

***Russula lentiginosa* spec. nov. from West Virginia, USA: a probable link between tropical and temperate *Russula*-groups**

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Abstract – *Russula lentiginosa* nov. sp. is described from West Virginia, USA, as a close relative to the European *R. viscida* and the American *R. ballouii* and related species, which should all be placed in the same subsection. The authors suggest that this group is a probable link between temperate *Russulae* and the predominantly tropical section *Fistulosae*.

Résumé – *Russula lentiginosa* nov. sp. est décrite de la Virginie de l'Ouest, E.U., comme une espèce proche de *R. viscida*, une russule européenne, et de *R. ballouii* et espèces proches. Toutes sont à transférer dans une même sous-section. Ce groupe autour de *R. viscida* est interprété comme un maillon entre les russules des régions tempérées et une section typiquement tropicale, les *Fistulosae*.

INTRODUCTION

A considerable number of collections made by the second author in the floristically interesting southern Appalachians belong to a well-characterized, yet still unknown *Russula*, which is described here as *Russula lentiginosa* sp.nov. Similarities in macro- and especially in microscopical features place it close to the European *R. viscida*, a species not yet recorded from the eastern United States, and some American species presently placed in subsection *Fistulosinae* (Heim) Singer such as *R. ballouii*.

MATERIAL AND METHODS

The color notations indicated in the descriptions are from Kornerup and Wanscher (1978). Microscopic features were examined and sketched by B. Buyck and compared with other species and/or type specimens. 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 $\times 2400$. In the figures, the long 10 μm scale line is for the basidiospores and the short one for the other elements. 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 dried material. 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. Measurements are given according to Heinemann and Rammeloo (1985) and are based on 20 spores (n) per specimen for each species. The measurements in italics represent the average among the measured 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.

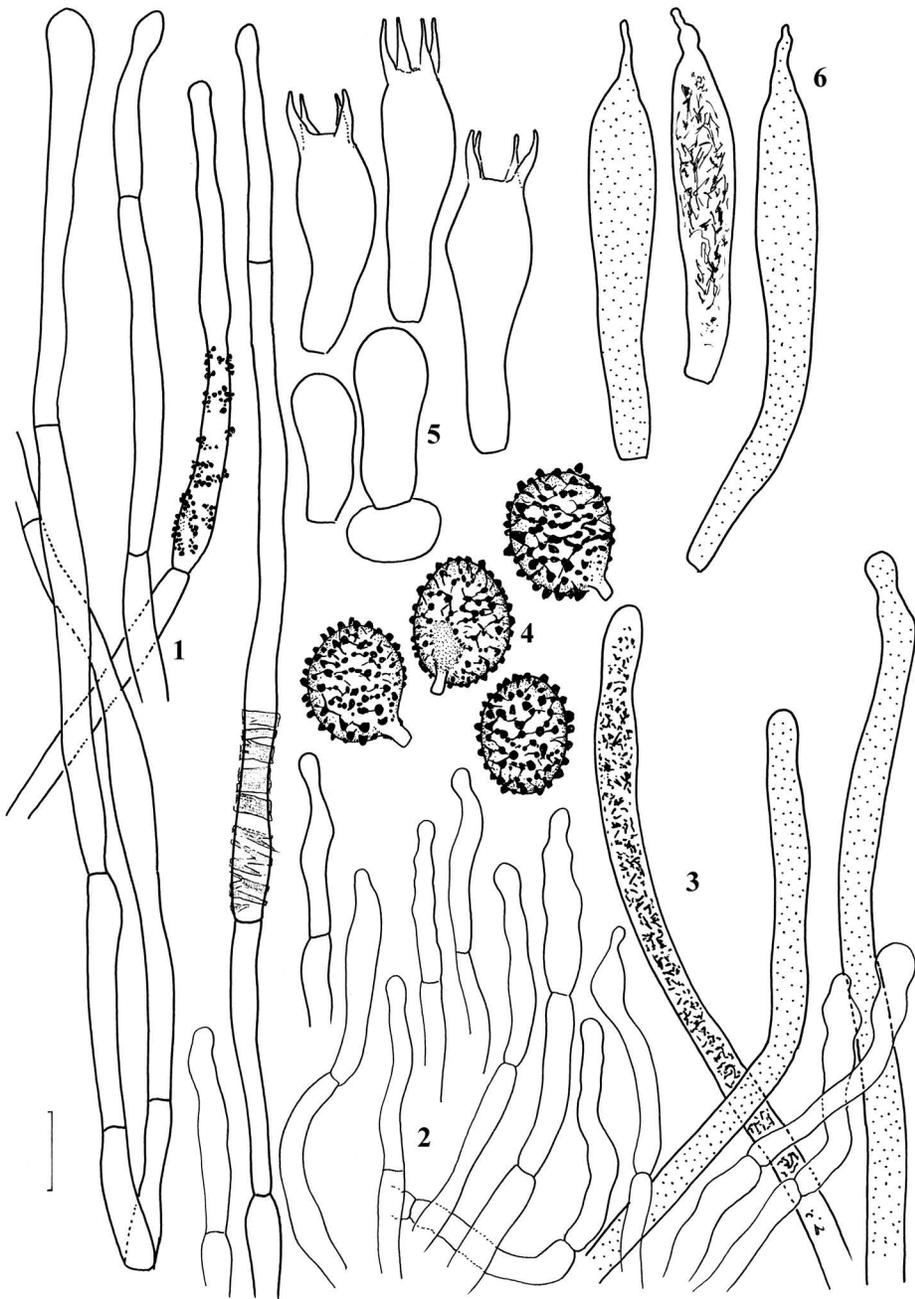
DESCRIPTION

Russula lentiginosa Buyck & Mitchell spec. nov. – Figs. 1-6, 13.

Russula viscida praecipue differt pileo velo brunneo-ochraceo valde distincte obfecto, colore pileo minor variabile, associatione arbore atque distributione geographica.

Holotypus DMWV 03-500, in herbario DEWV conservatus sub numero 3826, isotypus PC.

Pileus (2.7)4-8.5(11) cm diam., shape regular in outline, broadly convex to plane, then somewhat depressed or rarely funnel-shaped in age; margin plane to slightly incurved, obscurely striate for 2-5(13) mm, separable for 1/3-1/2 of the radius, dull or slightly shiny, deep red (9-10BCD6-8) with the center typically very dark, nearly black, sometimes discolored and more yellowish brown, with a very dense ochre, floccose to granular covering, particularly in the center of young specimens, more diffuse with age; trama (2)3-5(6) mm at mid-radius, firm, white, pink under the cuticle, unchanging when cut but slowly browning with age or from insect damage. **Gills** adnate, adnexed, or slightly decurrent, close to sub-distant with few lamellulae of varying length, not strongly narrowing towards the pileus margin, hardly to moderately forking near the stipe and occasionally elsewhere, 3-5(8) mm high at mid-radius, cream to whitish, brownish where insect damaged; edge entire, concolorous. **Stipe** central to slightly eccentric, usually not exceeding pileus diam. in length, 3-9 \times 1.2-2(2.7) cm, equal to subclavate or tapering towards the base, white to cream and with ochre to brownish scales or floccules toward the base, velvety elsewhere, firm; trama stuffed, very slowly browning when exposed. **Chemical reactions** stipe trama salmon to pink with FeSO_4 . **Taste** quickly and strongly acrid in gills, almost mild to tardily or moderately acrid in cap trama. **Smell** not distinctive. **Spore print** whitish (between Romagnesi code I(a)b, Dagron code 3). **Exsiccatum** retaining the pigmentation of the cap of the fresh fungus without much alteration.



Figs 1-6. *Russula lentiginosa* (holotype) 1. pigment-incrusted hyphae of the pileipellis, with indication of pigment scales and bands in part of one element as seen in cresyl blue. 2. terminal elements of the suprapellis. 3. cystidioid hyphae of subpellis and trama with contents indicated in one element as seen in Congo red. 4. spores in Melzer's reagent 5. basidia and basidiola. 6. hymenial cystidia. Scale bar = 5 μ m for spores, 10 μ m for the other elements.

Spores ellipsoid, (7.3) 7.8 – 8.30 – 8.8 (9.4) × (6.2) 6.4 – 6.71 – 7.0 (7.6) μm (Q = 1.12 – 1.24 – 1.36); ornamentation dense, composed of many, blunt, distinctly amyloid warts, up to 1 μm high, mostly interconnected with very fine lines, sometimes catenulated, resulting in a dense, complete to incomplete reticulum; suprahilar spot large, graying in Melzer's reagent. **Basidia** 27-38 × 10-12 μm, short and distinctly clavate, four-spored; sterigmata stout, 5-8 × 1.5 μm. **Hymenial cystidia** both on edge and sides of the gills, moderately numerous (pleurocystidia 1200-1500/mm²), slender and quite short, hardly emerging beyond the hymenium level, originating mostly in the subhymenium, sometimes from within the lamellar trama and then up to 140 μm long, fusiform to clavate, mostly appendiculate, 51-69 × 7-9 μm, thin-walled; contents abundant, crystalline, turning black with SV. **Marginal cells** not differentiated. **Subhymenium** relatively large-celled. **Lamellar trama** mainly composed of sphaerocytes intermixed with abundant, slender, cylindrical, mostly unbranched, cystidioid elements up to 5 μm diam., not breaking up easily when squashed. **Pileipellis** orthochromatic in cresyl blue with very distinct pigment-incrusted hyphae on top of the gelified pellis, obscurely two-layered; subpellis hyphae mostly 2-4 μm wide, often irregularly sinuose or inflated, thin-walled, slightly separated by gelification, forming a loose tissue; suprapellis a dens trichodermal layer of more or less gelified, sinuous, irregular endings of the subpellis-hyphae and long, cylindrical and slender, sparsely septate extremities with yellowish brown incrusted walls and refringent contents, at least towards the apical cells, these generally lying dispersed or aggregated in tufts on top of the smaller elements, only 2-4(6) μm wide but up to several hundred μm long, rounded at the apex, rarely moniliform or capitate. Pileocystidia in suprapellis not observed, present and abundant in subpellis and continuing as 'lactifer'-like hyphae in trama underneath, with coarsely granular to crystalline contents. **Stipitipellis** lacking any particular structure, composed of undifferentiated endings of hyphae encrusted with a yellowish brown pigment near the base and abundant cylindrical caulocystidia. **Clamps** absent.

Etymology

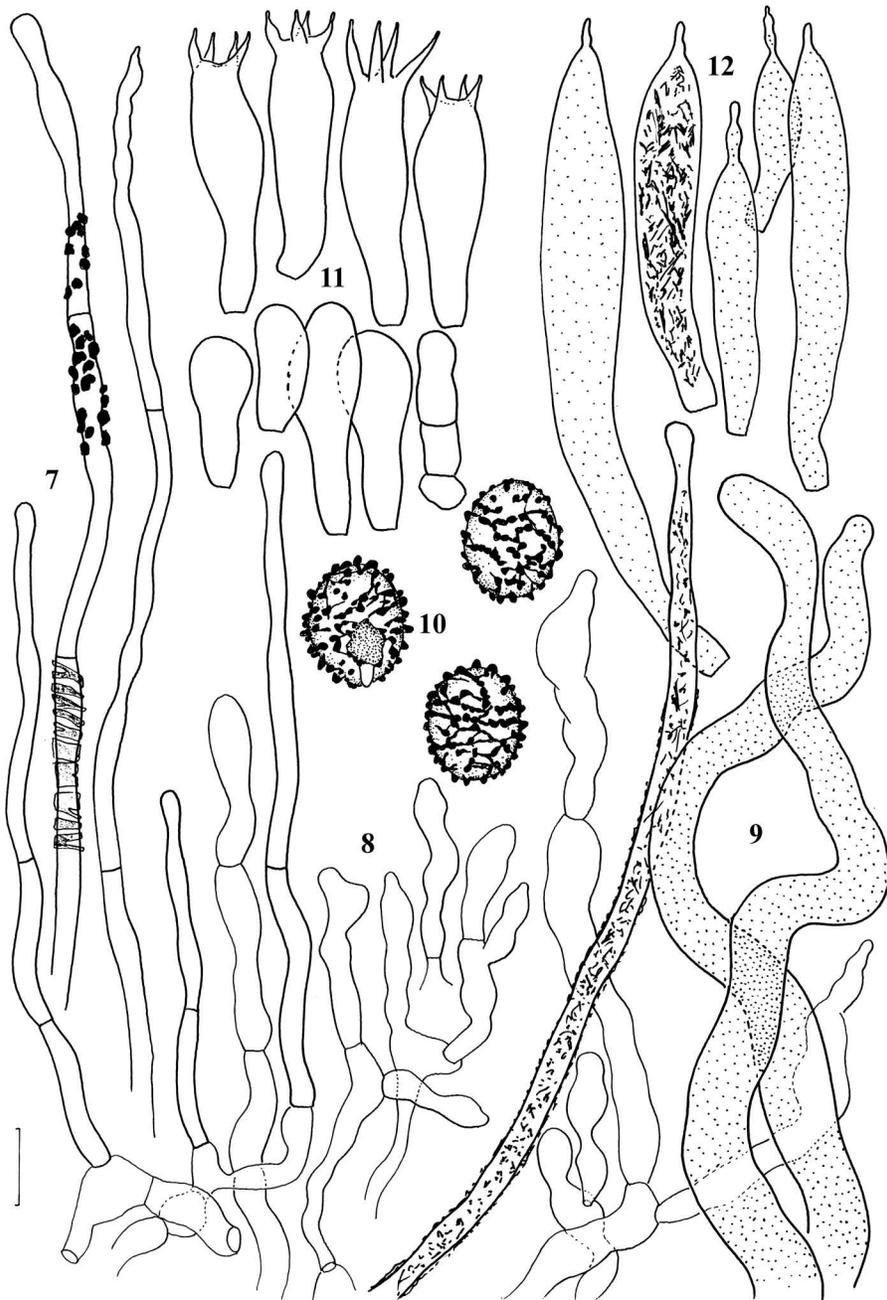
The term "*lentiginosus*" means "covered with minute dots, as from dust" (Stearn, 1983), referring to the ochraceous floccose covering of the cap and stipe base.

Habitat

Mostly scattered among forest litter in broadleaf or mixed broadleaf/*Tsuga canadensis* woods.

Distribution

WEST VIRGINIA: **Randolph Co.**, Three Springs Trail, Glady Fork, in forest of *Betula alleghaniensis*, *Tsuga canadensis*, *Liriodendron tulipifera*, *Prunus serotina*, *Acer rubrum* and *Fagus grandifolia*, 18 Sept. 2000, Donna Mitchell WV00-1028 (1221, 1222); Conely Run, in forest of *Quercus rubra*, *Q. prinus*, *Carya* sp., 21 Aug. 2000, Donna Mitchell WV00-995 (1266); Shaver's Mt., in forest of *Picea rubens*, *Quercus rubra*, *Fraxinus americana*, 20 July 2000, Donna Mitchell WV00-480 (1208); Stuart Memorial Drive, in forest of *Quercus rubra*, *Q. alba*, *Tsuga canadensis*, *Rhododendron maximum*, 21 July 2000, Donna Mitchell WV00-488 (1209); Stuart Park, 7 Sept. 2003, in forest of *Quercus* sp. and *Tsuga canadensis*, Don Yeltman WV03-1229. **Barbour Co.**, Laurel Mt., in forest of *Quercus rubra*, *Q. prinus*, *Fagus grandifolia*, *Betula lenta*, *Liriodendron tulipifera*, *Acer rubrum*, 15 Sept. 2001, Donna Mitchell WV01-1218 (2445); 4 July 2000, Donna Mitchell WV00-279 (1202); 19 Aug. 2000, Donna Mitchell WV00-779 (1219); 24 Sept. 2000, Donna Mitchell WV00-1053



Figs 7-12. *Russula ballouii* (D.Lewis 5801). 7. pigment-incrusted hyphae of the pileipellis, with indication of pigment scales and bands in part of one element as seen in cresyl blue. 8. terminal elements of the suprapellis. 10. cystidioid hyphae of subpellis and trama with contents indicated in one element as seen in Congo red. 11. spores in Melzer's reagent 5. basidia and basidiola. 12. hymenial cystidia of edge (2 smaller ones) and sides of the gills. Scale bar = 5 μ m for spores, 10 μ m for the other elements.



Fig. 13. *Russula lentiginosa* (holotype (top), DEWV 1222 (bottom) copyright W.Roody).



Fig. 14. *Russula ballouii* (DEWV 984 (top), DEWV 1720 (bottom) copyright W.Roody).

(1224); 24 July 2003, Donna Mitchell WV03-500 (3826, holotype), 6 Sept. 1997, Donna Mitchell WV97-9 (1306). **Tucker Co.**, Left Fork of Clover Run, in forest of *Tsuga canadensis*, *Quercus rubra*, *Q. alba*, *Betula lenta*, *Carpinus caroliniana*, *Liriodendron tulipifera*, 21 Sept. 2001, Donna Mitchell WV01-1399 (2582) (all collections at DEWV with herbarium numbers indicated between brackets).

DISCUSSION

This species can be characterized as a very acrid, pale-spored *Russula* with a deep red pileus and white stipe, having a characteristic ochre floccose to squamulose covering, both near the stipe base and on the cap. Microscopically, the structure and composition of the pellis and the features of basidia and hymenial cystidia remind one immediately of some tropical species in section *Fistulosae* (Heim ex Singer) Buyck, in particular some African species of subsection *Testaceoaurantiacinae* Buyck (see Buyck 1993 for discussion and illustration of features), but also of the European *R. viscida* Kudrna. The latter is one of those taxonomically isolated and therefore very interesting species in Europe. It is traditionally placed in subsection *Melliolentinae* Singer, but was recently transferred by Sarnari to a group of its own, *Viscidinae*, first at subsectional and later at sectional level (Sarnari, 1991, 1998) in subgenus *Russula* Romagnesi.

Viscidinae, as proposed by Sarnari (l.c.), was created for a very limited number of European species, essentially *R. viscida* with some satellite taxa (often

considered as forms, varieties or even as synonyms by many authors) and *R. ochroleuca* Fr. Their principal common characteristic is the presence of yellow-brown pigmented hyphae near the stipe base and in the pileipellis. The pigment is encrusting and stains bright blue in cresyl blue, making these hyphae look like strongly incrusting primordial hyphae. In the European species, these hyphae may form a macroscopically visible scurf near the stipe base, but not on the cap as in *R. lentiginosa*.

Although refuted by other contemporary authors, in particular Reumaux (1996), this grouping of *R. viscida* and *R. ochroleuca* was a few years later supported by a molecular study of ITS sequences of European *Russula* that placed both species next to *R. atropurpurea* in a basal position within the clade corresponding to subgenus *Russula*, whereas *R. melliolens* was situated in a very different clade (Miller & Buyck, 2002). Additional molecular support for subsection *Viscidinae* is found in two other studies (Eberhardt 2002, Beenken 2003) that also introduce new morphological-anatomical data for ectomycorrhiza to back up Sarnari's view.

In the field, *R. atropurpurea*, *R. ochroleuca* and *R. viscida* may resemble each other greatly, up to a point that they cannot be distinguished without microscopical examination, not even by experienced mycologists. Especially *R. atropurpurea* and *R. viscida* are very versatile in color and possess yellow forms that are easily taken for *R. ochroleuca* in the field. All three are type-species of infrageneric taxa at various levels in *Russula*, and *R. viscida* has no priority over both other type-species when grouped together; e.g. Beenken (2003) adopts *Ochroleucinae* (Romagn.) Bon as the correct name for subsection *Viscidinae*, including *R. viscida* and *R. ochroleuca*.

R. atropurpurea, which is provisionally best separated from these species in *Atropurpurinae*, lacks pigmented hyphae in the pellis, but shares with *R. ochroleuca* the strongly graying context, a phenomenon neither observed in *R. viscida*, nor in *R. lentiginosa*.

We propose to place *R. lentiginosa* provisionally in subsection (rather than section) *Ochroleucinae* (= *Viscidinae*) until a more comprehensive molecular phylogeny including also tropical and North American taxa becomes available. Indeed, *Russulas* with very similar characters in North or Central America include the acrid, orange-brown *R. ballouii* Peck and *R. tennesseensis* Singer, a likely synonym of the former (Bills, 1984), as well as the hardly acrid to mild *R. matoubensis* Pegler, all placed in subsection *Fistulosinae* of Section *Ingratae* Quélet in American literature (e.g. Singer 1986, Bills 1984). Subsection *Fistulosinae*, however, was originally described (Heim 1938) for some tropical African taxa that are quite different from these American species, both in the field and under the microscope. We propose, therefore, to place *R. ballouii* (Figs. 7-12, 14) and closely allied species provisionally in the same subsection as *R. viscida* with which they share similar features of hymenium and spores as well as a similar pellis-structure in stipe and cap. There are some minor differences between the taxa from both continents: the European *R. viscida* and *R. ochroleuca* possess slightly longer basidia, denser lamellar trama, and *R. viscida* also more differentiated pileocystidia and small, but recognizable marginal cells. There are also some interesting differences in the general aspect of the pigment incrustations between the various species, especially when observed in cresyl blue, but more specimens need to be compared in order to give it some diagnostic value. Spore ornamentations are very similar in all species and the small variations here observed, such as the frequently catenulate warts for *R. ballouii* when compared to more connected, somewhat larger warts of *R. lentiginosa* need to be confirmed on a larger number of collections.

Certain tropical species-groups of section *Fistulosae*, such as *Testaceo-aurantiacinae* Buyck, *Brunneofloccosinae* Buyck or *Pachycystidinae* Singer possess probably enough differences in type of spore ornamentation and some other characters to remain separate, but are certainly also very close to the discussed species.

According to the above scenario, subsection *Ochroleucinae* (= *Viscidinae*) links a pale-spored and principally acrid species-group of predominantly tropical distribution (section *Fistulosae*) to the core group of *Russula* in temperate regions (subgenus *Russula* sensu Romagnesi), a hypothesis already suggested by Buyck 15 years ago (1989b, fig. 92).

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REFERENCES

- BEENKEN L., 2003 — *Die Gattung Russula: Untersuchungen zu ihrer Systematik anhand von Ektomykorrhizen*. PhD. Dissertation, Univ. Muenchen, Germany.
- BILLS G., 1984 — Southern Appalachian Russulas. I. *Mycologia* 76 (6): 975-1002.
- BUYCK B., 1989 — Utilité taxonomique du bleu de crésyl dans le genre *Russula* Persoon. *Bulletin de la Société mycologique de France* 95: 1-6.
- BUYCK B., 1989b — Révision du genre *Russula* Persoon en Afrique Centrale: partie générale: 318 p., 98 fig.; partie descript. 590 p., 582 fig., iconogr. M.E. B. 444 pl. Rijksuniversiteit Gent.
- BUYCK B., 1991 — The study of microscopic features in *Russula*. 2. Sterile cells of the hymenium. *Russulales News* 1: 62-85.
- BUYCK B., 1992 — Checklist for tropical Russulae and their type specimens. *Russulales News* special issue 1: 1-99.
- BUYCK B., 1993 — *Russula* I (*Russulaceae*). In: *Flore illustrée des champignons d'Afrique Centrale* 15:337-407, pl. 55-68.
- EBERHARDT U., 2002 — Molecular kinship analyses of the agaricoid Russulaceae: correspondence with mycorrhizal anatomy and sporocarp features in the genus *Russula*. *Mycological Progress* 1 (2): 201-223.
- HEIM R., 1938 — Les lactario-russulés du domaine oriental de Madagascar: essai sur la classification et la phylogénie des Astérosporales. *Prodrome à une flore mycologique de Madagascar et dépendances*. 1196 p., 59 fig., 4 pl. Paris.
- HEINEMANN P & RAMMELOO J., 1985 — De la mesure des spores et de son expression. *Agarica* 6 (12): 366-380.
- KORNERUP A. & WANSCHER J.H., 1978 — *Methuen handbook of colour*. 3rd ed. London: Methuen & Co. Ltd. 252 p, 30 pl.
- MILLER S.L. & BUYCK B., 2002 — Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. *Mycological Research* 106 (3): 259-276.
- PEGLER D. N. & SINGER R., 1980 — New taxa of *Russula* from the Lesser Antilles. *Mycotaxon* 12: 92-96.
- REUMAUX P., 1996 — *Russules rares et méconnues*: 294 pp. Ed. Fédération mycologique Dauphiné-Savoie.
- SARNARI M., 1991 — *Russula* nuove o interessanti dell'Italia centrale e mediterranea. XV contributo: La sottosezione *Melliolentinae*. *Micologia et Vegetatio Mediterranea* 6 (2): 111-132.
- SARNARI M., 1998 — *Monographia illustrata del genere Russula in Europa*. Tomo primo. 799 p. Associazioni Micologica Bresadola. Trento.

- SINGER R., 1986 — *The Agaricales in modern taxonomy*. 4th ed. Königstein, Germany: Koeltz Scientific Books. 981 p.
- SINGER R., ARAUJO I. & IVORY M.H., 1983 — The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. *Beihefte zur Nova Hedwigia* 77: 1-352.
- STEARNS W.T., 1983 — *Botanical Latin*. Third ed., revised. 566 pp. David & Charles publishers, Newton Abbot, Devon.