obtuse warts, locally fused in short ramifying crests, up to 0.5 μm high, distinctly amyloid; suprahilar spot large but not amyloid. **Basidia** 61-70 (77) × 10-11 μm, clavate-pedicellate, 4-spored, exceptionally 2-spored; sterigmata stout, 6-7 × 1-1.5 μm. **Cystidia** appearing as very abundant on gill sides but, when counting SV+, turn out to be only rather abundant (800/mm²), hardly emergent or if so, then only up to 20 μm, 76-95(130) × 6-9 μm, mostly subcylindrical to narrowly fusiform and often with one or more constrictions near the apex, very thin-walled, distinctly greying in sulfovanillin although contents not abundant and often poorly visible, refringent-granular to finely crystalline. **Marginal cells** occupying the whole edge, which is sterile, terminal cell measuring 30-70 × 5-9 μm, resembling the extremities of stipiti – and pileipellis, also subcylindrical to more irregularly inflated, typically originating from a shortly cylindrical cell which is often inserted on a more inflated, sometimes ramified basal cell, not pigmented and optically empty, thin-walled. **Subhymenium** small-celled. **Lamellar trama** with conspicuously abundant, narrow, oleiferous hyphae, mainly composed of chains of inflated cells, rather than with loosely arranged sphaerocytes. **Pileipellis** orthochromatic in cresyl blue, a one-layered, narrow cutis lying immediately on the sphaerocytes rosettes, composed of narrow to moderately inflated, thin-walled hyphal extremities, the latter also more or less aggregated in tufts towards the cap margin, mostly 4-5 μm diam., many hyphae with conspicuous zebroid incrustations, some filled with brown diffuse pigment, especially in the cap center; endcells often irregularly inflated or sinous, towards the cap margin mostly subulate or narrowing upwards. Pileocystidia not observed. **Stipitipellis** with a remarkably abundant network of oleiferous hyphae or fragments of hyphae; covered with sinuous, more or less aculeate to fusiform, optically empty cells, reminiscent of empty hymenial cystidia. No caulocystidia with differentiated contents. **Clamps** absent.

Specimens examined

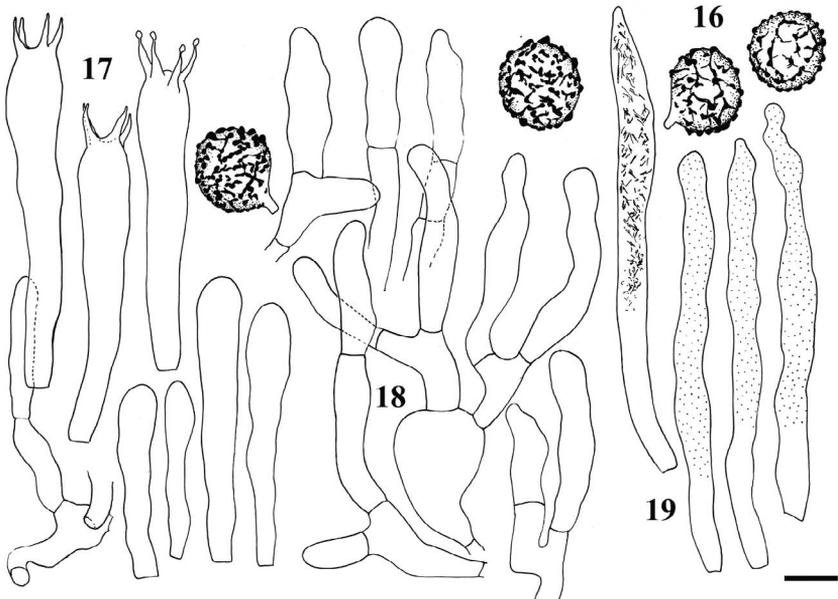
COSTA RICA: **San José prov.**, La Chonta, S. of Interamerican highway towards Laguna/ Cerro Chonta, in *Quercus seemannii* woodland mixed with *Quercus rapurahuensis* and *Q. copeyensis*, 9° 41' 56" N, 83° 56' 31" W, 2340-2400 m, 11 June 2001, in small groups of a few dispersed individuals, on soil, Buyck 01.219 (holotype PC, isotypes F, USJ), *ibid.*, 10 June 2003, Halling 8369 (paratype, NYBG, USJ)

Comments

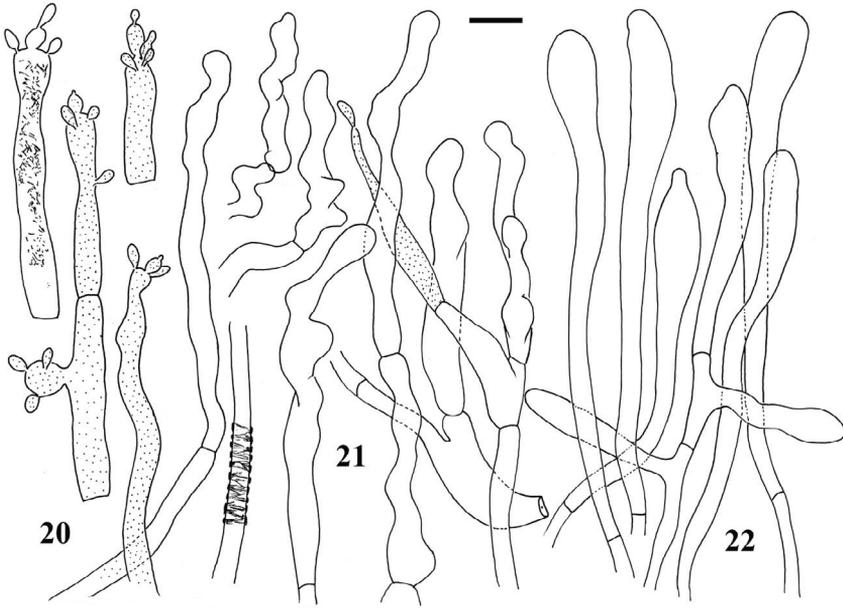
A coloured illustration of this species is provided on the New York Botanical Garden website for Costa Rican macrofungi (<http://www.nybg.org/bsci/res/hall/>)



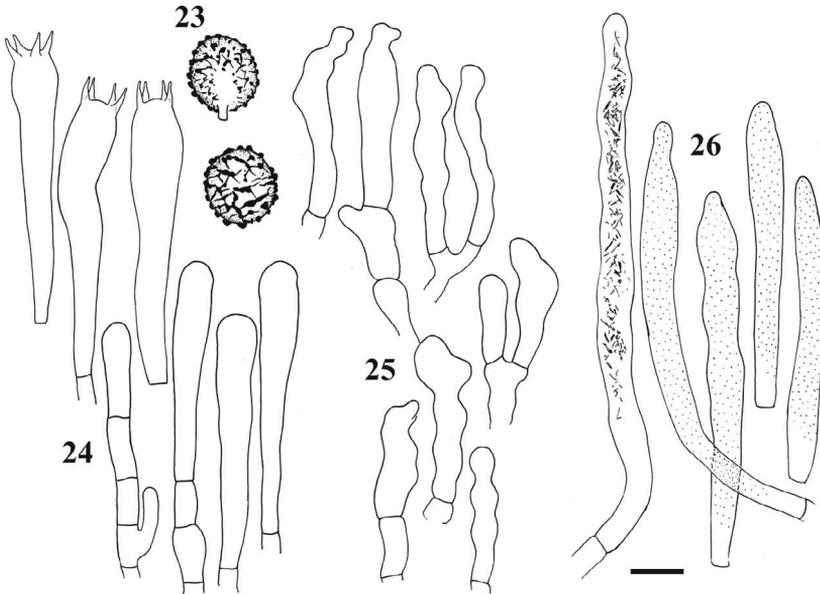
Figs 14-15. *Russula quercophila* (HOLOTYPE) 14. Extremities of the pileus. 15. Terminal elements of the stipitipellis. Scale bar = 10 μ m.



Figs 16-19. *Russula quercophila* (HOLOTYPE).16. Basidiospores. 17. Basidia and basidiola. 18. Marginal cells of the gill edge. 19. Hymenial pleurocystidia. Scale bar = 5 μ m for spores, 10 μ m for other elements.



Figs 20-22. *Russula imitatrix* (R. Fatto 271, from type locality) 20. Pileocystidia. 21. Extremities of the pileipellis with indication of incrustations in part of one fragment. 22. Terminal elements of the stipitipellis. Scale bar = 10 μ m.



Figs 23-26. *Russula imitatrix*. (R. Fatto 271, from type locality). 23. Basidiospores. 24. Basidia and basidiola. 25. Marginal cells of the gill edge. 26. Hymenial pleurocystidia. Scale bar = 5 μ m for spores, 10 μ m for other elements.

Russula quercophila is in Costa Rica associated with endemic *Quercus*. Using the existing American literature for identification, it would key out to the northeastern North American *Russula imitatrix* Homola & Shaffer, placed in section *Compactae* s.l.. This is yet another extremely rare fungus, which is – to our knowledge – only known from the type locality near Milford in Maine, USA, and from one other collection near Kentville (Nova Scotia, Canada). It was only quite recently discovered (Homola & Shaffer, 1975) and seems to be associated with gymnosperms, most likely hemlock (*Tsuga canadensis* Carr.) or white pine (*Pinus strobus* L.).

Under the microscope, *R. quercophila* is strongly similar to *R. imitatrix*. (figs. 20-26) because of more or less tortuous elements in the pellis and on the gill edge; both species also have a small-celled, almost filamentous subhymenium, both have a remarkably abundant network of slender (often fragments of) oleiferous hyphae under the surface of the stipe, and in both species, pellis of cap and stipe is covered with optically empty cells, reminiscent of empty hymenial cystidia.

When looking more closely, however, *R. imitatrix* has pileocystidia, which – although small and having very poorly differentiated, granular-refringent contents – are easily found because of the presence of several minute apical appendices. Its pileipellis is also composed of even more strongly irregular, contorted terminal elements. We were unable to demonstrate the presence of pileocystidia in the Costa Rican *quercophila*-collections.

Russula quercophila can also very easily be mistaken for the North American *R. eccentrica* (for comparison see comments under *R. cartaginis* above) and especially for the Costa Rican *R. cartaginis* itself because of the brown coloured cap, pale stipe, spaced, pinkish ochraceous gills and mild taste. *R. cartaginis*, however, has a more sulcate cap margin and the cap is not woolly-pubescent (only the stipe), gills are not anastomosing towards the margin and are white when young (pinkish ochraceous from the start in *R. quercophila*), and the stipe is much more irregularly veined and often wider near the base, instead of narrowing downwards as in *R. quercophila*. The former species turns also very dark in the herbarium, whereas *R. quercophila* remains pale upon drying, especially in the gills. Under the microscope, differences in spore characters and pileipellis composition exclude any confusion.

Because of its features, both macro- and microscopical, *R. quercophila* fits perfectly in the subsection *Albospissinae* Buyck as described and illustrated in Buyck (1993), until now still monospecific and only known from tropical Africa. *R. albospissa* differs essentially by features of the spores and the presence of long, cylindrical dermatocystidia in the pileipellis having an incrustated wall exactly as observed in the zebroid-encrusted – but empty – hyphae in the cap of *R. quercophila*.

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