Plant Systematics and Evolution

Blum versus Romagnesi: checking possible Russula (Russulaceae, Basidiomycota) synonymies of the two contemporary authors

--Manuscript Draft--

Manuscript Number:	PLSY-D-17-00195
Full Title:	Blum versus Romagnesi: checking possible Russula (Russulaceae, Basidiomycota) synonymies of the two contemporary authors
Short Title:	Blum versus Romagnesi: checking possible Russula synonymies
Article Type:	Original Article
Keywords:	type studies, multi-locus phylogeny, Russula blumiana, R. decipiens var. ochrospora, R. roseobrunnea
Corresponding Author:	Miroslav Caboň Plant Science and Biodiversity Centre SAS, Institute of Botany Bratislava, SLOVAKIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Plant Science and Biodiversity Centre SAS, Institute of Botany
Corresponding Author's Secondary Institution:	
First Author:	Miroslav Caboň
First Author Secondary Information:	
Order of Authors:	Miroslav Caboň
	Soňa Jančovičová
	Jean Michel Trendel
	Pierre-Arthur Moreau
	Felix Hampe
	Miroslav Kolařík
	Annemieke Verbeken
	Slavomír Adamčík
Order of Authors Secondary Information:	
Funding Information:	Agentúra na Podporu Výskumu a Vývoja (15-0210) — Mr. Slavomír Adamčík
	bilateral scientific mobility project SAV-AV ČR (16-06) — Mr. Slavomír Adamčík
	Visegrad fund (51400484) — Mr. Miroslav Caboň
Abstract:	Among 45 Russula species and infraspecific taxa described by J. Blum, only few are currently accepted. Here we present a case study on R. formosa nom. illeg. (homotypic synonym of R. blumiana), R. decipiens var. ochrospora nom. inval. and R. roseobrunnea. The study is based on sequences and morphological observations of authentic herbarium material determined by J. Blum. The sequence data demonstrated that R. decipiens var. ochrospora is probably identical with the type variety and R. roseobrunnea is probably conspecific with R. rutila. Russula blumiana is recognized and described in detail as a good species related to R. badia.
Suggested Reviewers:	Jorinde Nuytinck jorinde.nuytinck@naturalis.nl skilled in morphology of the Russulaceae

	Brian P. Looney blooney@vols.utk.edu Skilled in bioinformatics and phylogeny
	Anna Ronikier a.ronikier@botany.pl

Blum versus Romagnesi: checking possible *Russula* (Russulaceae, Basidiomycota) synonymies of the two contemporary authors

Miroslav Caboň[1*] (orcid.org/0000-0002-2255-3816), Soňa Jančovičová[2], Jean Michel Trendel[3], Pierre-Arthur Moreau[4] (orcid.org/0000-0003-4783-3643), Felix Hampe[5], Miroslav Kolařík[6] (orcid.org/0000-0003-4016-0335), Annemieke Verbeken[5] (orcid.org/0000-0002-6266-3091) & Slavomír Adamčík[1] (orcid.org/0000-0003-2156-5767)

[1]*Plant Science and Biodiversity Centre SAS, Institute of Botany, Dúbravská cesta 9, SK-845 23, Bratislava, Slovakia; SA: slavomir.adamcik@savba.sk*
[2] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Botany, Révová 39, SK-811 02 Bratislava, Slovakia; jancovicova@fns.uniba.sk*
[3] *7 rue des Coquilles, FR-67500 Haguenau, France; jmtrendel@free.fr*
[4] *Université de Lille, Faculte des Sciences Pharmaceutiques et Biologiques, laboratoire IMPECS, F-59000 Lille, France; pierre-arthur.moreau@univ-lille2.fr*
[5] *Ghent University, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium; felix.hampe@email.de; mieke.verbeken@UGent.be*
[6] *Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ-142 20 Praha 4, Czech Republic; miroslavkolarik@seznam.cz*
** Corresponding author's e-mail and telephone number: miroslav.cabon@savba.sk; 00421 259426108*

Abstract

Among 45 *Russula* species and infraspecific taxa described by J. Blum, only few are currently accepted. Here we present a case study on *R. formosa* nom. illeg. (homotypic synonym of *R. blumiana*), *R. decipiens* var. *ochrospora* **nom. inval.** and *R. roseobrunnea*. The study is based on sequences and morphological observations of authentic herbarium material determined by J. Blum. The sequence data demonstrated that *R. decipiens* var. *ochrospora* is probably identical with the type variety and *R. roseobrunnea* is probably conspecific with *R. rutila*. *Russula blumiana* is recognized and described in detail as a good species related to *R. badia*.

Key words: type studies, multi-locus phylogeny, *Russula blumiana*, *R. decipiens* var. *ochrospora*, *R. roseobrunnea*

Acknowledgement

Curator and staff of the herbarium PC are acknowledged for the loan of the material from Blum's herbarium. H. Marxmüller is acknowledged for the collection of *R. badia*. The studies of S. Adamčík, M. Caboň and S. Jančovičová were funded by the Slovak Research and Development Agency APVV-15-0210. The studies of M. Caboň and M. Kolařík were supported by bilateral scientific mobility project SAV-AV ČR 16-06 and Intra-Visegrad scholarship no. 51400484.

Conflict of interest

The authors declare that they have no conflict of interest.

Running title: Blum versus Romagnesi: checking possible *Russula* synonymies

Introduction

Jean Blum was a French mycologist who published 101 new names of agarics and bolets, among them 45 species and infraspecific names in the genus *Russula* published between 1951–1968 (http://www.mycobank.org) (Table 1). A large part of his *Russula* names (29) are invalid, mainly because he did not provide Latin diagnosis (Bon 1986) (Table 1). His work on *Russula* resulted in the monographic publication "Les Russules" (Blum 1962) but it was soon overshadowed by raise of a phenomenal and innovative monograph "Les Russules d'Europe et d'Afrique du Nord" by Henri Romagnesi (1967). There is a parallel in the mycological career of both French mycologists, substantial part of Romagnesi's publications is also about the genus *Russula* and among 524 of his validly published names of species and infraspecific taxa of agarics and bolets are 78 of *Russula* published between 1937–1997 (http://www.mycobank.org). It was the Romagnesi's influential monograph (Romagnesi 1967) that has a negative impact on accepting of Blum's russulas in the current literature. Among 43 Blum's species and lower-rank names already described at that time, Romagnesi (1967) adopted, re-described and accepted only few without any hesitation: *R. carminipes* J. Blum, *R. pseudoromellii* J. Blum ex Bon, *R. tinctipes* J. Blum ex Bon, *R. pallidospora* J. Blum ex Romagn. and *R. flavispora* J. Blum ex Romagn. In most cases, Romagnesi was not aware of invalid or illegitimate status of Blum's names, and he was probably rather unable to assign Blum's names to particular species he recognised.

Bon (1986) validated 15 of Blum's names and proposed three more species names that replace Blum's illegitimate names. He provided all validly published names by Latin diagnosis, type designation and his microscopic observations on Blum's authentic collections (among which he selected the types). The validation of Blum's *Russula* names has only little impact on their treatment in the current literature, e.g. Sarnari (1998, 2005) and Marxmüller (2014) accepted only *R. blumiana* J. Blum ex Bon, *R. pseudomelitodes* J. Blum ex Bon and *R. pseudoromellii* among russulas re-described by Bon. Concept of the majority of Blum's *Russula* names is currently treated as ambiguous or unknown. In this study, we seek to check possible synonymies of Blum's names with other widely used *Russula* names based on a case study on three taxa described by Blum: *R. formosa* J. Blum **nom. illeg.** (Art. 53; homotypic synonym of *R. blumiana*), *R. decipiens* var. *ochrospora* J. Blum **nom. inval**. and *R. roseobrunnea* J. Blum.

All three studied taxa are defined by acrid taste, yellow spore print and red pilei and placed by J. Blum in one "Section O" (Blum 1962), but interpretation of their concept and nomenclature developed very variously in the literature. None of these names is applied for a common and widely accepted species and was not recognized as a good species in the important *Russula* monographs (Romagnesi 1967; Sarnari 1998). The most frequently accepted and described is *R. blumiana* (Bon 1986; Reumaux et al. 1996; Sarnari 2001; Tassi 2003; Marxmüller 2014), but Bon (1988), contrary to all other authors, observed incrustations on pilecystidia, which allow very different opinions about its relationships and classification. *Russula roseobrunnea* was validly published by Blum (1953) and soon was considered to be a synonym of *R. pseudoemetica* Singer (Singer 1962), the latter is currently treated as another species with dubious concept and affinity to *R. vinosopurpurea* Jul. Schäff. (Romagnesi 1967; Sarnari 1998). This opinion was adopted by majority of authors, except of Bon (1979, 1988) who described it as a good species similar to *R. blumiana*. *Russula decipiens* var. *ochrospora*, **nom. inval.** is an abandoned name that is even not listed in the nomenclatural databases (http://www.mycobank.org, http://www.indexfungorum.org, http://www2.muse.it/russulales-news). Romagnesi (1967) reported and described his personal collection that was similar to the Blum's description, but he was not certain about its placement, status and relationships. Reumaux et al. (1996) presented the only later description and record of Blum's variety, but

they attributed it the species rank and proposed a new name *Russula ochrosperma* Moënne-Locc. with a new type (collected by these authors, not by J. Blum).

Materials and methods

Sampling

This study is based on specimens from the Blum's herbarium deposited in PC representing the authentic material of three selected taxa: *R. formosa* **nom. illeg.** (homotypic synonym of *R. blumiana*), *R. decipiens* var. *ochrospora* **nom. inval.** and *R. roseobrunnea.* Only for *R. blumiana*, the type specimen designated by Bon (1986) was studied. To identify phylogenetic relationships of sequences retrieved from Blum's material, we used sampling from previous study (Caboň et al. 2017) supplemented by a blast result against GenBank (Kõljalg et al. 2013) and our dataset with 97% similarity and higher. All specimens and sequences are listed in the Supplementary Tab. 1. Herbarium material collected by the authors is deposited in the herbaria: SAV and LIP.

Molecular analysis

Total genomic DNA was extracted from dried material using the methods previously described by Adamčík et al. (2016). Three molecular markers were amplified: the internal transcribed spacer regions of the ribosomal DNA (ITS), (2) partial mitochondrial small subunit ribosomal DNA (mtSSU), (3) the region between domains six and seven of the nuclear gene encoding the second largest subunit of RNA polymerase II (*rpb2*). The ITS region was amplified using the primers ITS1F – ITS4 or alternatively ITS1F – ITS2 and ITS3 – ITS4 (White et al. 1990; Gardes & Bruns 1993). The mtSSU region was amplified using the primer pair MS1 – MS2 (White et al. 1990). Both molecular markers were amplified with polymerase PerfectTAQ (5 PRIME, Hilden, Germany) or Hot Start Firepol Polymerase (Solis Biodyne, Tartu, Estonia) in accordance with the manufacturers recommendation using the same cycling protocol followed of Eberhardt (2012) or Knebelsberger & Stöger (2012) with longer denaturation and annealing times of up to 1 min instead of 30 sec. For amplification of *rpb2* we used a forward primer A-Russ-F (Caboň et al. 2017) and a reverse primer frpb2-7CR (Matheny 2005). The *rpb2* was amplified with Hot Start Firepol Polymerase, following cycling protocol of Caboň et al. (2017).

The PCR products were purified using the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) or Exo-Sap enzymes (Thermo Fisher Scientific, Wilmington, Delaware, USA). Samples were sequenced directly with BigDye 3.1 technology (Applied Biosystems, now Thermo Fisher Scientific, Wilmington, USA), sent to Macrogen Europe (Amsterdam, the Netherlands) or to SEQme (Dobříš, Czech republic) respectively.

Raw sequences were edited in the BioEdit Sequence Alignment Editor version 7.2.5 (Hall 2013), Geneious version R10 (Kearse et al. 2012) or Sequencher version 4.8 (Gene Codes Corporation). Intra-individual polymorphic sites having more than one signal were marked with NC-IUPAC ambiguity codes.

Phylogenetic analysis

Final datasets were aligned by MAFFT version 7 using the strategy E-INS-i (Katoh & Standley 2013) and manually improved in Geneious version R10 (Kearse et al. 2012). All three single-locus datasets were concatenated into one dataset using Seaview version 4.5.1 (Gouy et al. 2010). The concatenated final alignment has been deposited in TreeBASE (21097). The multi-locus dataset was analysed using two different methods: Bayesian inference (BI) and the Maximum Likelihood method (ML). Maximum Likelihood was computed in PhyML using SeaView (Gouy et al. 2010) using GTR+GAMMA substitution

model with 6 rate classes and 1000 bootstrap replications. For BI, the dataset was divided into 6 partitions: ITS, mtSSU, intronic region 7 of *rpb2*, and the 1st, 2nd, and 3rd codon positions of *rpb2*. The best substitution model for each partition was computed jointly in MEGA version 6 (Tamura et al. 2013). The BI was computed independently twice in MrBayes version 3.2.6 (Ronquist et al. 2012) with four MCMC chains for 10 000 000 iterations until the standard deviation of split frequencies reached below the 0.01 threshold. The convergence of runs was visually assessed using Trace function in Tracer version 1.6 (Rambaut et al. 2013).

Morphological observations

Micromorphological characteristics were observed using Olympus CX-41 with oil-immersion lenses at a magnification of 1000×. All drawings of microscopic structures, with the exception of spores, were made with a 'camera lucida' using an Olympus U-DA drawing attachment at a projection scale of 2000×. The contents of hymenial cystidia and pileocystidia were illustrated as observed in Congo red preparations from dried material, with the exception of some pileocystidia for which the contents are indicated schematically (dotted). Spores were observed on the lamellae with Melzer's reagent. All other microscopic observations were made in ammoniacal Congo red, after a short treatment in warm, aqueous KOH solution to dissolve the gelatinous matrix and improve tissue dissociation. All tissues were also examined in Cresyl blue to verify the presence of ortho- or metachromatic reactions as explained in Buyck (1989). Trama and cystidia were examined in a sulfovanillin solution. Acidoresistant incrustations of the primordial hyphae were stained with carbolfuchsin and observed in distilled water after incubation for a few seconds in a 10% solution of HCl (cf. Romagnesi 1967). Spores were scanned with an Artray Artcam 300MI camera and measured by the Quick Micro Photo version 2.1 software with an accuracy of 0.1 μm. Spore measurements excluded ornamentation and their line drawings were made using enlarged, scanned pictures. The Q value indicates the length/width ratio of the spores. The spore ornamentation density was estimated following Adamčík & Marhold (2000). The cystidia density estimates follow Buyck (1991). Statistics for the measurements of microscopic characteristics were based on 30 measurements per specimen and are based on all examined material of the described species. The range of measured values is expressed as the mean ± standard deviation; in the parenthesis are 5 and 95 percentiles.

Results

Three specimens from Blum's herbarium representing the authentic material of *R. formosa* (PC0084516, *J. Blum 1617*), *R. decipiens* var. *ochrospora* (PC0084509, *J. Blum 1933*) and *R. roseobrunnea* (PC0084512, *J. Blum 1395*) were successfully sequenced. The final dataset was composed of 18 specimens with a high sequence similarity to these three samples, 41 samples of 38 different species of the Crown clade and three of the outgroup. In summary, 62 specimens were included in the analysis. Of these specimens, majority (38) encompassed three genetic markers, 18 encompassed two markers and only six had one marker (five with only ITS and one with mtSSU). The final topologies of the ML and BI analyses show generally similar results as in our previous study (Caboň et al. 2017): they were not congruent and there is no significant clustering across the backbone of the Crown clade (Fig. 1).

We confirmed moderate statistical support for the Integrae clade (70/1), which contain two of Blum's specimens: *R. formosa* and *R. roseobrunnea*. Blum's specimen of *R. formosa* is clustered with another four specimens of *R. blumiana* from France and an unidentified specimen from USA in strongly supported (95/1) terminal clade, further nested in newly recognised and strongly supported Badia clade. Blum's specimen identified as *R. roseobrunnea* is placed in the species clade of *R. rutila* Romagn. in the Rubrinae clade.

Blum's specimen identified as *R. decipiens* var. *ochrospora* is clustered in the strongly supported clade together with other specimens identified as typical variety of the species (including the type specimen). The species clade of *R. decipiens* (Singer) Svrček shows relatively isolated position out of the Integrae clade.

Discussion

Some Blum's names as probable synonyms

Two sequenced collections from Blum's herbarium suggest that two studied collections are probably conspecific with another currently accepted species. The collection of *R. roseobrunnea* is conspecific with current concept of *R. rutila* (Caboň et al. 2017) and the collection of *R. decipens* var. *ochrospora* is identical with the type of the typical variety of *R. decipiens* (Fig. 1). Our results are against opinions of mycologists who dealt with these Blum's names.

Blum (1962) recognised and described *R. rutila* and *R. roseobrunnea* as two separate species. Singer (1962) treated *R. roseobrunnea* as synonym of *R. pseudoemetica*, a species related to or identical with *R. vinosopurpurea*, but the latter species has multi-septate pileocystidia (Adamčík & Jančovičová 2013) contrary to pileocystidia of *R. roseobrunnea* described as "vaguement septées en général" in the protologue (Blum 1953). Our morphological observation on the Blum's specimen of *R. roseobrunnea* confirmed presence of incrustations typical for *R. rutila* (Caboň et al. 2017), but this is contrary to Bon's interpretation (Bon 1988) who recognised Blum's species from *R. rutila* based on absence of the incrustations.

In our knowledge, *R. decipiens* var. *ochrospora* was only accepted and re-described by Reumaux et al. (1996) as a new species *R. ochrosperma*. The new species is typified by material collected by authors of the name and the pileocystidia are described as having 0-1 septum. Our morphological revision of the Blum's studied specimen did not confirmed presence of septa on pileocystidia and this is in agreement with the type study of *R. decipiens* var. *decipiens* (Adamčík & Jančovičová 2012) that shows exclusively not septate (one-celled) pileocystidia. In our opinion, *R. ochrosperma* in sense of Reumaux et al. (1996) represents a species different from *R. decipiens*.

Circumscription of R. blumiana

This study does not demonstrate synonymy coincidence of *R. formosa* J. Blum = *R. blumiana* with any other name. We did not sequenced successfully the type specimen of *R. blumiana* designated by Bon (1986), but we got the sequence from another Blum´s specimen (J. *Blum 1617*, PC0084516) as well as our recent collection (LIP *PAM 97090701*) originated from the same site as the type collection (Rambouillet, Etang d'Or, 40 km from Paris). The morphology of the type collection agrees well with other sequenced material, too. We did not confirmed presence of the incrustations on the pileocystidia reported by Bon (1986) in his type study and such incrustations are absent also in descriptions by Sarnari (2001, 2005), Tassi (2003) and Marxmüller (2014).

Russula blumiana belongs to the strongly supported Badia clade together with *R. badia* Quél. and *R. quercilicis* Sarnari. These three species are traditionally classified in two morphologically defined groups based on presence of incrustations on pileocystidia: *R. quercilicis* with incrustations in *R.* subsect. *Rubrinae* (Melzer & Zvára) Singer and other two species without incrustations in *R.* subsect. *Urentes* Maire (Sarnari 1998, 2005). The Badia clade is another example of disagreement between traditional morphological concept and lineages recognised by phylogenetic studies (Adamčík et al. 2016, Caboň et al. 2017). All three species of Badia clade are characterised by acrid taste, yellow spore print and contents of pileocystidia turning grey to black in sulfovanillin. However, this combination of

characters is present in several other unrelated groups of the crown clade. The Badia clade differs from the Rubrinae clade by absence of pink incrustation on pileocystida in sulfovanillin (Caboň et al. 2017) and from the Maculatinae clade by absence of yellow-brownish spots on surface of basidiomata. *Russula decipiens* differs by exclusively one-celled pileocystidia (Adamčík & Jančovičová 2012). *Russula intermedia* P. Karst. has smaller (up to 8 µm long) spores (Ruotsalainen & Vauras 1994) and *R. adulterina* Fr. larger (longer than 10 µm) spores (Sarnari 1998). *Russula cuprea* Krombh., *R. vinosopurpurea, R. gigasperma* Romagn., *R cupreola* Sarnari, *R. cupreoaffinis* Sarnari and *R. aurantioflammans* Ruots., Sarnari & Vauras have all more densely septate (with often more than 2 cells) pileocystidia with frequent diverticules (Sarnari 1998).

Our observations suggest that a possible combination of characters distinguishing the Badia clade from other members of *Maculatinae* sensu lato might be relatively dense spore ornamentation (6-10 elements in the 3 µm circle on the spore surface) and one to two celled pileocystidia (0-1 septate). *Russula blumiana* differs from *R. badia* by very flexuous and usually apically obtuse hyphal terminations in pileipellis near the pileus margin and from *R. quercilicis* by narrower and not incrusted pileocystidia. Sequence of North American (USA) origin very similar to European collections of *R. blumiana* possibly represents a closely related species (Fig. 1).

Taxonomic treatment

Russula blumiana Bon, Cryptogamie, Mycologie 7(4): 299. 1986

≡ *Russula formosa* J. Blum, Bull. Soc. Mycol. France 69: 60. 1953. [**nom. illeg.**, Art. 53: later homonym of *R. formosa* Kučera]

—TYPE: France: Rambouillet, Étang d´Ór, *s.d., s.col. J. Blum 6529* (PC)

Field aspect (Fig. 2): Basidiomata of intermediate size. Pileus 5-7 cm in diam., relatively fleshy, at first hemispherical, than plano-convex, when mature with broad, but not very deep central depression, margin usually deflexed, sometimes when old also inflexed, not or only shortly striated; cuticle relatively matt, slightly rugulose, at first lemon-yellow, soon developing to bright-yellow and orange, finally brick-red often near the margin darker (orange or bright red), than with a paler zone near the centre or between the margin and the centre, often variegated with darker small dots or bumps on the paler background. Stipe 3-4.5 × 1-1.3(-1.8) cm, cylindrical, often wider near the lamellae, with obtuse base, surface more or less longitudinally striated, white, hardly changing colour but sometimes with a pink to red shade or a pink flush. Lamellae moderately distant, slightly ventricose, up to 7 mm wide, towards the cap margin obtuse, adnate-emarginated, cream to pale yellow, edges sometimes red near the pileus margin. Context firm, odour indistinct, taste acrid, reaction to guaiac negative. Spore print yellow, IVa according to the scale of Romagnesi (1967).

Microscopic characters (Fig. 3, 4): Spores broadly ellipsoid, (7.6-)8.3-8.8-9.3(-9.9) × (6.2-)6.8-7.2-7.6(-8.3) µm, Q=(1.11-)1.19-1.22-1.25(-1.34); ornamentation composed of small, dense [(5-)6-9(-10) spines in a 3 µm diam. circle] amyloid spines, 0.5-0.8(-1) µm high; connected with occasional, fine, short line connections [0-2(-3) in the circle], often fused in pairs or short chains (1-6 fusions in the circle), isolated spines frequent; suprahillar plage amyloid, large. Basidia (33-)40.2-46.5-52.8(-59) × (10-)10.6-11.6-12.5(-14.5) µm, 4-spored, clavate, pedicellate; basidiola first cylindrical or ellipsoid, then clavate, ca. 5-10 µm wide (some equally wide as basidia, but shorter). Subhymenium pseudoparenchymatic. Lamellar trama mainly composed of large sphaerocytes. Pleurocystidia dispersed, ca. 500-900/mm^2, measuring (74-)83.7-100.8-117.9(-145) × (9.5-)10.5-11.7-12.9(-17) µm, clavate or fusiform, pedicellate, apically mainly acute, mucronate and with 3-13(-20) µm long appendage, thin-walled, with heteromorphous (mainly granular or crystalline) contents, slowly turn grey in

sulfovanillin. Cheilocystidia clavate, measuring (45-)61.9-73.2-84.5(-101) × (7-)9-10.4-11.8(-14) µm, with obtuse or acute tips, usually without an appendage, with heteromorphous (granular or sometimes also banded) contents. Marginal cells in shape similar to basidioles but smaller and narrower, narrowly clavate to subcylindrical, measuring (8-)15.9-20.9-25.8(-31) × (3-)3.6-4.3-5.1(-7) µm, apically obtuse. Pileipellis orthochromatic in Cresyl blue, not sharply delimited from the underlying sphaerocytes of the context, strongly gelatinized throughout, 120-190 µm deep; vaguely divided in 50-90 µm deep suprapellis of slender, intricate, ascending and up to 5 µm wide hyphae; and ca. 70-110 µm deep subpellis of mainly parallel, filamentous but frequently inflated, up to 8 µm wide hyphae. Acidoresistant incrustations absent. Terminal cells of hyphae in pileipellis near the cap margin measuring (18-)23-32.1-41(-65) × (3-)3.1-3.6-4.2(-5) µm, cylindrical, strongly moniliform-flexuous, apically mainly obtuse, occasionally with slightly constricted tips; subterminal cells equally wide and usually also equally long, often with lateral nodules or branches, intricate. Terminal cells of hyphae in pileipellis near the cap centre shorter, cylindrical, measuring (17-)20.6-26.3-32(-42) × (2.5)-3-3.5-3.9(-4.5) µm, frequently nodulose; subterminal cells shorter than those near the cap margin. Pileocystidia narrowly clavate or subcylindrical, mainly one-celled, occasionally or rarely of two or three cells, originating deep in suprapellis or in subpellis, terminal cells near the cap margin measuring (30-)58.3-74.7-91(-140) × (5-)6.5-7.3-8(-9.5) µm, apically obtuse, towards the basal part often strongly narrowed (3.5-5 µm), thin-walled, in Congo red with heteromorphous (mostly granular) contents, hardly react to sulfovanillin; pileocystidia near the cap centre more frequently septate, with shorter terminal cells measuring (22-)44-64.3-84.6(-111) × (6.5-)6.8-7.5-8.2(-9.5) µm. Pileus trama without cystidioid hyphae. Clamp connections absent in all parts.

Additional material examined: France, Rambouillet: Étang d´Ór, *s.d.*, *s.col.*, *J. Blum 6529* (PC); ibid., *s.d.*, *s.col.*, *J. Blum 1617* (PC0084516); ibid., 7 Sep 1997, *R. Chalange*, *PAM 97090701* (LIP); France, Bas-Rhin, Gunstett: Forêt de Gunstett, 25 Jul 2014, *J.M. Trendel*, *JMT 14072513* (SAV).

Conclusions

Our study suggests that a large part of taxa described by J. Blum are synonyms of other widely used *Russula* names. Among three studied taxa, only *R. blumiana* is confirmed to be a good species. This study also suggests a high probability to obtain sequences of ribosomal DNA from authentic material collected by J. Blum that may help to interpret the concept of his taxa. It is also possible that the description and concept of some of Blum's taxa correspond to more than one species, but this is difficult to check, because there is a limited number of specimens determined by J. Blum available in his herbarium (http://cobaye.mnhn.fr/institution/mnhn/collection/pc/).

References

Adamčík S, Jančovičová S (2012) Type studies in *Russula* subsection *Maculatinae*: *R. decipiens* and related taxa as interpreted by H. Romagnesi. Cryptogamie Mycol 33: 411–420. doi: 10.7872/crym.v33.iss4.2012.411

Adamčík S, Jančovičová S (2013) Type studies in *Russula* subsection *Maculatinae*: four species typified by H. Romagnesi. Sydowia 65: 201–222

Adamčík S, Marhold K (2000) Taxonomy of the *Russula xerampelina* group. I. Morphometric study of the *Russula xerampelina* group in Slovakia. Mycotaxon 76: 463–479

Adamčík S, Caboň M, Eberhardt U, Saba M, Hampe F, Slovák M, Kleine J, Marxmüller H, Jančovičová S, Pfister DH, Khalid AN, Kolařík M, Marhold K, Verbeken A (2016) A

molecular analysis reveals hidden species diversity within the current concept of *Russula maculata* (Russulaceae, Basidiomycota). Phytotaxa 270: 71–88. doi: 10.11646/phytotaxa.270.2.1

Blum J (1953) Les Russules âcres à sporée jaune. Bull Trim Soc Mycol Fr 69: 57–76

Blum J (1962) Les Russules. Flore Monographique des Russules de le France et des Pays Voisins. Lechevalier, Paris

Bon M (1979) Fungorum Rariorum Icones Coloratae, Pars XI. J. Cramer, Vaduz

Bon M (1986) Validations et typifications des Russules de Blum. Cryptogamie Mycol 7: 295–309

Bon M (1988) Clé monographique des russules d'Europe. Doc Mycol 18: 1–120

Buyck B (1989) Valeur taxonomique du bleu de crésyl pour le genre *Russula*. Bull Trim Soc Mycol Fr 105: 1–6

Buyck B (1991) The study of microscopic features in *Russula* 2. sterile cells of the hymenium. Russulales News 1: 62–85

Caboň M, Eberhardt U, Looney B, Hampe F, Kolařík M, Jančovičová S, Verbeken A, Adamčík S (2017) New insights in *Russula* subsect. *Rubrinae*: phylogeny and the quest for synapomorphic characters. Mycol Prog: submitted

Eberhardt U (2012) Methods for DNA barcoding of fungi. In: Kress WJ, Erickson DL (eds) DNA Barcodes Methods and Protocols, Humana Press, New York, pp 183–205. doi: 10.1007/978-1-61779-591-6_9

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2: 113–118

Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27: 221–224. doi: 10.1093/molbev/msp259

Hall T (2013) BioEdit, Biological sequence alignment editor for Windows 95/98/NT/2000/XP/7, version 7.2.5. Avaliable at: http://www.mbio.ncsu.edu/bioedit/bioedit.html, Accessed 20 February 2014

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software, version 7: improvements in performance and usability. Mol Biol Evol 30: 772–780. doi: 10.1093/molbev/mst010

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. doi: 10.1093/bioinformatics/bts199

Knebelsberger T, Stöger I (2012) DNA extraction, preservation, and amplification. In: Kress WJ, Erickson DL (eds) DNA Barcodes Methods and Protocols, Humana Press, New York, pp 311–338. doi: 10.1007/978-1-61779-591-6_14

Kõljalg U, Nilsson RH, Abarenkov K et al. (2013) Towards a unified paradigm for sequence-based identification of Fungi. Mol Ecol 22: 5271–5277. doi: 10.1111/mec.12481

Marxmüller H (2014) Russularum Icones, Vol. 2. Anatis Verlag, München

Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Mol Phylogenet Evol 35: 1–20. doi: 10.1016/j.ympev.2004.11.014

Reumaux P, Bidaud A, Moënne-Loccoz P (1996) Russules rares ou méconnues. Fédération Mycologique Dauphiné-Savoie, La Roche-sur-Foron

Rambaut A, Suchard MA, Xie D, Drummond AJ (2013) Tracer version 1.6. Avaliable at: http://beast.bio.ed.ac.uk/software/tracer/. Accessed 30 March 2015

Romagnesi H (1967) Les Russules D'Europe et D'Afrique du Nord. Bordas, Paris

Ronquist F, Teslenko M, van der Mark P, Avres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice, across a large model space. Syst Biol 61: 539–542. doi: 10.1093/sysbio/sys029

Ruotsalainen J, Vauras J (1994) Novelties in *Russula*: *R. olivobrunnea*, *R. intermedia* and *R. groenlandica*. Karstenia 34: 21–34

Sarnari M (1998) Monographia Illustrata del Genere *Russula* in Europa Vol. 1. Associazioni Micologica Bresadola, Trento

Sarnari M (2001) Sottogenere *Rusula* sez. *Russula* sottosezione *Urentes* (supplemento) serie Formosa ad interim. Rivista Micol 44: 63–70

Sarnari M (2005) Monographia Illustrata del Genere *Russula* in Europa Vol. 2. Associazioni Micologica Bresadola, Trento

Singer R (1962) Four interesting European Russulae of subsections *Sardoninae* and *Urentinae*, sect. *Russula*. Sydowia 16: 289–301

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725–2729. doi: 10.1093/molbev/mst197

Tassi G (2003) Étude de quelques récoltes intéressantes de russules. Bull Trim Soc Mycol Fr 119: 195–215

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, pp 315–322

Figures

Fig. 1 Maximum Likelihood phylogeny inferred from three loci (ITS, mtSSU and *rpb2*) with three target species-level clades highlighted. Supported superclades comprising the target species are indicated by arrows. Basidiomata samples are labelled by herbarium code and collections number in parenthesis, sequences of environmental samples are labelled with GenBank accession numbers in italics. Countries of origin are provided for the target species. Bootstrap values followed by Bayesian posterior probabilities are indicated above nodes.

Fig. 2 Field appearance of *R. blumiana*. **a**–**b** *JMT 14072513* (SAV), photo by J.M. Trendel. **c**–**d** LIP (PAM *97090701*), photo by P.A. Moreau.

Fig. 3 Hymenial elements of *R. blumiana, J. Blum 1617* (PC0084516, as *R. formosa*) **a** Cheilocystidia. **b** Pleurocystidia. **c** Basidiola. **d** Basidia. **e** Marginal cells. **f** Spores in Melzer's reagent. Contents of cystidia are shown as observed in Congo red for some elements only, the plus sign indicates the contents of the others schematically. Scale bar equals 10 μm, but only 5 μm for spores. Drawings by: S. Jančovičová.

Fig. 4 Pileipellis of *R. blumiana* **a** Pileocystidia near the pileus centre, SAV (*JMT 14072513C*). **b** Pileocystidia near the pileus margin, *J. Blum 1617* (PC0084516, as *R. formosa*). **c** Hyphal terminations near the pileus centre, SAV (*JMT 14072513C*). **d** Hyphal terminations in the pileus margin, *J. Blum 1617* (PC0084516, as *R. formosa*). Contents of cystidia are shown as observed in Congo red for some elements only, the plus sign indicated the contents of the others schematically. Scale bar equals 10 μm. Drawings by: S. Jančovičová.

Table 1 List of all species and infraspecific *Russula* names described by J. Blum arranged chronologically. Abbreviations: BSMF – Bulletin trimestriel de la Société mycologique de France, L – Latin diagnosis in the protologue, T – type specimen designated in the protologue

Blum's names	status	validations
R. fuscorubra var. *olivovirens* J. Blum, BSMF 67: 166. 1951	valid (L)	
R. amoena var. *intermedia* J. Blum, BSMF 68: 255. 1952	invalid	
R. caeruleomalva J. Blum, BSMF 68: 238. 1952	valid (L)	
R. grisea var. *leucospora* J. Blum, BSMF 68: 257. 1952	invalid	*R. leucospora* Bon, Cryptogamie Mycol 7: 301. 1986
R. lilacinicolor J. Blum, BSMF 68: 253. 1952	valid (L)	
R. maculata var. *globispora* J. Blum, BSMF 68: 232. 1952	valid (L)	
R. roseicolor J. Blum, BSMF 68: 224. 1952	valid (L)	
R. adulterina f. *frondosae* J. Blum, BSMF 69: 70. 1953	invalid	*R. frondosae* Reumaux, Russules rares ou méconnues: 284. 1996
R. formosa J. Blum, BSMF 69: 60. 1953	illeg. (L)	*R. blumiana* Bon, Cryptogamie Mycol 7: 299. 1986
R. roseobrunnea J. Blum, BSMF 69: 64. 1953	valid (L)	
R. carminipes J. Blum, BSMF 69: 449. 1953	valid (L)	
R. cupreoviolacea J. Blum, BSMF 69: 440. 1953	valid (L)	
R. lepida subsp. *flavescens* J. Blum, BSMF 69: 435.1953	invalid	
R. pseudolilacea J. Blum, BSMF 69: 434. 1953	valid (L)	
R. pseudorosea J. Blum, BSMF 69: 436. 1953	valid (L)	
R. robertii J. Blum, BSMF 69: 443. 1953	valid (L)	
R. variecolor J. Blum, BSMF 69: 445. 1953	illeg. (L)	*R. blumii* Bon, Cryptogamie Mycol 7: 309. 1986
R. xerampelina var. *abietum* J. Blum, BSMF 69: 429. 1953	valid (L)	
R. fulva J. Blum, BSMF 70: 406. 1955	invalid	*R. mustelina* var. *fulva* Bon, Cryptogamie Mycol 7: 299. 1986
R. fuscorosea J. Blum, BSMF 70: 406. 1955	invalid	*R. fuscorosea* Bon, Cryptogamie Mycol 7: 300. 1986
R. integra f. *fulvidula* J. Blum, BSMF 70: 394. 1955	invalid	
R. pseudoromellii J. Blum, BSMF 70: 399. 1955	invalid	*R. pseudoromellii* Bon, Cryptogamie Mycol 7: 305. 1986
R. subintegra J. Blum, BSMF 70: 395. 1955	invalid	*R. subintegra* Bon, Cryptogamie Mycol 7: 307. 1986
R. tinctipes J. Blum, BSMF 70: 401. 1955	invalid	*R. tinctipes* Bon, Cryptogamie Mycol 7: 308. 1986
R. crawshayriana J. Blum, BSMF 72: 152. 1956	invalid	*R. joannis* Bon, Cryptogamie Mycol 7: 297. 1986
R. luteotacta var. *semitalis* J. Blum, BSMF 72: 143. 1956	invalid	*R. luteotacta* var. *semitalis* Bon, Cryptogamie Mycol 7: 303. 1986
R. luteotacta var. *serrulata* J. Blum, BSMF 72: 142. 1956	invalid	
R. persicina var. *montana* J. Blum, BSMF 73: 268. 1957	invalid	
R. pyrenaica J. Blum, BSMF 73: 257. 1957	invalid	*R. pyrenaica* Singer, Collect. Bot. (Barcelona) 13: 687. 1982
R. speciosa J. Blum, BSMF 73: 264. 1957	invalid	*R. speciosa* Bon, Cryptogamie Mycol 7: 307. 1986
R. flavocitrina J. Blum, BSMF 76: 267. 1960	invalid	*R. flavocitrina* Bon, Cryptogamie Mycol 7: 298. 1986

Blum's names	status	validations
R. multicolor J. Blum, BSMF 76: 266. 1960	invalid	Russula multicolor Bon, Cryptogamie Mycol 7: 304. 1986
R. vesca f. *montana* J. Blum, BSMF 76: 253. 1960	invalid	
R. xerampelina var. *ochracea* J. Blum, BSMF 77: 162. 1961	invalid	
R. decipiens var. *ochrospora* J. Blum, Les Russules: 175. 1962	invalid	
R. brunneoviolacea var. *cristatispora* J. Blum, Les Russules: 94. 1962	invalid	*R. brunneoviolacea* var. *cristatispora* Bon, Cryptogamie Mycol 7: 297. 1986
R. delica var. *glutinosa* J. Blum, Les Russules: 207. 1962	invalid	*R. chloroides* var. *glutinosa* Bon, Cryptogamie Mycol 7: 300. 1986
R. maximispora J. Blum, Les Russules: 113. 1962	invalid	*R. maximispora* Bon, Cryptogamie Mycol 7: 303. 1986
R. pseudodelica var. *flavispora* J. Blum, Les Russules: 208. 1962	invalid	*R. flavispora* Romagn., Russules d'Europe Afr. Nord: 233. 1967
R. pseudodelica var. *pallidospora* J. Blum, Les Russules: 208. 1962	invalid	*R. pallidospora* Romagn. Russules d'Europe Afr. Nord: 233. 1967
R. pseudomelitodes J. Blum, Les Russules: 132. 1962	invalid	*R. pseudomelitodes* Bon, Cryptogamie Mycol 7: 305. 1986
R. sabulosa Heim & J. Blum,, Les Russules: 204. 1962	invalid	*R. adusta* var. *sabulosa* Bon, Cryptogamie Mycol 7: 306. 1986
R. werneri var. *europae* J. Blum, Les Russules: 128. 1962	invalid	*R. europae* Romagn., Russules d'Europe Afr. Nord: 834. 1967
R. delicatoides J. Blum, Rev Mycol (Paris) 33 (1): 113. 1968	valid (L, T)	
R. straminea var. *battouenii* J. Blum, Rev Mycol (Paris) 33 (1): 111. 1968	valid (L, T)	

Fig 1

Click here to download Figure Fig 1.tif

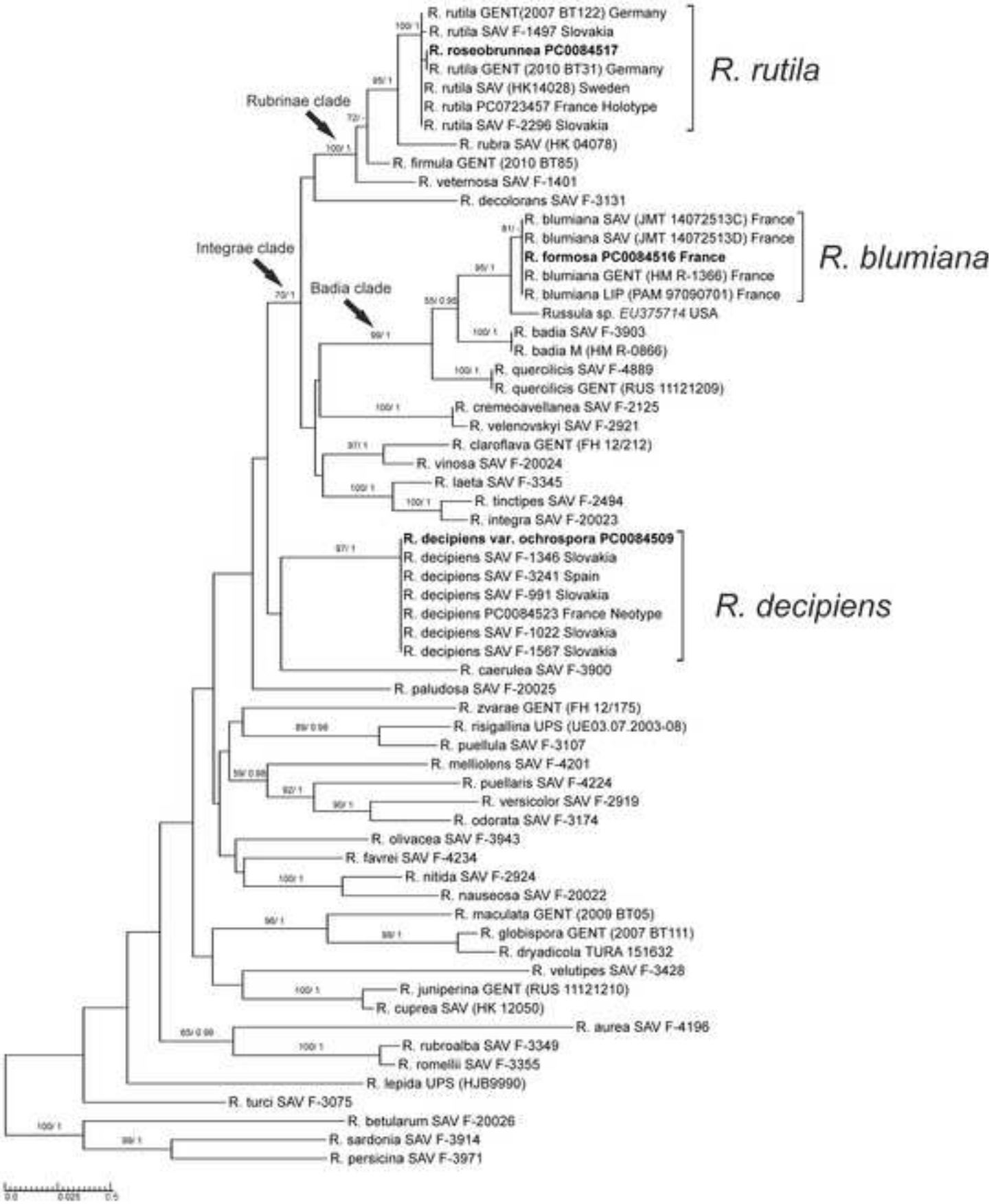


Fig 2

Click here to download Figure Fig2.tif

Fig 3

Click here to download Figure Fig3.tif

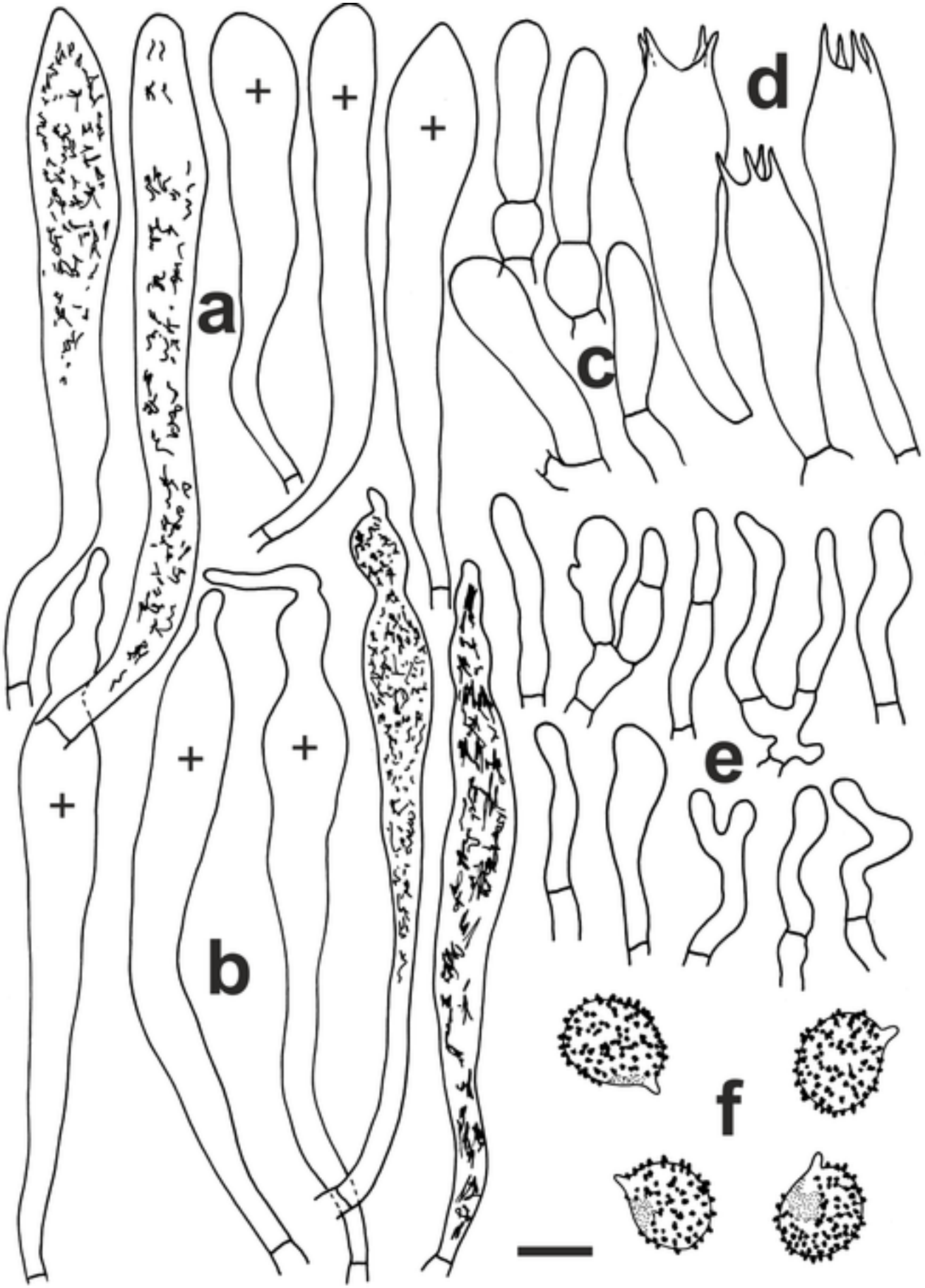
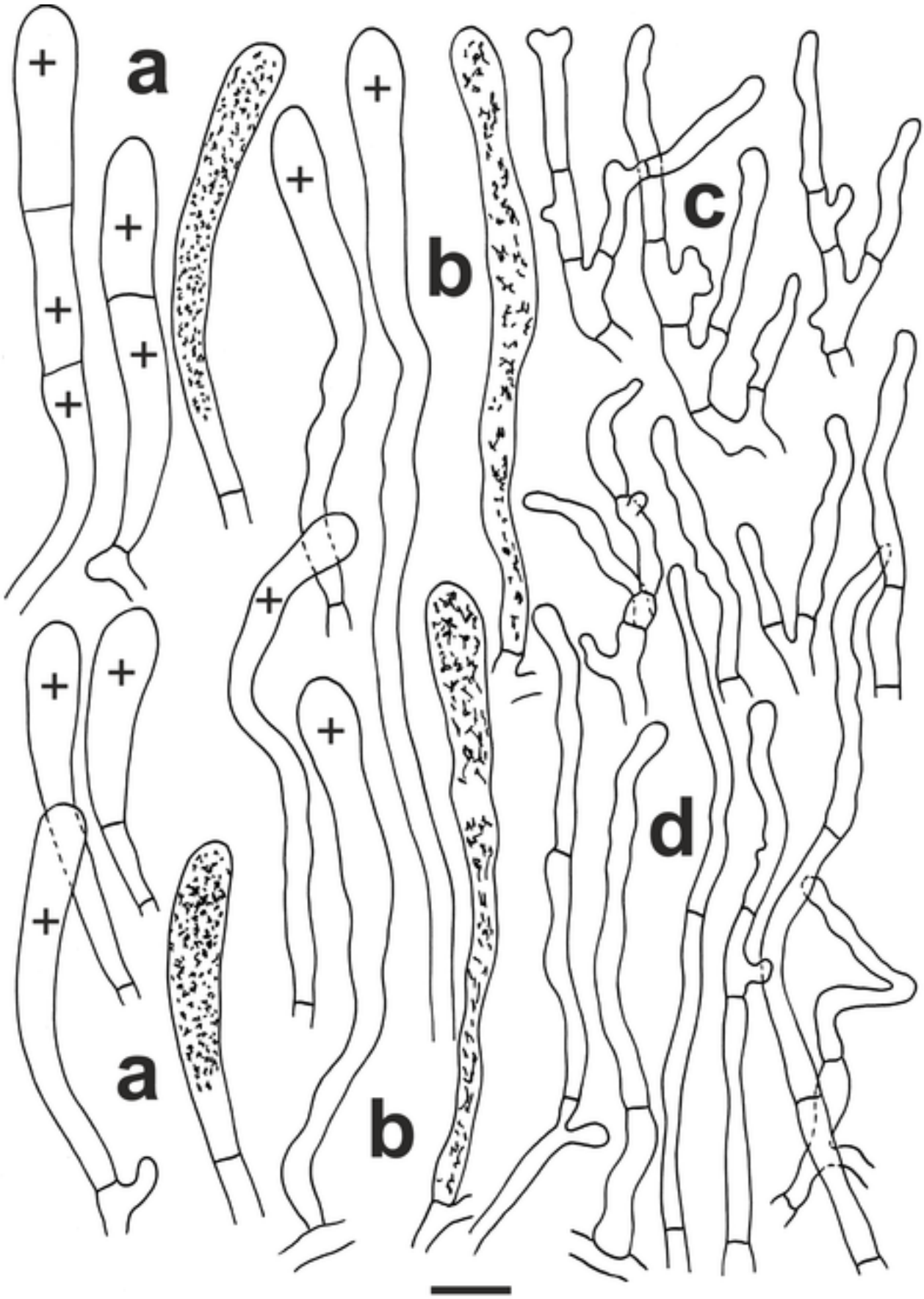


Fig 4

Click here to download Figure fig 4.tif



Covering letter

Dear Editor-in-Chief,

We are submitting the manuscript entitled "**Blum versus Romagnesi: checking possible *Russula* (Russulaceae, Basidiomycota) synonymies of the two contemporary authors**" prepared by Miroslav Caboň, Soňa Jančovičová, Jean Michel Trendel, Pierre-Arthur Moreau, Felix Hampe, Miroslav Kolařík, Annemieke Verbeken and Slavomír Adamčík for publication in your journal Plant Systematics and Evolution.

The manuscript contains original unpublished data on morphological observations and DNA sequences. All sequences published by authors are deposited in GenBank database and provided with accession numbers, the alignment is deposited in TreeBASE. All authors had possibility to check the text and agreed with its publication. Field work has been accomplished by MC, FH, SA, JMT, PAM and SJ. MC, SA and SJ prepared micro-morphological analyses, SJ and MC prepared line drawings. MC, MK and FH performed DNA extractions and molecular laboratory work, MC and SA edited sequence files and aligned sequences, MC and SA performed phylogenetic analysis. SA and MC wrote the manuscript but with significant contribution of all authors. All persons contributing with material and photographs agreed with using of them and are acknowledged.

Proposed reviewers:
Jorinde Nuytick jorinde.nuytinck@naturalis.nl
Brian P. Looney blooney@vols.utk.edu
Anna Ronikier a.ronikier@botany.pl

Thank you for considering of our manuscript for publication in your journal

Yours sincerely

Corresponding author:
Miroslav Caboň
Department of Cryptogams, Institute of Botany, Plant Science and Biodiversity Centre, Slovak Academy of Sciences
Dúbravská cesta 9, SK-845 23 Bratislava, Slovakia
miroslav.cabon@savba.sk