Type studies in *Russula* subsection *Maculatinae* and affiliated taxa: four species as interpreted by Henri Romagnesi

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Observations on the type material of four species classified in or affiliated to *Russula* subsect. *Maculatinae* selected by Henri Romagnesi are presented in this study: *R. badia*, *R. globispora*, *R. maculata* and *R. vinosopurpurea*. Micromorphological characters are extensively described and illustrated. Differences recognised on the type specimens are compared and discussed with those used in the literature. Epitypes for *R. badia* and *R. maculata* are designated.

Keywords: micromorphology, Russulaceae, fungi, Europe.

Research of the genus *Russula* Pers. (Russulales, Basidiomycota) has recently developed to modern standards (Buyck 1991 a, b) in Europe on European material. Species concepts and techniques used for taxa delimitation within the genus have been adopted mostly by accepting the European monographs by Romagnesi (1967, 1985) and Sarnari (1998, 2005). Despite the popularity and frequency of specialized *Russula* publications from the area (http://www.mtsn.tn.it/russulales-news/), European descriptions of most species lack not only several recently introduced characters, but also precise and statistically supported descriptions of widely used characters (Buyck 1995, p. 13–14). On the other hand, ongoing type studies in the genus *Russula* Pers. from the United States (e.g. Adamčík & Buyck 2011 a, b; Buyck & Adamčík 2011 a, b) used a precise and innovative approach for the description of micromorphological characters, which in many cases revealed a lack of reliable descriptions of European taxa for comparison with North American taxa. Such carefully elaborated studies are also known from other continents; e.g. type studies and descriptions on African members of *Russula*

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subsect. *Mamillatinae* Buyck (Buyck 2005), on Australian sequestrate species (Lebel & Tonkin 2007), and on some Indian species (Das *et al.* 2010).

The deficiency of European type studies and/or precise descriptions is illustrated e.g. by Buyck (2005) who compared the American species *R. xantho* Shaffer with the European *R. aurea* Pers.; he did not refer to any previously published description of the latter and used his own observations. Insufficient knowledge of microscopic structures and their variability in European *Russula* members results in ambiguous species concepts and the description of new taxa (e.g. Reumaux *et al.* 1996, Socha *et al.* 2011). The general problems mentioned above are also a current subject in *Russula* subsect. *Maculatinae* (Romagn.) Konrad & Joss. that is topic of this study. Our goal is to define sufficiently the species concept of selected and traditionally accepted taxa classified in or affiliated to this subsection based on type specimen studies.

Romagnesi (1967) characterized *Russula* subsect. *Maculatinae* (on the rank of section) based prevailingly on red caps, yellow spore print, acrid taste of the flesh, no diverticula on the hyphae in the pileipellis and pileocystidia with the contents turning grey in sulfovanilin. Sarnari (1998) classified most of the taxa accepted by Romagnesi (1967) in the *Maculatinae* in his *Russula* subsect. *Urentes* Maire, the group he had interpreted in broader taxonomic concept. Sarnari (1998) included in *Urentes* also the taxa with diverticula on the hyphae in the pileipellis (by Romagnesi classified in the "sect. *Urentinae* Maire"), as well as an additional species *R. badia* Quél. The last species differs from the other members of *Maculatinae* by its slightly paler yellow spore print and the preference for conifers as mycorrhizal partners, the features why Romagnesi (1967) classified it in *Russula* subsect. *Sardoninae* Singer. The delimitation of both these infrageneric groups as treated by Romagnesi and Sarnari has not been confirmed by a serious phylogenetic study; they have been typified by different species names, however, it is unclear if the species refer to the same phylogenetic group. Because of nomenclatural and taxonomic confusions in *Russula* subsect. *Urentes* (Sarnari 1998, p. 112), we prefer to use the name *Maculantinae* for the group studied here.

This paper is the part of an ongoing revision of the microscopic features of all existing type material of *Russula* subsect. *Maculatinae* and affiliated taxa. In the first part of our type studies, we have described microscopic characters of *R. decipiens* and three other taxa with one-celled pileocystidia: *R. deceptiva* Romagn., *R. decipiens* var. *vermiculata* nom. inval. and *R. romagnesii* Singer ex Romagn. (Adamčík & Jančovičová 2012). This study includes four species known as having septate pileocystidia; they were accepted and typified by Romagnesi (1967) (see the note on the typification below) and classified within *Russula* subsect. *Urentes* by Sarnari (1998): *R. badia*, *R. globispora* (Blum) Bon, *R. maculata* Quél. and *R. vinosopurpurea* Jul. Schaeff. All of these species were described before the publication of the monographic study by Romagnesi (1967), who introduced a new classification and sev-

eral novelties in the descriptions of *Russula* species, thereby especially improving the microscopy: *R. badia* was described by Quélet (1881), *R. globispora* as a variety of *R. maculata* by Blum (1952), *R. maculata* by Quél. & Roze in Quélet (1878), and *R. vinosopurpurea* by Schaeffer (1938). Neither of these species has a type specimen designated by the original author nor do they have authentic herbarium material connected to their protologues. Sarnari (1998), therefore, designated illustrations cited in protologues as lectotypes for the species described by Quélet and designated the neotype for *R. vinosopurpurea* (Romagnesi's specimen labelled as "type"). In his voluminous and comprehensive monograph, Romagnesi (1967) chose for each accepted species a representative specimen among his herbarium material and he labelled it as "type". Sarnari (1998, 2005) accepted Romagnesi's "types" as valid neotypes, if they did not contradict priority of existing original material and if he agreed with the concept used by Romagnesi, and this approach seems not to contradict with the rules of the International Code of Nomenclature for algae, fungi, and plants (according to personal communication with the nomenclatural experts D. L. Hawskworth, S. Redhead and K. Marhold). Because the recent concept of most widely accepted taxa refers to their interpretation by Romagnesi and because Quélet did not provide his species with relevant descriptions of microscopic structures, we are designating here two of Romagnesi's "types" as epitypes for species described by Quélet (*R. maculata* and *R. badia*).

There are four other red-capped species without diverticulate hyphae in the pileippelis that were accepted in the subsect. *Urentes* by Sarnari (they also fit to the concept of *Maculantinae* by Romagnesi 1967): *R. aurantioflammans* Ruotsalainen, Sarnari & Vauras; *R. dryadicola* Singer ex R. Fellner & Landa, *R. intermedia* P. Karst. and *R. veternosa* Fr. All these species, except the last one, do not have type material selected from the Romagnesi's "types" (Ruotsalainen & Vauras 1994, Sarnari 1998). Our repeated request for the loan of the epitype designated for *R. veternosa* (PC, Romagnesi 53-206) has not been successful and that is why we have not included it in this study.

In this contribution, we have compared differences in microscopic characters of the four type specimens. Although such a kind of type study is necessary for interpretation of species concepts, the delimitation of all included species will require statistically tested observations on recently collected material supplemented by a molecular analysis.

Materials and methods

Micromorphological characters were observed under an Olympus CX-41 light microscope with an oil-immersion lens at a magnification of 1000×. All drawings of the microscopical structures, except of the spores, were made by using an Olympus U-DA drawing attachment at a projection scale of 2000×. Spores were photographed with an Olympus Artcam camera and measured by the Quick Micro Photo (version 2.1) software. Enlarged pictures

of spores were used for measuring with an accuracy of 0.1 µm and for making line drawings. Q gives the length/width ratio of the spores excluding ornamentation. Statistics for measurements of microscopical characters are based on 30 measurements and given as average value ± standard deviation; values in parentheses give measured minimum or maximum values. An estimate for spore ornamentation density follows Adamčík & Marhold (2000). Density of pleurocystidia is estimated according to Buyck (1991 b). Spores were observed on the gills; vertical structure of the pileipellis halfway the radius of the cap.

Spores were observed in Melzer's reagent. All other microscopical observations were made in ammoniacal Congo red, after a short treatment in warm aqueous 10 % KOH to dissolve the gelatinous matrix and improve tissue dissociation; in this medium, contents of pleurocystidia and pileocystidia were also observed. 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 was colored in karbolfuchsin and observed in distilled water after staining for a few seconds in a 10 % solution of HCl (cf. Romagnesi 1967).

Herbarium abbreviations follow Holmgren *et al.* (1990). We accepted the infrageneric classification of the genus *Russula* by Romagnesi (1967, 1985).

Taxonomy

Russula badia Quél., Compt. Rend. Assoc. Franc. Avancem. Sci. 9: 668. 1881. – Figs. 1, 2, 9, 10, 17–21.

Original description. – Stipe spongieux, fragile, finement ridé en long, glabre, blanc, souvent rosé en bas. Chapeau convexe, plan ($0^{m}08$), puis déprimé au centre, uni sur le bord, un peu visqueux, bai foncé, légèrement purpuracé. Chair elastique, puis molle, blanche, violette sous la cuticule, très poivrée, odeur douce. Lamelles sinuées, souvent fourchues, minces, serrées, jonquille. Spore sphérique ($0^{mm}01$), aculéolée, citrine.

Automne. — Dans les forêts de conifères des montagnes. Jura et Vosges.

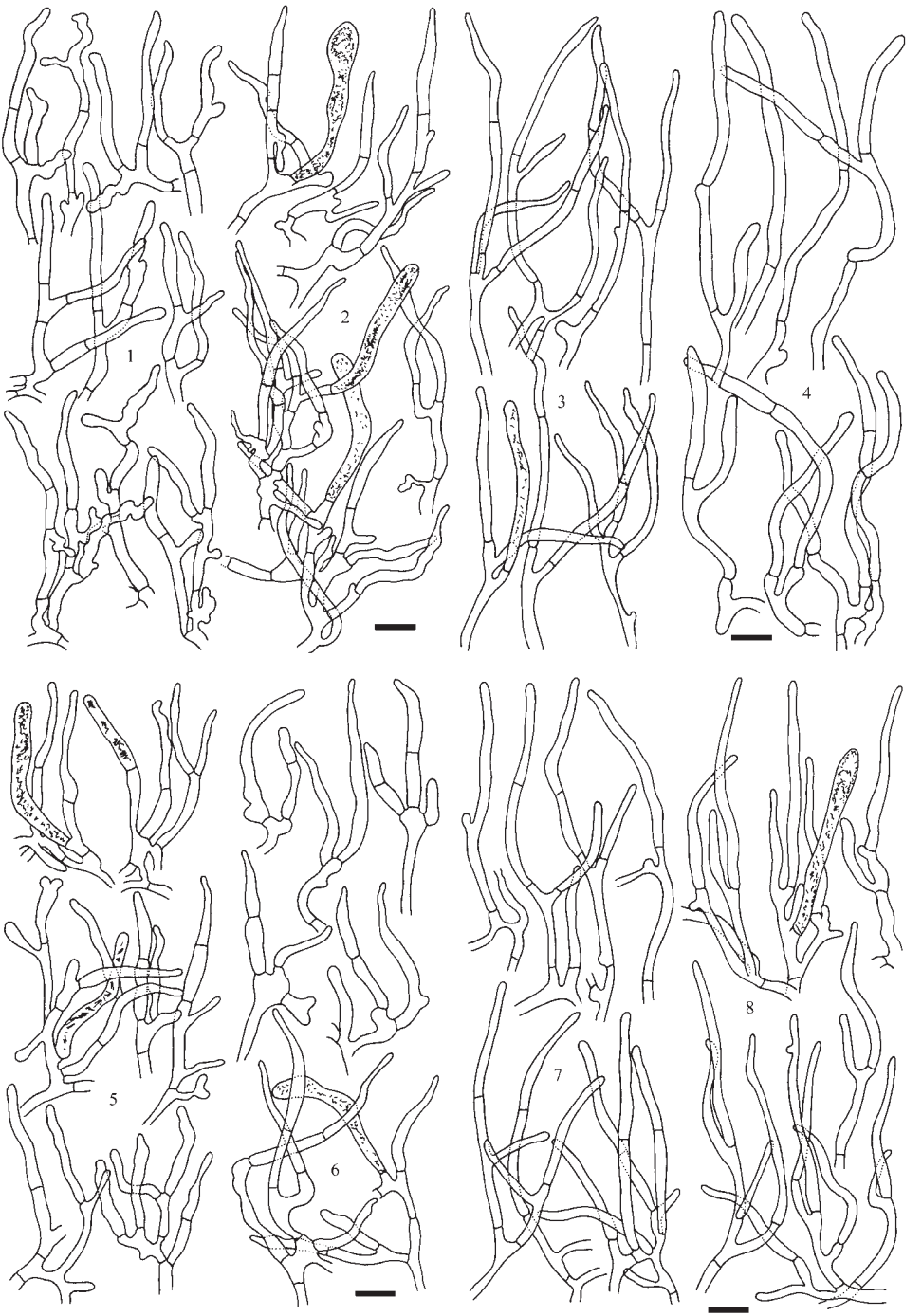
Il ressemble à xerampelina. (Pl. VIII, fig. 9).

Lectotypus [designated by Sarnari 1996, p. 686]. – Quél., Compt. Rend. Assoc. Franc. Avancem. Sci. 9: pl. 8, fig. 9. 1881.

Epitypus [designated here]. – SWITZERLAND, Zolingue, mixed forest with *Picea*, 14 Sep 1953, *leg.* H. Romagnesi (PC 84525, coll. Romagnesi 53.272).

Description of the micromorphological characters. – S p o r e s (7.5)7.9–8.8(9.3) × (6.1)6.5–7.3(7.5) µm, av. 8.4 × 6.9 µm, Q=(1.15)1.18–1.27(1.35), av.

Figs. 1–8. *Russula badia* (epitype, PC 84525): **1.** Hyphal endings in the cap centre. **2.** Hyphal endings near the cap margin with few pileocystidia. *Russula globispora* (neotype, PC 84520): **3.** Hyphal endings in the cap centre with one pileocystidium. **4.** Hyphal endings near the cap margin. *Russula maculata* (epitype, PC 84521): **5.** Hyphal endings in the cap centre with few pileocystidia. **6.** Hyphal endings near the cap margin with one pileocystidium. *Russula vinosopurpurea* (neotype, PC 84518): **7.** Hyphal endings in the cap centre. **8.** Hyphal endings near the cap margin with one pileocystidium. Bars 10 µm.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Q=1.22, ornamentation consisting of amyloid, 0.5–0.8 µm high, numerous warts [(5)6–8(9) warts in a 3 µm diam. circle on spore surface]; warts interconnected by fine, short to longer, occasional to frequent lines (0–3 line-connections in the circle), frequently fused (1–4 fusions in the circle) and both (line-connections and fusions) form abaxially oriented chains and zebra-like patterns that are occasionally branched, locally they form a reticulate structure; isolated warts absent. Suprahilar plage amyloid, very large. – Basidia (39)42.5–48(50) × (9)9.5–12.5(13.5) µm, av. 45.2 × 11.1 µm, 4-spored, clavate or fusiform, pedicellate; basidioles first cylindrical or ellipsoid, then widely clavate, ca. 5–12 µm wide (some equally wide as basidia, but shorter). – Subhymenium pseudoparenchymatic. – Lamellar trama mainly composed of large sphaerocytes. – Pleurocystidia moderately numerous to numerous, ca. 1100–1600/mm^2, measuring (60)66–83(94) × (8.5)9–9.5(11) µm, av. 74.5 × 9.8 µm, fusiform or clavate, pedicellate, on tips mostly acute to acute-pointed, mucronate or appendiculate, with a 2–10 µm long appendage, thin-walled or sometimes with slightly and indistinctly thickened walls (up to 0.5 µm), most often with a heteromorphous (mostly granular, some banded or crystalline in part) contents in major part of its volume, turning brown-grey in sulfovanilin. – Marginal cells similar to basidioles, but smaller and narrower, (14)17–27.5(33) × (3)4–7(8) µm, av. 22.3 × 5.5 µm, often flexuous, mostly narrowly clavate to subcylindrical, with obtuse tips; hidden among very numerous protruding cheilocystidia that are less voluminous than the pleurocystidia, (32)42.5–63.5(71) × (6)7–9(9.5) µm, av. 53 × 8.1 µm, clavate-pedunculate, with obtuse tips (not mucronate or appendiculate), thin-walled or indistinctly thick-walled (up to 0.5 µm), almost completely filled with heteromorphous (granular) contents. – Pileipellis orthochromatic in Cresyl blue, not sharply delimited from the underlying sphaerocytes of the context, vaguely divided in a 80–100 µm deep suprapellis and a 90–120 µm deep subpellis. Suprapellis strongly gelatinized (but not near the surface), a trichoderm of dense intricate hyphal endings and protruding pileocystidia, gradually passing to the dense and more gelatinized subpellis of intricate, irregularly oriented, ca. 3–7 µm wide hyphae. Incrustations absent. Hyphal endings in the pileipellis near the cap margin with terminal cells originating in various depths of the pileipellis, measuring (19)25–37(43) × 2.5–3.5 µm, av. 30.9 × 3.1 µm, always with narrowed and distinctly attenuated tips, subcylindrical or fusiform, centrally often more inflated than near the septum, slightly or distinctly moniliform and/or flexuous, especially those originating in the deeper layer, basal cells equally wide, mostly branched, irregular-nodulose-flexuous, often with very irregular lateral projections, intricate, subapical cells sometimes regularly unbranched and shorter than the terminal cells. Hyphal endings in the cap centre more moniliform and flexuous, sometimes also nodulose or with lateral branches, towards apices less attenuated and sometimes not narrowed, measuring (20)22–32.5(40) × 2.5–4 µm, av. 27.3 × 3.3 µm, subapical cells mostly very irregular and branched and frequently with lateral and moniliform-flexuous

lateral projections that form a very dense intricate structure in the deeper layer. – Pileocystidia usually clavate, very numerous, often in clusters and protruding from the layer of other hyphal endings, some one-celled and originating in the suprapellis or in the upper part of subpellis, others originating in the deeper layer and then with two or three septa near the terminal part, distinctly narrowed (2.5–5 µm) near the basal septum, thin-walled, terminal cells measuring (21)22–92.5(125) × (4.5)6–9(10.5) µm, av. 57.3 × 7.7 µm, if multi-celled then cylindrical or ellipsoid; without acidoresistant incrustation after karbolfuchsin treatment, in Cresyl blue orthochromatic, in Congo red with heteromorphous contents in all parts, in sulfovanilin distinctly greying. – Trama composed of large sphaerocytes and scattered cystidioid hyphae. – Clamp connections absent in all parts.

Russula globispora (J. Blum) Bon, Doc. Mycol. 65: 55. 1986. – Figs. 3, 4, 11, 12, 22–26.

≡ *Russula maculata* var. *globispora* J. Blum, Bull. Soc. Mycol. France 68(2): 232, 1952.
≡ *Russula straminea* f. *globispora* (J. Blum) C. Lej., Annales XI Journées Mycologiques de la CEMM 2003: 66. 2005.

Original description. – A typo differt sporis sphaericis, majoribus, 9–13 µ, echinulatis, aculeis 1–1,5 (2) µ altis.

Neotypus [designated by Sarnari 1996, p. 700]. – [France], Noailles (Oise), under *Fagus*, 18 Aug 1956, *H. Romagnesi* (PC 84520, coll. Romagnesi 56.104, as *R. maculata* var. *bresadoliana*).

Description of the micromorphological characters. – Spores (9.7)10.1–11.4(12.4) × (8.1)8.6–9.5(9.7) µm, av. 10.8 × 9 µm, Q=(1.13)1.15–1.23(1.28), av. Q=1.19, ornamentation consisting of amyloid, (0.8)0.9–1.1 µm high, distant spines (4–5 spines in a 3 µm diam. circle on the spore surface); spines mostly isolated, line-connections usually absent or very rare, infrequently fused in pairs, rarely in triplets [0(–2) fusions in the circle]. Suprahillar plage amyloid, moderately large. – Basidia (41)42.5–48.5(53) × (12)14–16.5(17) µm, av. 45.3 × 15.5 µm, 4-spored, broadly clavate; basidioles first subcylindrical, then clavate, ca. 6–14 µm wide. – Subhymenium pseudoparenchymatic. – Lamellar trama mainly composed of large sphaerocytes. – Pleurocystidia dispersed, ca. 300–600/mm^2, measuring (60)66.5–88(95) × (12)13.5–16(16.5) µm, av. 77.4 × 14.8 µm, fusiform or widely clavate, pedicellate, on tips acute to acute-pointed and mostly with a 2–7 µm long appendage, thin-walled, with heteromorphous (mostly granular-crystalline) contents in major part of its volume, turning dark-grey in sulfovanilin. – Marginal cells very small, (11)15–20.5(25) × 4–5.5(6.5) µm, av. 17.7 × 4.8 µm, irregular in shape: moniliform and flexuous, narrowly clavate, fusiform to subcylindrical, mostly with obtuse tips, but some apically constricted; mixed with dispersed cheilocystidia that are less voluminous than the pleurocystidia, (34)48–73(91) × (7)8–12(13.5) µm, av. 60.4 × 10 µm, clavate or fusiform, pedunculate, with obtuse or acute tips, not mucronate, but some appendiculate, thin-walled, almost completely filled with a heteromorphous (granular-crystalline or granular-banded) contents. – Pileipellis orthochromatic in Cr-

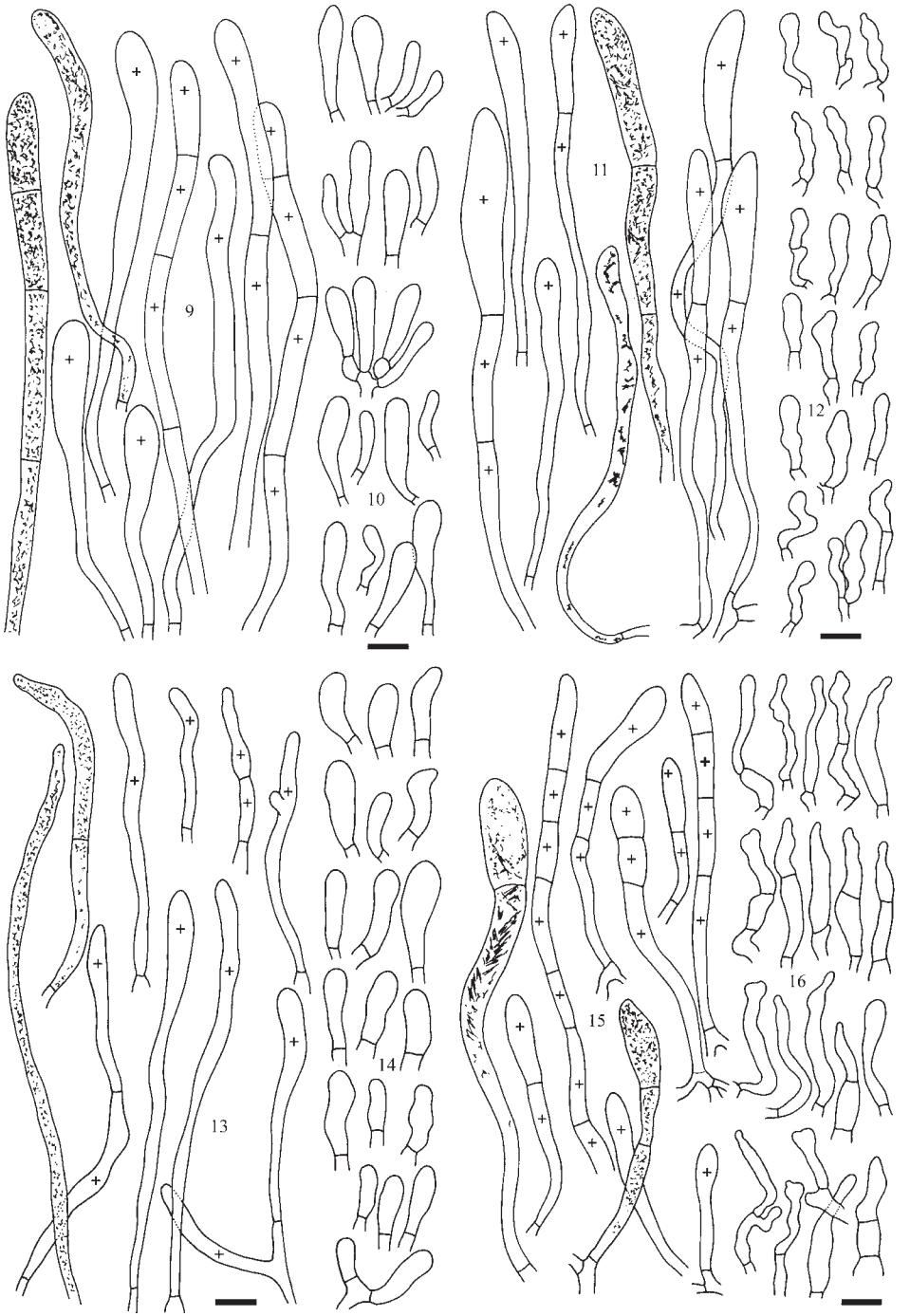
esyl blue, not sharply delimited from the underlying sphaerocytes of the context, vaguely divided in a 120–170 µm deep suprapellis and a 100–130 µm deep subpellis. Suprapellis strongly gelatinized, on some places covered in addition with an up to 40 µm deep transparent gelatinous matter, a trichoderm of erect, aeriferous hyphal endings and dispersed pileocystidia, gradually passing to the dense, gelatinized subpellis of intricate, horizontally oriented, ca. 2–4.5 µm wide hyphae. Incrustations absent. Hyphal endings in the pileipellis near the cap margin with terminal cells measuring (27)32–52(69) × 3–4(5) µm, av. 42.2 × 3.5 µm, narrowly cylindrical and long, with obtuse tips, frequently moniliform and/or flexuous, basal cells equal, branching (sometimes with shorter lateral branches) or unbranching, occasionally anastomosing. Hyphal endings in the cap centre similar to those near the margin, but frequently narrower and often with constricted tips, less moniliform or flexuous, measuring (25)29.5–46.5(56) × 2.5–3 µm, av. 38 × 2.6 µm. – Pileocystidia usually narrowly clavate, rarely fusiform, dispersed, mostly two-celled with wider and shorter terminal cells and long narrower flexuous basal cells, frequently also one-celled, thin-walled, terminal cells measuring (30)33–81.5(111) × (3.5)5.5–9(11) µm, av. 51.2 × 7.4 µm; without acidoresistant incrustation after karbolfuchsin treatment, in Cresyl blue distinctly orthochromatic (but with pale green-blue intracellular pigment), in Congo red with a heteromorphous contents in all parts, in sulfovanilin strongly greying, in water or KOH with a yellowish contents. – Trama mostly composed of large sphaerocytes, without cystidioid hyphae, but with dispersed oleipherous hyphae with a yellowish refringent contents, 2–5 µm wide and constricted on the septa. – Clamp connections absent in all parts.

Russula maculata Quél. & Roze, Bull. Soc. Bot. France 24: 323. 1878 ("1877"). – Figs. 5, 6, 13, 14, 27–31.

Original description. – Stipe court, épais, dur en dehors, spongieux, strié-reticulé, poli, blanc, rarement rosé, taché à la fin de roux ou de bistre. Chapeau épais, dur, convexe plan (0^{m},06–9), visqueux, rouge incarnat pâle, puis décoloré, ochracé ou blanchâtre, tacheté de pourpre ou de brun; marge festonnée unie et ordinairement plus colorée. Chair fragile, puis spongieuse, blanche, poivrée au bout de quelques istants de mastication et exhalant une agréable odeur de rose ou de pomme. Lamelles atténuées-adnées, bifurquées-rameuses, pruineuses, jonquille clair, puis jaune abricot avec un reflet aurore. Spore (0^{mm},01) sphérique, aculéolée et citrine. (Pl. V, f. 8).

Été. — Dès le mois de juin, dans les bois secs du Jura. Retrouvé dans les bois sablonneux des environs de Paris par M. E. Roze.

Figs. 9–16. *Russula badia* (epitype, PC84525): **9.** Pileocystidia with contents indicated in two elements as seen in Congo red. **10.** Marginal cells on the gill edge. *Russula globispora* (neotype, PC 84520): **11.** Pileocystidia with contents indicated in two elements as seen in Congo red. **12.** Marginal cells on the gill edge. *Russula maculata* (epitype, PC 84521): **13.** Pileocystidia with contents indicated in two elements as seen in Congo red. **14.** Marginal cells on the gill edge. *Russula vinosopurpurea* (neotype, PC 84518): **15.** Pileocystidia with contents indicated in two elements as seen in Congo red. **16.** Marginal cells on the gill edge. Plus sign indicates contents of pileocystidia schematically. Bars 10 µm.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Lectotypus [designated by Sarnari 1996, p. 695]. – Quél., Bull. Soc. Bot. France 24: pl. 5, fig. 8. 1877.

Epitypus [designated here]. – [France], Boran sur Oise (Oise), deciduous forest on calcareous soil (*Quercus, Corylus, Betula*), 14 Jul 1954, *leg.* H. Romagnesi coll. 54.43 (PC 84521).

Description of the micromorphological characters. – Spores (8.3)8.6–9.7(10.2) × (6.5)7–8(8.6) µm, av. 9.1 × 7.5 µm, Q=(1.16)1.18–1.26(1.35), av. Q=1.22, ornamentation consisting of amyloid, 0.6–0.7 µm high, relatively distant warts [4–6(7) warts in a 3 µm diam. circle on spore surface]; warts interconnected by fine, short to longer, occasional to frequent lines [(0)1–3 line-connections in the circle], occasionally fused in short chains or crests [(0)1–3 fusions in the circle] that are often branching, they rarely form a reticulate structure; isolated warts absent. Suprahillar plage amyloid, very large. – Basidia (39)42.5–54.5(60) × (12)12.5–14.5(15) µm, av. 48.5 × 13.6 µm, 4-spored, widely clavate, pedicellate; basidiola first cylindrical or ellipsoid, then clavate, ca. 5–10 µm wide. – Subhymenium pseudoparenchymatic. – Lamellar trama mainly composed of large sphaerocytes. – Pleurocystidia dispersed, ca. 500–600/mm^2, measuring (59)70–84(–90) × (11)11.5–13.5(14) µm, av. 77 × 12.7 µm, fusiform or rarely clavate, pedicellate, on tips acute to acute-pointed and mostly with a 2–7 µm long appendage, thin-walled, with a heteromorphous (mostly granular-crystalline) contents in major part of its volume, turning dark-grey in sulfovanilin. – Marginal cells similar to the basidioles but shorter, (12)15–22.5(28) × (4.5)5–7.5(9) µm, av. 18.9 × 6.3 µm, narrowly clavate to subcylindrical, with obtuse tips; mixed with dispersed cheilocystidia that are less voluminous than the pleurocystidia, (56)64–85.5(93) × (8)9.5–12 µm, av. 75 × 10.8 µm, clavate or fusiform, pedunculate, with obtuse tips (not mucronate or appendiculate), thin-walled, almost completely filled with heteromorphous (granular-crystalline) contents. – Pileipellis orthochromatic in Cresyl blue, not sharply delimited from the underlying sphaerocytes of the context, vaguely divided in a 70–100 µm deep suprapellis and a 70–90 µm deep subpellis. Suprapellis strongly gelatinized, covered in addition with a 50–80 µm deep transparent gelatinous matter, a trichoderm of ascending to erect, dense hyphal endings and dispersed pileocystidia, gradually passing to the dense gelatinized subpellis of intricate, irregularly oriented, ca. 3–6 µm wide hyphae. Incrustations absent. Hyphal endings in the pileipellis near the cap margin with terminal cells originating in various depths of the pileipellis, measuring (10)18–38.5(50) × (2.5)3–4.5(5) µm, av. 28.3 × 3.7 µm, always with narrowed and distinctly attenuated tips (mostly narrowed to 2 µm or more), very variable in length and often very short, mostly subulate or fusiform, occasionally subcylindrical, centrally often more inflated than near septum, often slightly or distinctly moniliform and/or flexuous, especially those originating in the deeper layer; basal cells equally wide or occasionally locally nodulose-inflated, ca. 3–10 µm wide, mostly branched, irregular-nodulose-flexuous, occasionally with lateral branches, intricate, subapical cells of longer hyphal endings frequently regular-unbranched. Hyphal endings in the cap centre

similar to those near the margin, but frequently moniliform and flexuous, sometimes also nodulose or with lateral branches, with less attenuated tips and sometimes not narrowed near the tips, measuring (15)19.5–29(32) × 2.5–4 µm, av. 24.4 × 3.3 µm. – Pileocystidia usually narrowly clavate, dispersed, mostly one-celled and originating in the suprapellis or in the upper part of the subpellis, others originating in the deeper layer and then with two or more septa, distinctly narrowed (2.5–4.5 µm) near the basal septum, thin-walled, terminal cells measuring (24)27.5–95(171) × 4–6(6.5) µm, av. 61.4 × 4.9 µm, very variable in length, often flexuouse and slightly moniliform; without acidoresistant incrustation after karbolfuchsin treatment, in Cresyl blue orthochromatic, in Congo red with heteromorphous contents in all parts, in sulfovanilin distinctly but weakly greying, in water or KOH with yellowish contents. – Trama mostly composed of large sphaerocytes, cystidioid hyphae absent. – Clamp connections absent in all parts.

Russula vinosopurpurea Jul. Schaeff., Ann. Mycol. 36(1): 27. 1938. – Figs. 7, 8, 15, 16, 32–36.

Original description. – Magna ochrospora moderate acris, Russulae integrae respondens coloribus obscure rubris in deflorescendo variegatis, stipite albo dure corticato, carne seniorum ± grisescente, sporis mediis isolate verrucosis distincta, sub quercubus nascens.

Neotypus [designated by Romagnesi 1967, p. 890]. – [France], Forêt de Compiégne (Oise), under *Fagus* and *Carpinus*, 22 Aug 1959, *leg.* H. Romagnesi (PC 84518, coll. Romagnesi 59.128).

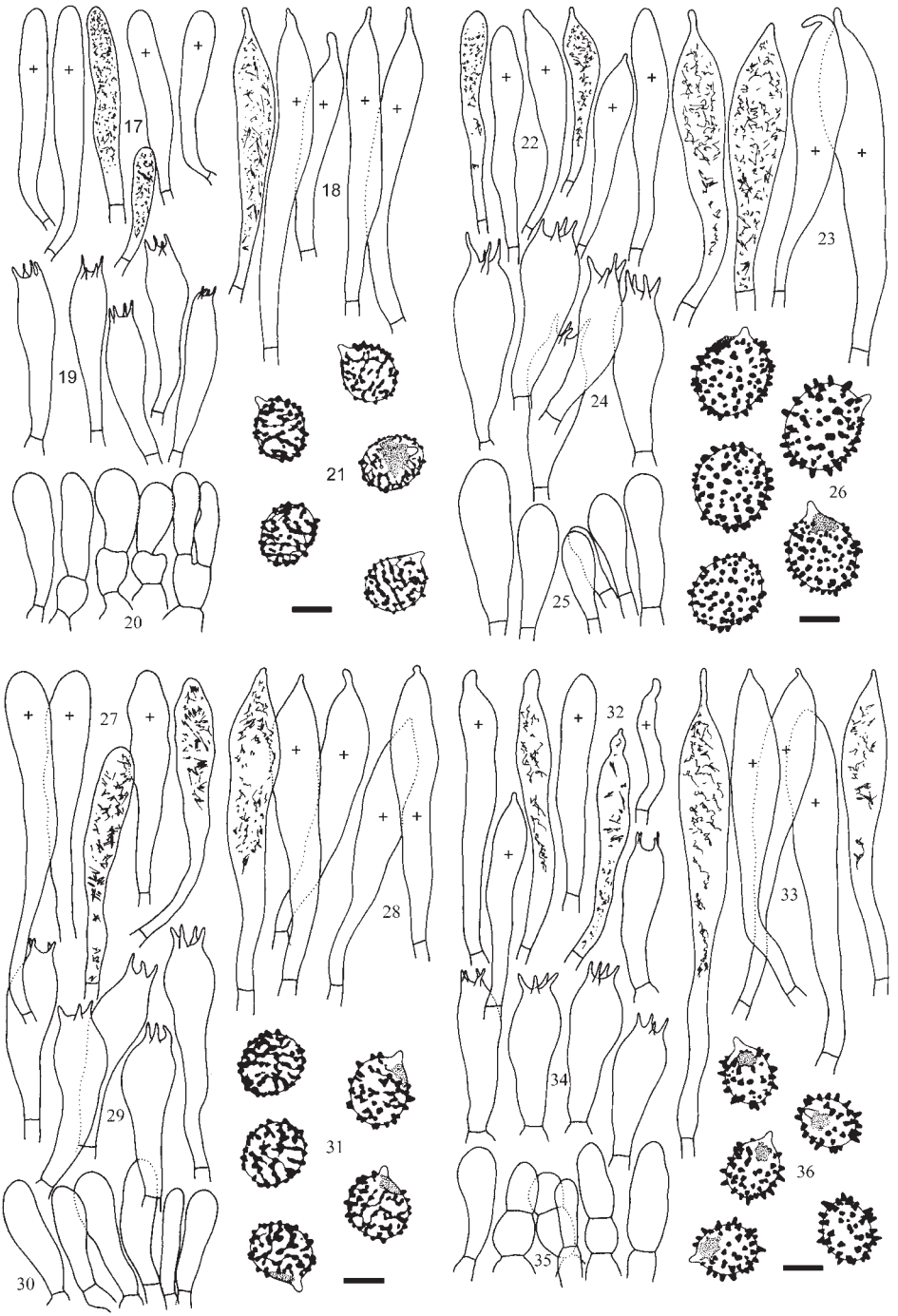
Description of the micromorphological characters. – Spores (7.4)7.8–8.5(8.8) × (5.5)6.1–6.8(7.1) µm, av. 8.2 × 6.5 µm, Q=(1.2)1.22–1.32(1.4), av. Q=1.27, ornamentation consisting of amyloid, 1.1–1.6 µm high, distant spines [(3)4–6 spines in a 3 µm diam. circle on spore the surface]; spines mostly isolated, line-connections usually absent or very rare, infrequently fused in pairs, rarely in triplets [0–2 fusions in the circle]. Suprahilar plage amyloid and distinctly smaller compared to other members of subsect. *Maculatinae*. – Basidia (34)35.5–43.5(52) × (11)13–16 µm, av. 39.5 × 14.5 µm, 4-spored, widely clavate; basidiola first ellipsoid or subcylindrical, then clavate, ca. 5–11 µm wide. – Subhymenium pseudoparenchymatic. – Lamellar trama mainly composed of large sphaerocytes. – Pleurocystidia dispersed, ca. 500–700/mm^2, measuring (66)74–113(145) × (11)12–15(16) µm, av. 93.4 × 13.5 µm, fusiform or widely clavate, pedicellate, on tips acute to acute-pointed and mostly with 3–9 µm long appendage, thin-walled, with heteromorphous (mostly granular-banded) contents in major part of its volume, turning dark red-grey in sulfovanilin. – Marginal cells small, (16)18–32.5(36) × (3)3.5–5.5(6) µm, av. 25.3 × 4.6 µm, very irregular in shape: moniliform and flexuous, mostly fusiform to subcylindrical, some in shape looking like a transition to cystidia but optically empty, with obtuse or constricted tips; mixed with dispersed cheilocystidia being less voluminous than pleurocystidia, (27)37.5–68(78) × (5)7–10(10.5) µm, av. 52.7 × 8.5 µm, clavate or fusiform, pedunculate, mostly with acute, mucronate or appendiculate

tips, thin-walled, almost completely filled with heteromorphous (granular-crystalline or granular-banded) contents. – Pileipellis orthochromatic in Cresyl blue, sharply delimited from the underlying sphaerocytes of the context, vaguely divided in a 55–60 µm deep suprapellis and a 35–50 µm deep subpellis. Suprapellis gelatinized, composed of aeripherous, ascending or repent hyphal endings and dispersed pileocystidia, gradually passing to the dense, gelatinized subpellis of intricate, horizontally oriented, ca. 2–4 µm wide hyphae. Incrustations absent. Hyphal endings in the pileipellis near the cap margin with terminal cells measuring (25)33–55.5(66) × (2.5)3–4(4.5) µm, av. 44.3 × 3.4 µm, mostly attenuated, subulate,rarely subcylindrical, with attenuated tips (constricted to 1.5–2.5 µm), usually not moniliform, slightly flexuous, basal cells equal, mostly branched, often with shorter regular lateral branches. Hyphal endings in the cap centre shorter, less attenuated and narrower than those near the margin, measuring (16)22.5–38.5(54) × 2.5–3.5 µm, av. 30.5 × 2.9 µm, basal cells often unbranched. – Pileocystidia usually clavate, rarely fusiform, dispersed, mostly multi-celled and arising in subpellis, with wider and shorter terminal cells and often with long narrower flexuous basal cells, sometimes also one-celled and these arising in the suprapellis, thin-walled, terminal cells measuring (14)18–40(55) × (4)5.5–9.5(12) µm, av. 28.9 × 7.6 µm; without acidoresistant incrustation after karbolfuchsin treatment, in Cresyl blue orthochromatic (but with pale green-blue intracellular pigment), in Congo red with heteromorphous crystalline-granular contents in all parts, in sulfovanilin strongly greying. – Trama mostly composed of large sphaerocytes, with dispersed cystidioid hyphae. – Clamp connections absent in all parts.

Discussion

Note on the designation of the epitypes. – In this study, we have designated the epitypes for the two species described by L. Quélet: *R. badia* and *R. maculata*. As both epitypes were collected in the years 1953–1954, they might be of not good quality for extractions of DNA and molecular studies. Despite the argument that recently collected specimens may serve as better material for molecular studies, our designation of the epitypes has several reasons. First, we are following the trend introduced by Sarnari (1998) who accepted "Romagnesi's types" as valid neotypes and in

Figs. 17–36. *Russula badia* (epitype, PC 84525): **17.** Cheilocystidia. **18.** Pleurocystidia. **19.** Basidia. **20.** Basidiola. **21.** Basidiospores in Melzer's reagent. *Russula globispora* (neotype, PC 84520): **22.** Cheilocystidia. **23.** Pleurocystidia. **24.** Basidia. **25.** Basidiola. **26.** Basidiospores in Melzer's reagent. *Russula maculata* (epitype, PC 84521): **27.** Cheilocystidia. **28.** Pleurocystidia. **29.** Basidia. **30.** Basidiola. **31.** Basidiospores in Melzer's reagent. *Russula vinosopurpurea* (neotype, PC 84518): **32.** Cheilocystidia. **33.** Pleurocystidia. **34.** Basidia. **35.** Basidiola. **36.** Basidiospores in Melzer's reagent. Contents of cystidia indicated in some elements as seen in Congo red (schematically replaced with plus sign in others). Bars 10 µm, only 5 µm for spores.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several cases also used them for designation of epitypes (e.g. in *R. veternosa*); these "types" are labeled with red stickers indicating its status of "Type" in the herbarium PC. Second, the original concept of both species is not interpretable by the protologue and we do not know any better publication for explanation of the recent concept than Romagnesi's monograph referring to "Romagnesi's types". Third, our studies on types have revealed several characters not described in the literature. The importance of these characters must be verified on more material, which suggests that the morphological delimitation of the species studied here may be insufficient, and the designation of recent collections means a risk of adopting a concept different from that of Romagnesi.

The number on the specimen designated here as the epitype of *R. maculata* differs from that on the specimen selected by Romagnesi (1967). Romagnesi referred to the collection 54.53 as the "type" and some of his line drawings are based on the collection 54.43. We requested the loan of 54.53 from the herbarium PC, but we received the collection 54.43; it was labelled as "Type" and provided with a note that the specimen's number in the book is a typing error.

Characters used in the literature for species delimitation. – To our knowledge, the most precise descriptions of the studied species are those included in the most widely used monographs by Romagnesi (1967) and Sarnari (1998). Both authors used a combination of macro- and micromorphological characters for species delimitation within *Russula* subsect. *Maculatinae*. According to their identification keys, *R. badia* is a species easily recognizable in field (associated with conifers, with a pale yellow spore print, reddish tints on the stem, a delayed acrid taste, the smell of cedar wood), but the three other species require microscopic observations. Among the most important micromorphological characters for species delimitation, Romagnesi (1967) and Sarnari (1998) used the size and ornamentation of the spores and the septation of the pileocystidia.

Verification of diagnostic characters used in literature. – The estimation of the variability of spore size in both monographs agrees largely with our observations on the types (for an overview and comparison of all micromorphological characters observed on the types in this study see Tab. 1). Both monographers recognized *R. globispora* as a species with distinctly larger spores while the three other species bear intermediate-sized spores. Romagnesi (1967) gives a somewhat longer spore size for *R. badia* ("8–11 × 6.5–8.25 µm") than for *R. maculata* ("8.2–10 × 7–8.7 µm"), but Sarnari (1998), on the contrary, estimated that the latter has slightly larger spores ("7.8–9.6 × 6.7–8 µm" for *R. badia* vs. "8–10 × 7–9 µm" for *R. maculata*). Our observations on the types of both species rather confirm Sarnari's values, but the difference does not seem to be useful for species recognition. The minimum spore width estimated by both authors for *R. vinosopurpurea* suggests that this species has slightly narrower (but otherwise similar in size) spores than *R. badia* and *R. maculata* and this is congruent with our meas-

urement of Q value (Tab. 1). In the literature, the shape of spores is interpreted for all species as more or less similar, but according to our measurements *R. globispora* has smaller Q values than *R. vinosopurpurea* (see Q values in Tab. 1).

According to spore ornamentation, the types of *R. globispora* and *R. vinosopurpurea* differ from two the other species by very prominent isolated spines. The type of *R. globispora* has lower (0.9–1.1 µm) spore ornamentations than *R. vinosopurpurea* (1.1–1.6 µm) (Tab. 1). Romagnesi (1967) provided similar values to our observations from the types, but Sarnari (1998) gave similar prominence for both species ("0.75–1.2 µm" for *R. globispora* and "0.8–1.3 µm" for *R. vinosopurpurea*). The spore ornamentation observed on the types of *R. maculata* and *R. badia* is composed of spines or warts with frequent fine lines, the ornamentation is equally prominent in both species – this is in agreement with Romagnesi, but Sarnari described the ornamentation in the spores of *R. maculata* as higher ("0.6–1.1 µm" for *R. badia* vs. "0.8–0.1 µm" for *R. maculata*). The description of the connections in the spore ornamentation by both authors is in agreement with our measurements of density of the line-connections; they are frequent in *R. badia* and *R. maculata* and rare or absent in the two other species.

The number of septa (or cells) in the pileocystidia is considered to be an important character, e.g. *R. decipiens* (Singer) Svrček has no septa in the pileocystidia (one-celled), while other species frequently have one or more septa (two- to multi-celled). Sarnari (1998) described all four studied species as bearing 1–3 septa. Romagnesi (1967) recognized the pileocystidia of *R. vinosopurpurea* as multi-septate, while in *R. badia* as not always multi-septate and he shows mostly one-celled pileocystidia on the drawings of the two other species. According to our observations on the type material, only the type of *R. vinosopurpurea* has prevailingly multi-celled pileocystidia (often with more than 3 cells), the other species very frequent have one-celled pileocystidia restricted to the suprapellis.

Other characters used in literature. – There are several other characters that are included in the published descriptions (Romagnesi 1967, Sarnari 1998), but that are not used or discussed for species delimitation. Among them, the elements of the hymenium (except for the spores) are usually not used for species delimitation and this is true also for our four studied species. Both monographic studies gave overlapping values for the size of the basidia and pleurocystidia, however, we observed distinctly narrower basidia and pleurocystidia in the type of *R. badia* (Tab. 1).

The difference in the structure of the pileipellis near the cap margin and cap centre (e.g. demonstrated by Adamčík & Marhold 2000 and Adamčík 2001) is usually not implemented in published description and drawings. Published descriptions mostly refer to both structures or to the hyphal endings halfway the radius of the cap (the position of the described elements is usually not specified). Hyphal endings in the pileipellis are usually without specification of terminal and basal cells. This is the reason of frequent over-

Tab. 1. Comparison of selected characters observed on the type material of *Russula badia*, *R. globispora*, *R. maculata* and *R. vinosopurpurea*. All values of microscopical characters are averages of 30 measurements. TC = terminal cells of hyphae in pileipellis. In shaded boxes are the distinguishing characters.

		R. badia	*R. maculata*	*R. globispora*	*R. vinosopurpurea*
Spores	**Size**	8.4 × 6.9 µm	9.1 × 7.5 µm	10.8 × 9 µm	8.2 × 7.5 µm
	Q	1.22	1.22	1.19	1.27
	Ornamentation	0.5–0.8 µm high	0.6–0.7 µm high	0.9–1.1 µm high	1.1–1.6 µm high
	Warts	6–8	4–6	4–5	4–6
	Lines	1–3	1–3	0	0(–1)
Pileipellis near margin	**TC margin size**	30.9 × 3.1 µm	28.3 × 3.7 µm	42.2 × 3.5 µm	44.3 × 3.4 µm
	TC margin shape	fusiform, subulate or subcylindrical, constricted or attenuated on tips	mostly attenuated, constricted or attenuated on tips	cylindrical, mostly obtuse on tips	subulate, occasionally subcylindrical, attenuated on tips
	Branching of subapical cells	mostly branched	mostly branched	mostly unbranched	mostly branched
	Difference in width of TC	1.6 µm	1.85 µm	0.5 µm	1.5 µm
	Lateral branches	frequent, in centre irregular	in centre frequent, not very irregular	occasional, not irregular	rare to occasional, not irregular
TC centre	**Size**	27.3 × 3.3 µm	24.4 × 3.3 µm	38 × 2.6 µm	30.5 × 2.9 µm
	Shape	subcylindrical with constricted tips	in major part subcylindrical, frequently attenuated	mostly subcylindrical, frequently attenuated, mostly constricted on tips	cylindrical
	Suprapellis	80–100 µm	70–100 µm	120–170 µm	55–60 µm
	Subpellis	90–120 µm	70–90 µm	100–130 µm	35–50 µm
	Delimitation from trama	sharp	not sharp	not sharp	sharp
	Cystidioid hyphae in trama	scattered	absent	absent	present

Tab. 1. Continued.

		R. badia	*R. maculata*	*R. globispora*	*R. vinosopurpurea*
Pileocystidia	**Size**	57.3 × 7.7 µm	61.4 × 4.9 µm	51.2 × 7.4 µm	28.9 × 7.6 µm
	Cell number	1–3	mostly 1	mostly 1, frequently 2 or more	mostly 2–6, occasionally 1
Marginal cells	**Size**	22.3 x 5.5 µm	18.9 × 6.3 µm	17.7 × 4.8 µm	25.3 × 4.6 µm
	Shape	like basidioles	like basidioles	moniliform, flexuous, small	moniliform, flexuous, irregular
Cheilocystidia	**Size**	53 × 8.1 µm	75 × 10.8 µm	60.4 × 10 µm	52.7 × 8.5 µm
	Tips	obtuse	obtuse	obtuse or acute, some mucronate	mostly acute and appendiculate
Basidia	**Size**	45.2 × 11.1 µm	48.5 × 13.6 µm	45.3 × 15.5 µm	39.5 × 14.5 µm
Pleurocystidia	**Size**	74.5 × 9.8 µm	77 × 12.7 µm	77.4 × 14.8 µm	93.4 × 13.5 µm
	Density	1100–1600/mm^2	500–600/mm^2	300–600/mm^2	500–700/mm^2

looking characters useful for species delimitation. In general, Sarnari and Romagnesi used most frequently the narrowing of the hyphal tips as a reliable character. In the case of our four studied species, Sarnari did not mention any distinct differences in this character, but Romagnesi described the tips of *R. badia* as obtuse and of *R. vinosopurpurea* as attenuated. According to our type studies, only the type specimen of *R. globispora* has mostly obtuse tips of the hyphae in pileipellis and the three remaining species have tips constricted or attenuated. Concerning the dimensions measured on the hyphal endings in the pileipellis, both monographic publications give only the width of the hyphae. According to Romagnesi, *R. badia* has hyphal endings in pileipellis that are 1.7–2.7 µm wide and the three other species have hyphae that are 2–4 µm wide. Sarnari gave a range of width for all species that is more or less similar (between 2–4 µm), which is in agreement to our observations on the types (this is valid only for the width of the terminal cells of the hyphae near the cap margin, see comments on the pileipellis bellow).

Both monographs describe a similar range of the values for width of the pileocystidia, but our type studies show a clear difference: *R. maculata* has the longest and the narrowest terminal cells of its pileocystidia and *R. vinosopurpurea* the shortest and the widest.

Characters not described in literature. – Compared to literature, we have improved our observation of the pileipellis by distinguishing the structure of the pileipellis near the margin and in the centre. In addition, we have recognized the shape and the size of the terminal and basal cells. Some of these characters show distinct differences among the studied type specimens (Tab. 1). *Russula badia* and *R. maculata* have shorter terminal cells of the hyphae near the margin than the two other species. The terminal cells near the margin are mostly attenuated in *R. maculata* and *R. vinosopurpurea*, mostly cylindrical in *R. globispora* and of various shapes in *R. badia*. *Russula globispora* differs from the three other species in not having constricted obtuse tips of the hyphae near the cap margin; the difference between the width at the tips and the widest part of the terminal cells of the hyphae near the cap margin is in average only 0.5 µm, while other type specimens have this a difference of at least 1.5 µm. *Russula globispora* has mainly unbranched subapical cells of the hyphae of the pileipellis near the cap margin (branching mostly at the second basal cell), while *R. vinosopurpurea* has subapical cells that are mostly branched. *Russula badia* has frequent lateral branches on the hyphae in the pileipellis that become irregular, nodulose-flexuous in the centre, while *R. vinosopurpurea* has rare and regular lateral branches.

The terminal cells of the hyphae of the pileipellis in the cap centre have a similar subcylindrical shape in all studied type specimens. They are equally long, but narrower than those near the cap margin in *R. globispora* and, on the contrary, almost equally wide, but shorter than near the margin in *R. vinosopurpurea*. The two other species have the terminal cells near the margin and in the centre of cap that are of similar size.

The pileipellis structure on vertical section shows distinct differences among species. The type of *R. globispora* has the thickest pileipellis (220–290 µm) and *R. vinosopurpurea* has the thinnest (90–110 µm). The types of *R. maculata* and *R. globispora* have a pileipellis surface that is covered by additional transparent gelatinous matter. *Russula badia* and *R. vinosopurpurea* have a sharply delimited pileipellis from the underlying sphaerocytes of the cap trama, which is roughly in agreement with the description of the possibility of peeling of the pileus cuticle in the literature.

Presence and form of the oleipherous hyphae in pileipellis and trama are described in both monographs, but we have not found distinct differences among species. However, *R. vinosopurpurea* has pileocystidia that descend to the trama (as cystidioid hyphae) and such hyphae are also scattered in *R. badia*; they are absent in the trama of the two remaining species.

Neither Romagnesi nor Sarnari described the elements on the edge of the gills. Our observations confirmed in all species the presence of marginal cells mixed with scattered to numerous cheilocystidia. These cheilocystidia are smaller (narrower and shorter) than the pleurocystidia, often have yellowish heteromorphous content and are usually obtuse and not appendiculate or mucronate. The only exception is *R. vinosopurpurea* with mostly acute and appendiculate cheilocystidia. Marginal cells of *R. badia* and *R. maculata* are similar to the basidioles on the sides of the gills, but the two other species have developed marginal cells of irregular shape (moniliform and flexuous).

Most of the species have dispersed pleurocystidia, except for *R. badia* that has numerous pleurocystidia.

Conclusion

Our type studies of *R. badia*, *R. globispora*, *R. maculata* and *R. vinosopurpurea* show the most distinct differences in these characters:

Spores. – *R. globispora* and *R. vinosopurpurea* have similar spore ornamentation composed of prominent isolated spines, but the spores of *R. globispora* are larger and more subglobose. *R. badia* and *R. maculata* have spores of similar size and shape, ornamented by lower warts or spines that are connected by frequent lines, but they are not reticulate. *Russula badia* differs in denser spines (warts).

Structure of the pileipellis near the margin of the cap. – All species have slender hyphal endings (not exceeding 4 µm in av.). *Russula globispora* and *R. vinosopurpurea* have distinctly longer terminal cells than the two other species. *Russula globispora* is the only species with cylindrical terminal cells of hyphae that are mostly obtuse and not constricted near the tips and have mainly unbranched subapical cells.

Structure of the pileipellis in the cap centre. – All species have similar subcylindrical terminal cells. *Russula globispora* has narrower and more constricted terminal cells in the centre than those near the margin; *R. vinosopurpurea* has wider and less constricted cells in the centre than

near the margin; and *R. badia* and *R. maculata* have terminal cells in the centre and near the margin of similar size.

Vertical structure of the pileipellis. – *Russula globispora* has a pileipellis that is not sharply delimited from the trama and is thicker than 200 µm. *Russula vinosopurpurea* has a pileipellis that is sharply delimited from the trama and that is thinner than 100 µm. *Russula badia* and *R. maculata* both have a pileipellis that is 150–200 µm thick and they differ in sharply delimited trama of the first.

Pileocystidia. – In all species one-celled pileocystidia are present and they arise in the upper part of the pileipellis (suprapellis). They all have also two- or multi-celled pileocystidia, those mostly arising in the subpellis. Compared to other species, two- or multi-celled pileocystidia are more frequent in *R. vinosopurpurea*, and the terminal cells of the pileocystidia are distinctly shorter (up to 40 µm long). *Russula maculata* has distinctly narrower terminal cells of pileocystidia (up to 6 µm) compared to other species.

Elements in hymenium. – Among the studied species, *R. badia* has the narrowest basidia and pleurocystidia and the most numerous pleurocystidia.

Cystidioid hyphae in trama. – Only two species, *R. badia* and *R. vinosupurpurea*, have cystidioid hyphae in trama.

Elements on gill edge. – *Russula globispora* and *R. vinosopurpurea* have irregular, flexuous-nodulose marginal cells well distinguished from the basidioles. *Russula vinosopurpurea* is the only species with mainly acute and appendiculate tips of the cheilocystidia, the remaining three species have mainly blunt cheilocystidia without appendage.

The differences in the microscopic structure recognized among the types possibly reflect the distinguishing characters for the four species studied, but they must be verified by statistically supported observations on additional material and molecular studies.

Notes on the delimitation of the four studied species and on related species. – Considering the combination of macro- and micromorphological characters, *R. badia* is considered to be a well delimited and well known species. Romagnesi (1967) classified this species in *Russula* subsect. *Sardoninae* Singer, but our type study shows this is in many characters similar to *R. maculata* – the type species of subsect. *Maculatinae*. On the type of *R. badia* we observed some unique characters (e.g. narrow basidia and numerous pleurocystidia), but their importance for classification and species delimitation must be verified by additional phylogenetic studies.

Russula globispora was first treated as an infraspecific taxon of *R. maculata* (Romagnesi 1967, Blum 1952), but our type studies show that it is morphologically rather similar to *R. vinosopurpurea* (e.g. similar isolated spines on spores, long terminal cells in pileipellis near the cap margin, distinctive marginal cells). *Russula dryadicola* is a very similar alpine species. Sarnari (1998) demonstrated a wide ecological gradient in the habitat of *R. globispora* and according to him, this species may grow in sub-Mediterra-

nean oak forests and also in boreal mixed forests. There is also an opinion of some authors (Lejeune 2005, Pérez de Gregorio 2006) of the collections from the Mediterranean area of Europe, who consider *R. globispora* to be a form of *R. straminea* Malençon being described from Morocco [*R. straminea* f. *globispora* (Blum) C. Lej.]. This suggests it may be a species complex and it needs further studies on material from various areas and habitats.

The type of *R. vinosopurpurea* originated from beech forest and in this character as well in spore characters it is similar to *R. veternosa* Fr. According to our observations (unpublished data), the latter has shorter and not attenuated terminal cells in the pileipellis near the margin. Anyway, *R. vinosopurpurea* is very rare (Sarnari 1998) and urgently needs critical comparison with *R. veternosa*.

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