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Walking the thin line… ten years later: the dilemma of above- versus below-ground features to support phylogenies in the *Russulaceae* (Basidiomycota)

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Abstract

For the very first time, morpho-anatomical features of both fruiting bodies as well as below-ground structures have been confronted with a newly produced multigene phylogeny of root symbiotic basidiomycetes using one of the most speciose genera of ectomycorrhizal fungi (*Russula*, Russulales) as an example. In this first of two papers, the authors focus more specifically on below-ground structures. Our five-gene phylogeny divides the genus in five main clades, here interpreted as representing seven subgenera, all significantly supported. Although more conserved than features of fruiting bodies, the anatomy of ectomycorrhiza does not allow for an unambiguous characterization of the main clades resolved by phylogenetic analysis, but the anatomy of ectomycorrhiza performs better to naturally classify the species of this genus. Features of fruiting bodies remain much more adequate for the delimitation of terminal clades and are irreplaceable for morphological species identification. Tropical taxa mostly nest in ancient lineages, but are also present in some terminal clades of otherwise temperate species groups. The shift from plectenchymatic to pseudoparenchymatic ECM outer mantle structures happened most likely already in the paleotropics, and is here hypothesized to have facilitated a major diversification of the genus with new hosts in the northern hemisphere. Available data as well as our own observations on below ground structures of several *Lactifluus* species suggests that this genus shares with *Russula* the absence of lactifers in ECM mantles and rhizomorphs, contrary to species of *Lactarius* where lactifers are always present. First observations on rhizomorphs of species in *Multifurca* confirm the presence of vessel-like and ladder-like hyphae, also found in the other agarioid genera of this family, while distinct lactifers are only present in the lactarioid, but not in russuloid members of this genus.

Keywords Ectomycorrhizal anatomy · *Lactifluus* · *Multifurca* · Multigene phylogeny · Rhizomorphs

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Introduction

The root-symbiotic, ectomycorrhizal (ECM), mushroom-forming fungi implicate a conservative estimate of ca. 25,000 fungi worldwide (Comandini et al. 2012). They comprise more than 250 genera that represent some 78–82 fungal lineages in Asco-, Basidio- and Zygomycota that independently acquired the ECM mode of nutrition without any obvious proof for reversals (Tedersoo et al. 2010; Tedersoo and Smith 2013). Among plant symbiotic fungi, the ECM life style is a very important ecological trait as these fungi shape most of the dominant types of woody vegetation on earth (Brundrett 2009; Tedersoo et al. 2010). With proportions of colonized fine root tips approaching in some cases 100% (Taylor et al. 2000), the host plant may become effectively isolated from the soil environment as any nutrients and water entering the root must first pass through a fungal mantle, and so must any material leaving the root. Ectomycorrhizal fungi therefore occupy and, most probably control, the interface between the soil environment and their host plants (Taylor and Alexander 2005).

Fungal fruiting bodies offer the traditional features that laid the basis for the first classifications of these mushroom forming fungi; they also provided the only features currently used in identification keys for the various groups of mushroom forming fungi. Anatomical features of the mycelium and its various specialized structures have mostly been—and most often still are—ignored when field mycologists collect mushrooms in the field or when new mushroom-forming taxa are described. As the large majority of the ECM fungi produce soft, fleshy fruiting bodies that are extremely short-lived, Agerer (1987–2012, 1995, 2001, 2006 and references therein) and collaborators therefore started some 30 years ago to explore other avenues allowing for the identification of these ecologically and economically important fungi by studying their below-ground structures. As such, they started to document the anatomy and morphology of the dual symbiotic organs that are related to this mutualistic life style, i.e. the ectomycorrhiza, and also of the specialized structures associated with nutrient transport and soil exploration, the fungal rhizomorphs. These studies not only demonstrated a surprising morpho-anatomical diversity among fungal below ground structures, but revealed also the existence of different strategies of soil exploration among different groups of ECM fungi (Agerer 2001) which are increasingly seen as crucial elements for a better understanding of the plant-fungus symbiosis (Lilleskov et al. 2011; Koide et al. 2014).

Russula Pers, the subject of this paper and the type genus of family Russulaceae, is certainly one of the largest and most frequently monographed genera of ECM Basidiomycetes (for a list of European monographs, see Sarnari 2005). The genus was demonstrated to represent one of four monophyletic clades in non-corticioid Russulaceae (Buyck et al. 2008, 2010), all of which are presently considered to be obligatory root-symbiotic species, even though this assumption remains—as for all ECM fungal genera—largely based on extrapolation of observations made for a limited number of species. This extrapolation seems justified or further supported by (1) circumstantial evidence that all known species are strictly confined to plant communities that are inhabited by known ECM hosts, and (2) that even those species that typically grow in more elevated positions on dead or living plant tissues have been shown to still form abundant ECM (Henkel et al. 2000).

Compared to several other groups of mushroom forming fungi, *Russula* fruiting bodies exhibit a high diversity of macroscopic, microscopic and chemical features, which resulted in very complex and highly structured, multilevel classifications (e.g. Sarnari 1998; Singer 1986; Romagnesi 1967; Bon 1988). Macroscopic features, such as spore print color, taste and smell, context firmness, cap color, context color changes and reactions to certain chemical reagents, as well as hymenophore configuration were among the main features that allowed for the subdivision of the genus in the earliest classifications (e.g. Singer 1932). Very rapidly, however, European monographs started to take considerable advantage of a wide range of microscopic features and microchemical reactions, in particular those related to the spores and the composition of the cap cuticle, culminating finally in the still widely authoritative subdivision of the genus into nine subgenera, each in turn subdivided in several sections and then further into subsections, series or stirps (Romagnesi 1967, 1987). Since then, recent molecular studies have demonstrated the poor phylogenetic signal of the principal macroscopical features (such as overall habit or exudation of milk) used for the delimitation of the different recognized genera in Russulaceae (see Buyck et al. 2008), leading thus to the modern concept of a genus *Russula* that includes not only the traditional epigeous, agaricoid species, but now also some pleurotoid species, as well as many species that exhibit a reduction to complete suppression of the stipe resulting in truffle-like fruiting bodies that remain hypogeous their entire life (Lebel and Tonkin 2007).

With regard to the anatomy of the below ground structures, *Russula* is certainly the best documented ECM genus in terms of availability of detailed published descriptions for ECM and rhizomorphs, in particular through the work of Beenken (2004). Confronting these below-ground features with genus phylogenies was until now hampered by either the lack of a strongly supported, representative genus phylogeny for ECM fungi, or by the unavailability of ECM descriptions for a representative number of species within a particular genus, or both. Recently produced multigene

phylogenies for the ECM genus *Cantharellus* Adans.:Fr. (Buyck et al. 2014, 2016; De Kesel et al. 2016) or for ECM family Inocybaceae (Matheny et al. 2009), for example, could solely discuss correlations with features of the fruiting bodies in the near absence of published anatomical-morphological studies of their ectomycorrhiza.

To the best of our knowledge, this is the first study that confronts ectomycorrhizal anatomy with a robust multigene phylogeny for a representative, worldwide sampling of ECM mushroom-forming basidiomycetes (Agaricomycotina). In this first of two papers, the authors will focus more specifically on features of below-ground structures.

Materials and methods

Sampling

Below-ground features

All in all, this study of family Russulaceae could benefit, based on available data and also on newly made observations, from information on below-ground structures for over 80 *Russula* taxa, as well as from those for 7 *Lactifluus* (Pers.) Roussel, 33 *Lactarius* Pers. and 4 *Multifurca* Buyck & V. Hofst. (for the names of these taxa, see Online Resource 1). As only part of our sampling did benefit from data on below-ground structures, the different features are not mapped on the phylogenetic tree but summarized separately (see Online Resource 2). Detailed descriptions for below-ground anatomy can be found for 64 Russula species on www.deemy.de, in particular through the PhD work of Beenken (2004). These constitute a more or less representative image for the European *Russula* species, but the various species groups that have a predominantly or exclusively tropical distribution remain largely undocumented. For the purpose of this paper, therefore, additional ECM structures for several (sub)tropical species of *Russula*, as well as for most of the outgroup taxa, have been studied by the first author (for the names of these taxa, see Online Resource 1; detailed descriptions will eventually be published elsewhere).

Taxon sampling, DNA isolation, amplification and sequencing

For this study 160 fungal collections were sampled from a pool of over 1000 extracted and sequenced agaricoid specimens belonging to family Russulaceae (as defined by Larsson and Larsson 2003). The final five-gene dataset minimizes missing data and represents 149 *Russula* species. Additionally, eleven outgroup taxa were selected following Buyck et al. (2008), including two species of *Multifurca*, three species of *Lactarius* and six species of *Lactifluus* (see Online Resource 3). All sequences were generated by the authors of this study, except for the three *Lactarius* species for which sequence data were sampled from GenBank (Van de Putte et al. 2012). For easier comprehension of the following paragraphs, names of well-known or otherwise discussed (sub)sections that were delimited on morphological basis, have been plotted onto the phylogeny (see Online Resource 4).

Fungal genomic DNA was isolated as described in Hofstetter et al. (2002) from fresh or dried material stored in cetyl-trimethyl-ammonium bromide buffer (CTAB 1x). Five loci were amplified and sequenced: 900–1400 base pairs of the ribosomal nuclear large subunit (nucLSU) using primers LROR and LR7 (or LR5 when unsuccessful with LR7, see http://www.biology.duke.edu/fungi/mycolab/primers.htm); 600 base pairs of the ribosomal mitochondrial small subunit (mitSSU) with primers MS1 and MS2 (White et al. 1990); 1300 base pairs of the largest subunit of the RNA polymerase II (*RPB*1) with primers *RPB*1-AF (Stiller and Hall 1997) and *RPB*1-CR (Matheny et al. 2009); 700 base pairs of the second largest subunit of the RNA polymerase II (*RPB*2) using primers *RPB*2-6F and f*RPB*2-7cR (Liu and Hall 2004) or other backward primers newly designed for this study: *RPB*2-7cRruss1 (5′-TGGTAYGTRTTTC-GAGG-3′) or *RPB*2-7cRruss2 (5′-GCCCATRGCY-GAYTGGTA-3′); and 900 base pairs of the translation elongation factor 1-alpha (*TEF1*) using primers EF1-F and EF1-R (Morehouse et al. 2003). Amplifications were performed under the conditions and with the reagents of the Taq PCR core kit (QIAGEN, Inc., Valencia, California, USA). Sequencing used the amplification primers, reagents and conditions of the BigDye®Terminator v3.1 Cycle sequencing Kit and an automated capillary sequencer ABI 3700 DNA analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses

Sequences were assembled and edited using the software package Sequencher TM 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Alignments were performed manually in MacClade v4.05 (Maddison and Maddison 2002). All best tree searches and bootstrap analyses were conducted in RAxML-VI-HPC version 7.7.7 (Stamatakis 2006) using the rapid bootstrap algorithm (RBS; option –f a) implemented and developed by Stamatakis et al. (2008). The general time-reversible substitution model with among site rate heterogeneity was selected (option –m GTRGAMMA) and 1000 runs with distinct heuristic starting trees (option –N 1000) were executed. Two

different partitionings of the data were tested for their ability to maximize likelihood tree value and RBS support: 12 partitions (nucLSU, mitSSU, *RPB*1 intron, *RPB*1 1st, *RPB*1 2nd, *RPB*1 3rd, *RPB*2 1st, *RPB*2 2nd, *RPB*2 3rd, *TEF1* 1st, *TEF1* 2nd and *TEF1* 3rd) or 9 partitions (nucLSU, mitSSU, *RPB*1 intron, *RPB*1 1st + 2nd, *RPB*1 3rd, *RPB*2 1st + 2nd, *RPB*2 3rd, *TEF1* 1st + 2nd and *TEF1* 3rd).

Combinability among the five datasets was examined conducting 1000 RBS heuristics for each single locus dataset and for the 5-loci used in combination. The module compat.py (available at: www.lutzonilab.net) was used to compare the resulting RBS values between single locus analyses versus combined analysis of the five loci. Conflict was assumed when single-locus and combined-locus analyses for the same set of taxa inferred two different relationships both with significant RBS support values (RBS $\geq$ 70%; Mason-Gamer and Kellog 1996).

After exclusion of conflicting sequence data, a search for the best tree and for RBS values were conducted on the five-locus combined dataset in RAxML with the same settings as for data combinability analyses. In addition, Bayesian analyses were completed on this combined dataset using a Bayesian Metropolis coupled Markov chain Monte Carlo algorithm (B-MCMCMC) as implemented in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003). Bayesian analyses were conducted with eight independent chains, sampling every 100th tree for 15 million generations using a GTR model of nucleotide substitution, with an estimated proportion of invariable sites and a gamma distribution of rate variation with four categories. To ensure that all runs converged to the same log-likelihood stationary level, we conducted three independent B-MCMCMC runs and verified that the average standard deviation of split frequencies stayed below 0.01 for each run after the 10 million generation burn-in phase. The 50,000 last trees of each run were collected to build a majority-rule consensus tree and to calculate Bayesian posterior probabilities (PP). Tree branches were considered significantly supported when RBS values were $\geq$ 70% and PP values were $\geq$ 0.95 (Alfaro et al. 2003).

Results

Phylogenetic analyses and combinability tests

A total of 739 sequences were newly generated for this study (156 nucLSU, 156 mitSSU, 155 *RPB*2, 135 *RPB*1 and 137 *TEF*-1) and deposited in GenBank (see Online Resource 3). The 160 taxa 5-locus full-length alignment was 4826 base pairs long. After exclusion of ambiguously aligned regions (spliceosomal introns in protein-coding genes and parts of the variable domains of mitSSU and nucLSU) the sequence data matrix used for phylogenetic analyses was 3514 base pairs long. Sequences that could not be obtained (see Online Resource 3) were coded as missing characters in the combined dataset. Using twelve partitions resulted in slightly higher RBS support values than using nine partitions. Twelve partitions were consequently implemented for further phylogenetic analyses (nucLSU, mitSSU, *RPB*1 intron, *RPB*1 1st, *RPB*1 2nd, *RPB*1 3rd, *RPB*2 1st, *RPB*2 2nd, *RPB*2 3rd, *TEF1* 1st, *TEF1* 2nd and *TEF1* 3rd).

Combinability tests revealed that two sequences (nuc*LSU* for *R. pelargonia* Niolle and *RPB*2 for *R. liberiensis* Singer) were incongruent (contaminant and obtained from the wrong taxon, respectively). These two sequences (accessible in GenBank, see Online Resource 3) were consequently removed from the 160 taxa 5-locus dataset.

Overall topology of this phylogeny

ML and Bayesian resolution and tree topologies were nearly identical. The most likely ML tree (-ln = 54,270.874549) is depicted in Fig. 1. Phylogenetic analyses infer with maximal support a monophyletic *Russula* (RBS = 100%, PP = 1) and the most likely tree splits this genus into five major clades.

Reading from base to top, the first and basal, fully supported monophyletic clade (RBS = 100%, PP = 1) is solved from the rest of *Russula* only by Bayesian analysis (RBS < 50%, PP = 0.97). This basal clade is composed of two subclades in a sister relationship, viz. subgenera *Archaea* (clade I; RBS = 59%, PP = 1) and *Compactae* (Fr.) Bon (clade II; RBS = 100%, PP = 1). The second main clade is also significantly supported (RBS = 94%, PP = 1) and is composed of one large subclade representing subg. *Heterophyllidia* Romagn. (clade IV; RBS = 100%, PP = 1) which clusters with a single species that represents subg. *Crassotunicata* Buyck & V. Hofst. (III in our phylogeny). The latter is a very small subgenus that is presently composed of three species worldwide (see Bazzicalupo et al. 2017 and discussion below). A handful of species representing subg. *Malodora* Buyck & V. Hofst. (clade V; RBS = 100%, PP = 1) constitute the third major *Russula* clade, while the fourth main clade corresponds to subg. *Brevipes* Buyck & V. Hofst. (clade VI; RBS = 97%, PP = 1). Finally, the fifth clade represents subg. *Russula* (RBS = 100%, PP = 1), a clade which includes nearly half of the species from this analysis and the vast majority of the northern hemisphere diversity of the genus. It is again subdivided in two significantly supported groups: clade VII (RBS = 78%, PP = 1) and clade VIII

(RBS = 99%, PP = 1). The relationships between these five major clades remain largely unresolved.

Discussion

Overall topology versus traditional classification proposals

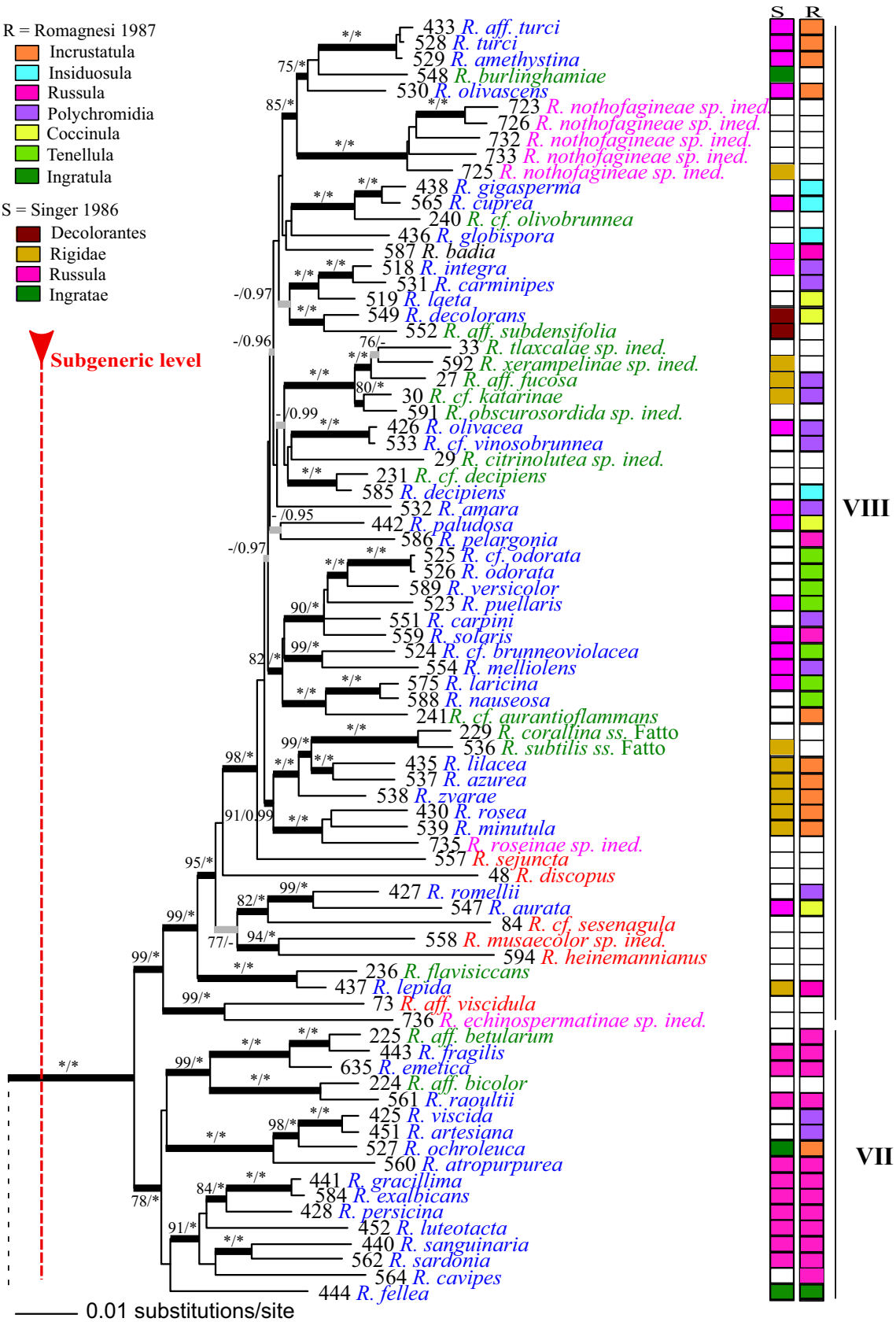
Influenced by a comprehensible desire to assist and facilitate the notoriously difficult identification of the various regional *Russula* mycota, the approach taken by past monographs was, quite understandably, rather practical when defining subgenera. As such, several subgenera were delimited on the basis of a single or a few characters that allowed to split off large species groups from the rest of the genus: e.g. subg. *Incrustatula* Romagn. for all species having primordial hyphae, or subg. *Compactae* for all species with unequal gills. Our phylogeny now clearly demonstrates that this kind of splitting results in completely artificial species groups, as also noted in other recent molecular studies (e.g. Cabón et al. 2017).

The principal macro- and micromorphological features of fruiting bodies will be discussed in more detail in a second paper. Yet, when summarizing the impressive variability of the main features for each of the major clades retrieved in our phylogeny (Online Resource 5), it is evident that all features of fruiting bodies are hopelessly variable within each of the clades with each feature being shared by at least one or more of the other major clades. As a result, even combinations of features of fruiting bodies cannot be used to define subgenera in *Russula* in an unambiguous manner, nor is it possible to construct an identification key leading to the individual subgenera. On the other hand, more terminal clades (viz. those corresponding in modern morphological classifications to subsections or to stirps and series) correspond to far more natural assemblages and many of the recognized subsections in the genus constitute apparently monophyletic clades in our phylogeny (Online Resource 4). This seems indeed logical as most of the previously recognized subsections have commonly been based on a large number of shared similarities allowing for a more natural grouping of species by combining macro- as well as microscopical characters, and sometimes even chemical (see e.g. Romagnesi 1967; Sarnari 1998 for definitions of subsect. *Cyanoxanthinae* Singer and *Xerampelinae* Singer) or host-related features (see e.g. Romagnesi 1967; Sarnari 1998 for definitions of subsect. *Betulinae* (Romagn.) Sarnari and *Laricinae* (Romagn) Bon). The subdivision of the individual subgenera in sections, subsections and their further subdivisions will, however, not be discussed in this paper as the sampling is not representative enough to allow a sound argumentation. Moreover, the finer topology will surely be influenced to a considerable degree by inclusion of more tropical taxa, in particular those from South America, Africa and Australia.

Fig. 1 Most likely tree inferred from phylogenetic analyses of a 5-locus/160 taxa data set. Branches significantly supported by both bootstrap values (BS) ≥ 70% and posterior probabilities (PP) ≥ 0.95 are in black bold, while branches that are only supported by a single method are in bold grey. Support values are reported along the branches (BS/PP) with maximum values for BS (= 100%) and PP (= 1.0) being replaced by an asterisk (*). The phylogeny is compared (on the right side) with the main subgeneric subdivision presented in the latest classifications of Romagnesi (1987) and Singer (1986) with categories corresponding to the highest level adopted in each classification, i.e. subgenera in Romagnesi (R), sections in Singer (S). Colors of taxon names correspond to their geographic origin: Europe (blue), North America (green, being interpreted here to include also mountainous regions of Mexico and Costa Rica—see Online Resource 3), Africa and Madagascar (red), New Caledonia (lilac)

The five main clades retrieved in our phylogeny (Fig. 1) are here interpreted to represent seven subgenera. Nomenclatural aspects of these subgenera have been dealt with elsewhere (Hongsanan et al. 2015; Das et al. 2017c). These major clades do not correspond to the currently recognized subgenera adopted in the latest *Russula* monographs, and even less so to earlier classifications that were strongly biased toward field characters (e.g. Singer 1932). When comparing, for example, our phylogeny with Romagnesi's authoritative classification (1987—see Fig. 1 column 'R') the species previously classified in subgenus *Compactae*—a subgenus recognized in all past monographs as an extremely natural and easily recognizable entity because of the presence of intercalary short gills among the normal, full length gills as in both genera of milk caps in the family—are here distributed over four of the seven accepted subgenera in this phylogeny (clades I, II, V and VI).

Inversely, five out of the nine currently recognized subgenera in Romagnesi's monograph (subgenera *Coccinula* Romagn., *Insiduosula* Romagn., *Polychromidia* Romagn., *Tenellula* Romagn. and *Incrustatula* Romagn.) constitute together clade VIII, a clade which represents here only part of subg. *Russula* in our phylogeny. Also clade IV in our phylogeny is composed of species that were, depending on the author, traditionally placed in three to five different subgenera. This clade IV is actually composed of traditional entities—whether sections (Singer 1986) or subgenera (Romagnesi 1987; Sarnari 1998, 2005)—that were each regarded as particularly natural entities, e.g. subg. *Heterophyllidia* for mostly mild, pale spored species having typically septate-inflated hyphal extremities containing a granular pigment (Romagnesi


R = Romagnesi 1987
Incrustatula
Insiduosula
Russula
Polychromidia
Coccinula
Tenellula
Ingratula
S = Singer 1986
Decolorantes
Rigidae
Russula
Ingratae
Subgeneric level
S
R
433 R. aff. turci
528 R. turci
529 R. amethystina
548 R. burlinghamiae
530 R. olivascens
723 R. nothofagineae sp. ined.
726 R. nothofagineae sp. ined.
732 R. nothofagineae sp. ined.
733 R. nothofagineae sp. ined.
725 R. nothofagineae sp. ined.
438 R. gigasperma
565 R. cuprea
240 R. cf. olivobrunnea
436 R. globispora
587 R. badia
518 R. integra
531 R. carminipes
519 R. laeta
549 R. decolorans
552 R. aff. subdensifolia
33 R. tlaxcalae sp. ined.
592 R. xerampelinae sp. ined.
27 R. aff. fucosa
30 R. cf. katarinae
591 R. obscurosordida sp. ined.
426 R. olivacea
533 R. cf. vinosobrunnea
29 R. citrinolutea sp. ined.
231 R. cf. decipiens
585 R. decipiens
532 R. amara
442 R. paludosa
586 R. pelargonia
525 R. cf. odorata
526 R. odorata
589 R. versicolor
523 R. puellaris
551 R. carpini
559 R. solaris
524 R. cf. brunneoviolacea
554 R. melliolens
575 R. laricina
588 R. nauseosa
241R. cf. aurantioflammans
229 R. corallina ss. Fatto
536 R. subtilis ss. Fatto
435 R. lilacea
537 R. azurea
538 R. zvarae
430 R. rosea
539 R. minutula
735 R. roseinae sp. ined.
557 R. sejuncta
48 R. discopus
427 R. romellii
547 R. aurata
84 R. cf. sesenagula
558 R. musaecolor sp. ined.
594 R. heinemannianus
236 R. flavisiccans
437 R. lepida
73 R. aff. viscidula
736 R. echinospermatinae sp. ined.
225 R. aff. betularum
443 R. fragilis
635 R. emetica
224 R. aff. bicolor
561 R. raoultii
425 R. viscida
451 R. artesiana
527 R. ochroleuca
560 R. atropurpurea
441 R. gracillima
584 R. exalbicans
428 R. persicina
452 R. luteotacta
440 R. sanguinaria
562 R. sardonia
564 R. cavipes
444 R. fellea
VIII
VII
0.01 substitutions/site

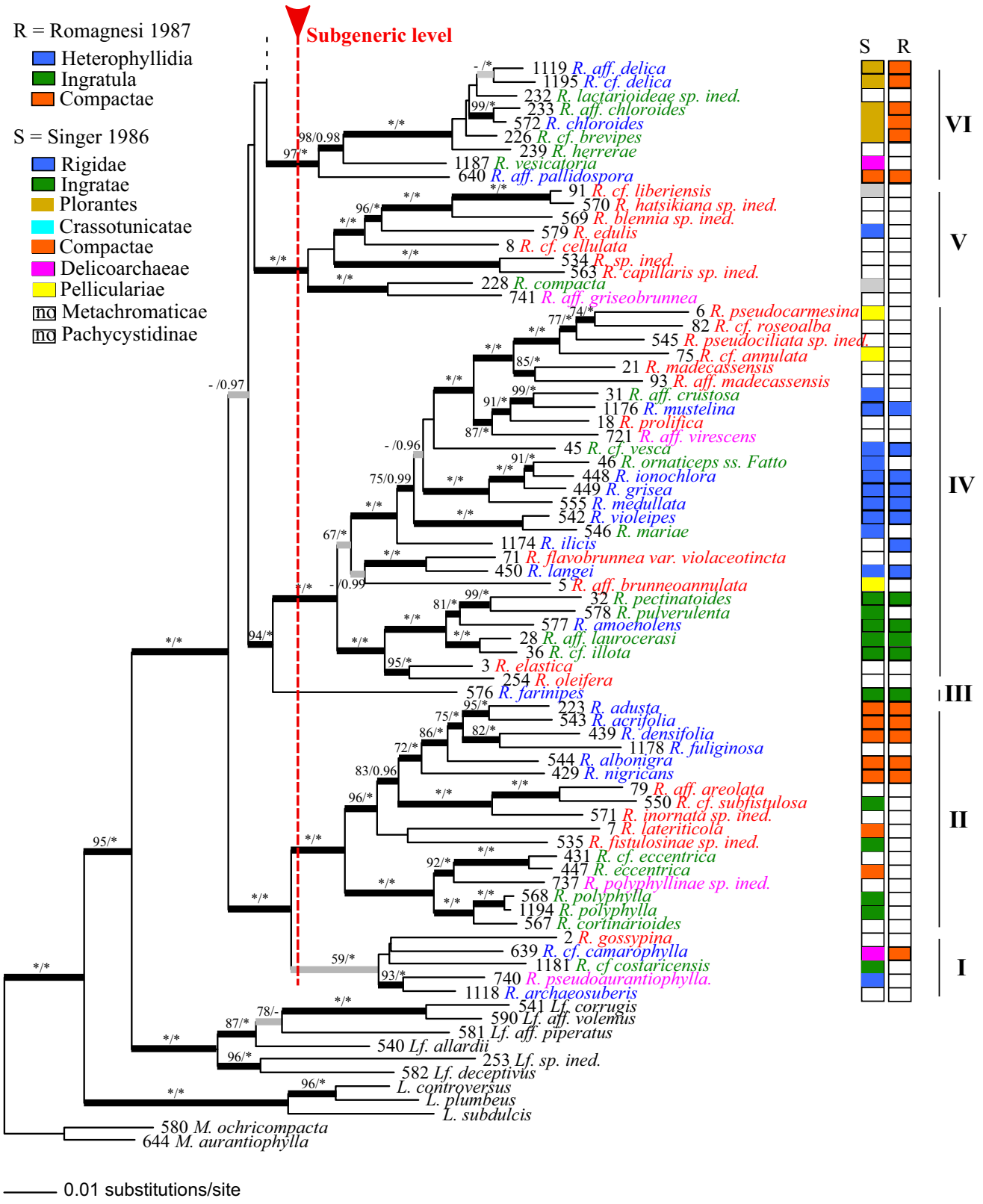


Fig. 1 continued

1967); subg. *Ingratula* Romagn. for brownish-yellowish, often acrid species with mostly encrusting pigment, or subg. *Amoenula* Sarnari as the only acystidiate clade of pale spored species sharing very similar microscopical features including those of spores and pileipellis structure (Sarnari 1998).

Compared with these European (or with the much older American) monographs that have a regional focus, Singer's latest classification (1986) reflects his more worldwide experience and knowledge of the genus. This results, for example, in a more even distribution of treated species across all clades in our phylogeny (see Fig. 1, column 'S'). Unfortunately, apart from Singer's initiative of separating *Plorantes* (Bataille) Singer and *Compactae*, this did not contribute to a more natural classification as all of Singer's monographic treatments of *Russula* remained very strongly influenced by field characters. Singer's concept of *Rigidae* Fr., *Decolorantes* Singer or of Heim's *Pellicullariae* (see Heim 1970), for example, are clearly artificial and ignore the existence of important microscopic differences between the various species that compose each of these large sections.

Overall, most of the main clades retrieved in this analysis have for the first time been retrieved (although with differences in internal topology) in the combined ITS/partial LSU/partial *RPB*2 phylogeny published by Buyck et al. (2008). The latter study included only 28 *Russula* species, all but two of European origin. These same clades—although again with different topologies—have most recently also been retrieved by Looney et al. (2016) using a combined ITS/partial LSU/partial *RPB*2 & *RPB*1 dataset), and by Bazzicalupo et al. (2017) using a combined ITS/partial LSU/partial *RPB*2/partial *TEF*-1 dataset), in both cases again based on an exclusively northern hemisphere sampling (see Fig. 2). These phylogenies exhibit some topological differences, significantly supported (Fig. 2), compared to the topology retrieved in the present study. Looney et al. (2016) retrieved/malodora supported as the sister group with/russula, while Bazzicalupo et al. (2017) recovered/compactae as sister group to/russula. The present study did not resolve these relationships even using more sequence data (mitSSU/partial LSU/partial *RPB*2 & *RPB*1, and partial *TEF*-1 dataset) but resolved the monophyly of/compactae and/archaea, as in Looney et al. (2016), and the monophyly of/heterophyllidia and/crassotunicata. Phylogenetic discrepancies between these studies are likely to result from taxon sampling, biased toward northern hemisphere taxa in Looney et al. (2016) and because of a poor taxon sampling for most subgenera except for/russula in Bazzicalupo et al. (2017).

The very short branch lengths at the backbone of our phylogeny suggest a very rapid diversification of the various major clades (i.e. those that are here equivalent to subgeneric level) and a lot more sequencing may be required before the precise relationships shared between these major clades can be significantly resolved. Clade III (subg. *Crassotunicata*) is here placed for the first time with significant support as sister to Clade IV (subg. *Heterophyllidia*), although this may be a case of long branch attraction (Felsenstein 1978).

Considering the almost exclusively European—North American sampling of the previously published phylogenetic studies, it is quite remarkable that the inclusion of tropical fungi did not dramatically change the overall topology in the sense that all tropical taxa positioned themselves within clades already recognized on the basis of northern hemisphere taxa, and this notwithstanding their sometimes very surprising and unusual field habit. Yet, the inclusion of more tropical as well as of more southern hemisphere taxa may profoundly affect biogeographic hypotheses (see below).

Based on the field experience of the first author and the results of the phylogenetic analyses, we will summarize below the principal features for each of the seven subgenera and shortly discuss their composition:

Clade I: Subg. *Archaea* Buyck & V. Hofst., in Hongsanan et al., Cryptog. Mycol. 36: 372. 2015

Moderately large to small species, compact to very thin-fleshed. Cap dull coloured, yellowish, brownish or gray. Annulus never present. Gills irregularly unequal, with lamellulae either more or less abundant than normal gills. Context yellowing, browning, greying or reddening; mild to acrid. Spore print white. Secotioid and gasteroid representatives unknown.

Spores very small, with inamyloid suprahilar spot. Primordial hyphae absent. Gloeocystidia in all parts of the fruiting body, mucronate to obtuse. Hyphal extremities of cap surface variably inflated or not.

Ectomycorrhizal mantle with a plectenchymatic outer layer, producing abundant, emergent, hyphal extremities. Gloeocystidia inconspicuous, terminal, one-celled, minutely capitate with mostly one terminal knob. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae unknown.

Type species: *R. archaea* R. Heim, Candollea 7: 382. 1938

Distribution: Europe (Sarnari 1998), North and South America (Buyck 1998; Buyck et al. 2003b), Africa (Buyck 1994), New Caledonia, New Zealand and Australia (Buyck et al. 2017, this study, Cooper and Leonard 2014), Asia (Das et al. 2017a)

Subdivision: Our phylogeny contains too few species to suggest any subdivision for the moment, although preliminary phylogenies based on ITS sequence data (e.g. Buyck

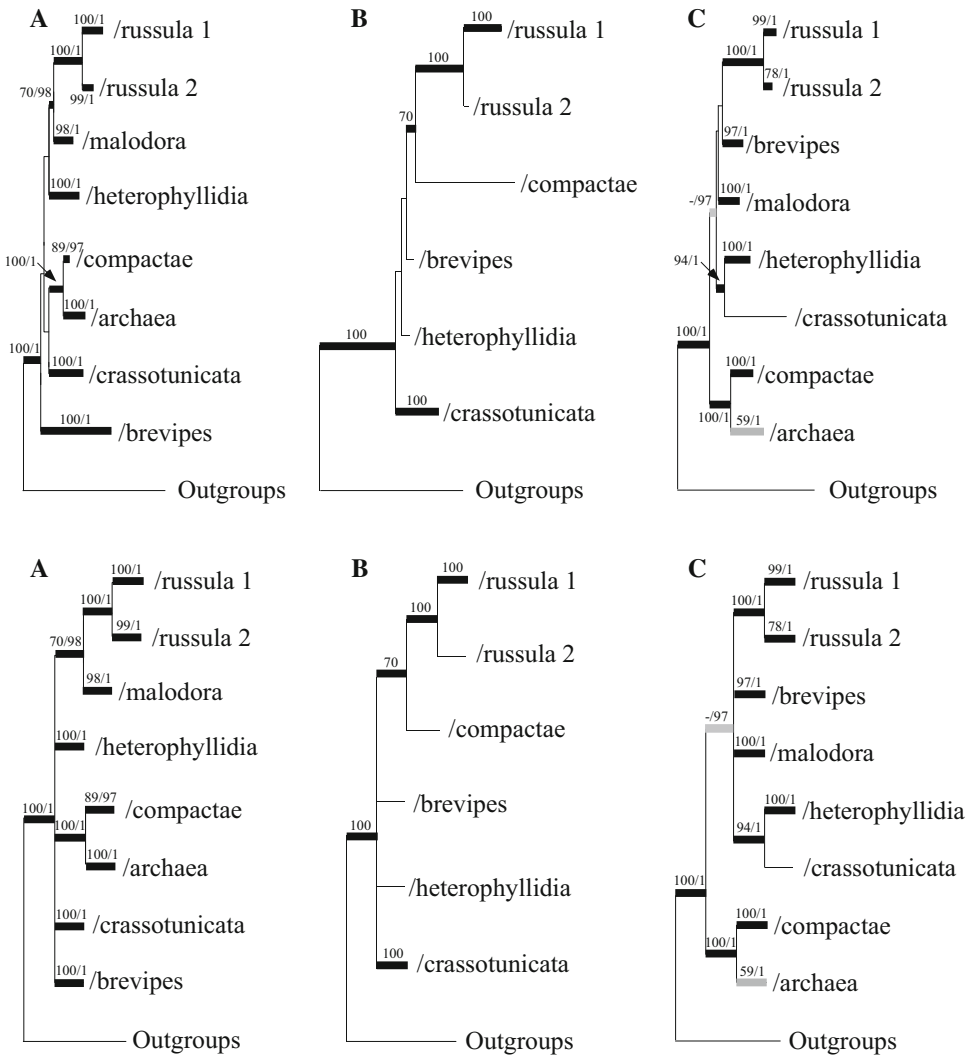


Fig. 2 Tree topologies recovered for *Russula* by recent phylogenetic studies: **a** from Looney et al. (2016), **b** from Bazzicalupo et al. (2017), **c** this study. Topologies reflecting obtained branch length, inclusive of not significantly supported branches (top line) and simplified topologies (below) after suppression of unsupported branches. Branches in bold black are significantly supported with indication of values for ML bootstrap (BS $\geq$ 70%) and Bayesian posterior probabilities (PP $\geq$ 0.95), when applicable (Looney et al. 2016, the present study). Branches in bold grey are only significantly supported by Bayesian analyses

et al. 2017) from more taxa suggest that the subgenus might need to be split in two sections, for which available existing names include sect. *Gossypinae* Buyck and *Archaeinae* R. Heim ex Buyck & Sarnari.

Notes: This subgenus harbors species that were placed in sections *Archaeinae* Heim ex

Buyck & Sarnari and the still monospecific *Gossypinae*. Although our phylogeny retrieved only significant Bayesian support, this subgenus has been constantly retrieved with significant support in all previous phylogenetic analyses of the genus (Bazzicalupo et al. 2017; Looney et al. 2016; Kong et al. 2015).

Subg. *Archaea* has always been suggested to represent one of the most ancient species groups in the genus. Originally, this hypothesis was based on the hygrophoroid aspect of the fruiting bodies of the type species (Heim 1938). Later, however, the emphasis was more on micromorphology of the very few species that compose this small subgenus: very small spores and short, narrow basidia, poor differentiation of tissues, cosmopolitan distribution but with only very few species on each continent (Buyck 1993, 1998). Another unique character of most species, which has also been illustrated and discussed by Beenken (2004) for below-ground structures of *R. gossypina*, is the presence of a thick mucus sheathing the walls of some hyphae (see Buyck 1999) rather than a gelatinous matrix that is present in between hyphae as observed in most other viscid *Russula*. To some extent, these mucus-sheathed structures of the fruiting body context morphologically resemble the ladder-like hyphae typical of the below-ground structures of Russulaceae (see Buyck 1999).

Clade II: Subg. *Compactae* (Fr.) Bon, emend. Buyck & V. Hofst. in Hongsanan et al., Cryptog. Mycol 36: 373. 2015

Fruiting bodies very large to very small, thick-fleshed. Cap dull-coloured, white, brown, grey to black. Annulus never present. Gills regularly unequal. Context reddening, greying, blackening, rarely browning, with or without

distinct, mostly disagreeable smell, mild to very acrid. Spore print white. Secotioid and gasteroid representatives unknown.

Spores with inamyloid suprahilar spot. Gloeocystidia present in all tissues or not, sometimes restricted to the hymenium only and there mostly minutely capitate with one central knob, elsewhere often with two excentrical knobs (the "Mickey Mouse type"), more rarely obtuse rounded. Hyphal extremities of cap surface inflated or not.

Ectomycorrhizal mantle with a plectenchymatic outer layer, covered with emergent, one-celled, flask-shaped gloeocystidia that are mostly mucronate with one central knob or, more frequently, two excentrical knobs. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae documented only for sect. *Nigricantinae* Bataille (see Kong et al. 2015).

Type species: *R. nigricans* Fr., Epicr. syst. mycol.: 350. 1838.

Distribution: Europe (Sarnari 1998), North and Central America (Looney et al. 2016; Kong et al. 2015), New Zealand, Australia (McNabb 1973; Cooper and Leonard 2014; Lebel and Tonkin 2007), Asia, Africa (Park et al. 2014; Buyck 1993; this study).

Subdivision: Our phylogeny suggests the recognition of at least two sections, including sect. *Nigricantinae* Bataille and *Polyphyllae* Buyck & V. Hofst. (in Das et al. 2017c). Whether or not the species-group around *R. fistulosa* Heim merits sectional rank [i.e. as *Fistulosae* (Heim ex Sing.) Buyck] remains uncertain at the moment.

Notes: Our phylogeny retrieved this subgenus as sister to subg. *Archaea* with significant support. Both subgenera differ principally in the regularly polydymous gills of all species in subg. *Compactae* versus the irregular and often poor presence of shorter lamellulae in most species composing subg. *Archaea*. Other subgenera with species having regularly polydymous gills (subg. *Brevipes* and to a much lesser extent subg. *Malodora*) are phylogenetically more distantly related.

Sect. *Nigricantinae* should here be interpreted 'sensu *lato*' as it still embraces tropical African species previously placed in sect. *Fistulosae* subsect. *Fistulosinae* Heim ex Sing (Buyck 1993) differing for the core group in the much more abundant and robust gloeoplerous elements in all parts of their fruiting bodies. Sect. *Polyphyllae* differs mainly in the color shift of the hymenophore (where it is most evident!) from whitish to distinctly pinkish without final blackening; neither do the pileus and stipe context turn black (Das et al. 2017c). Although presently known *Polyphyllae* have mostly been described from North and Central America (see Buyck and Hofstetter in Liu et al. 2015; Buyck et al. 2003a; Buyck and Halling 2004), the latter section is principally (sub)tropical in distribution and well represented in Africa (e.g. subsect. *Ingentinae* Buyck) and also widely distributed in Asia (Buyck unpubl.), but absent from Europe.

Ectomycorrhizal features of this subgenus are apparently less variable compared to those of the fruiting bodies and most species possess the typical "Mickey Mouse" type of gloeocystidia on their below ground structures (see Fig. 3c–d) even when gloeocystidia in the fruiting bodies are completely different (e.g. see Figs. 10–11 in Buyck et al. 2003a) and this both for *Nigricantinae* and *Polyphyllae*. This study provides first observations on below-ground structures for the latter section (see Fig. 3c–e).

Clade III: Subg. *Crassotunicata* Buyck & V. Hofst. in Das et al. Cryptog. Mycol. 38(4): 533, 2017

Medium-sized to rather small, robust to slender species, moderately fleshy. Cap pale coloured and white, cream to yellowish, becoming sometimes rapidly brownish in age or where injured, strongly gelatinous to almost dry. Annulus

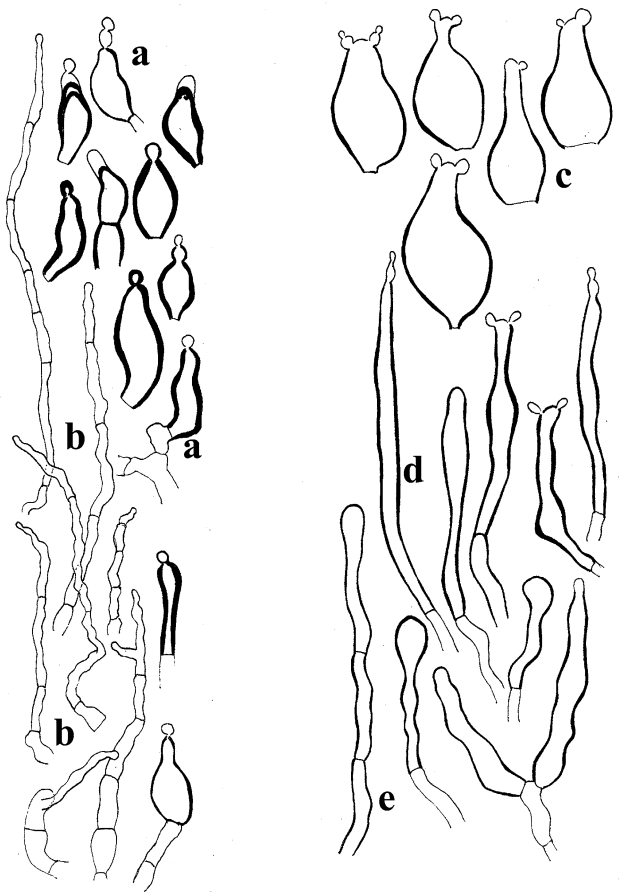


Fig. 3 Below-ground features. (*a–b*) *R. vesicatoria* (subg. *Brevipes*), emerging elements of the ECM outer mantle. (*a*) Gloeocystidia single knob. (*b*) Emanating hyphae (drawings from Buyck 07.009). (*c–e*) *R. polyphylla* (subg. *Compactae*, sect. *Polyphyllae*), emerging elements of the below-ground structures; (*c*) Gloeocystidia from the ECM outer mantle; (*d*) Gloeocystidia from the rhizomorph surface; (*e*) emanating hyphal tips from the rhizomorph surface. Scale bar = 10 μm

never present. Gills irregularly unequal, with lamellulae fairly frequent but mostly (not always) distinctly less abundant than full-length gills. Context faintly yellowing to strongly browning, medium to strongly acrid. Spore print white. Secotioid and gasteroid representatives unknown.

Spores small to quite large, never completely reticulate, with inamyloid suprahilar spot. Primordial hyphae absent. Gloeocystidia abundant and very conspicuous in all parts of the fruiting body, mostly mucronate with a single, terminal knob. Hyphal extremities of cap surface variably inflated or not.

Ectomycorrhizal mantle with a plectenchymatic outer layer and short emanating hyphal ends. Gloeocystidia conspicuous, emergent, one-celled, minutely capitate with one terminal knob, sometimes repeatedly constricted and moniliformous. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae unknown.

Type species: *R. crassotunicata* Singer, Bull Soc mycol France 54: 132. 1938

Distribution: Europe (Sarnari 1998), North America (Shaffer 1970; Roberts 2007), Asia (Buyck unpubl.)

Subdivision: Neither our phylogeny, nor the very limited number of known species argues in favor of a subdivision in sections for the moment. From a nomenclatural point of view, sect. *Crassotunicatae* Sing. is available, as well as subsections *Farinipedes* Sing. and *Crassotunicatinae* Sing. to name subclades.

Notes: This very small subgenus is represented by a single species in our phylogeny but some of our previous datasets for this paper indicated that the species of sect. *Ingratae* subsect. *Farinipedes* Sing. (*R. farinipes* and *R. pallescens*, see Sarnari 1998) and the type species of sect. *Crassotunicatae* Sing., *R. crassotunicata*, form a strongly supported clade that is obviously very ancient, something already suggested in several other recent phylogenetic analyses of *Russula* (Buyck et al. 2008, 2017; Looney et al. 2016; Bazzicalupo et al. 2017). This clade is here for the first time placed with strong support as sister to subg. *Heterophyllidia* as here emended (i.e. including also most species previously placed in subg. *Ingratula*). This position is in line with relationships suggested in most previous systematic treatments of *Russula* (e.g. Singer 1975; Sarnari 1998) that placed some or all of these species in subg. *Ingratula* (or equivalent groups). However, as we have only a single species included in our analyses, we cannot exclude a phenomenon of long branch attraction to place this subgenus here with high support as sister to subg. *Heterophyllidia*.

Presently, subg. *Crassotunicata* is restricted to the northern hemisphere where some species are even occurring at quite high latitudes, although the subgenus is also present in subtropical parts of Asia (Buyck unpubl.) and may be represented by several cryptic species in North America. The long lifetime of fruiting bodies of *R. crassotunicata* probably explains why it is a reputed host of fungal saprophytes such as *Dendrocollybia racemosa* (Machnicki et al. 2006) and *Collybia tuberosa*, to the same extent as a similar phenomenon can frequently be observed for parasites such as *Hypomyces lactifluorum* on *R. brevipes* (subg. *Brevipes*) or *Asterophora* species (see Redhead and Seifert 2001) on *R. nigricans* and allies (subg. *Compactae*).

This paper now also provides first observations on ECM morphology for this subgenus, in this case obtained from ECM of *R. crassotunicata* (Fig. 4d–e). These illustrate the very conspicuous, large gloeocystidia emerging from the ECM outer mantle; thereby resembling the dermatocystidia of these species. ECM outer mantle features are otherwise very similar to those described for *R. fuegiana* for example (Palfner and Godnoy 1996), the latter being a species in

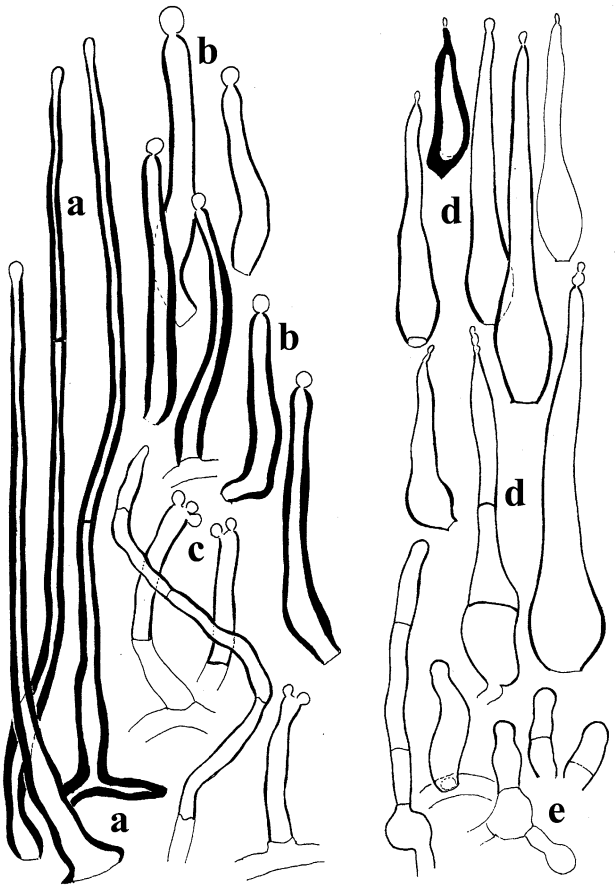


Fig. 4 Below-ground features. (*a–c*) *R. blennia sp. ined.* (subg. *Malodora*), emerging elements of the ECM outer mantle; (*a*) thick-walled aculeate hyphae; (*b–c*) Gloeocystidia, majority of single knob type with large, sphaerical knobs, some (in *c*) with two (exceptionally three) knobs (drawings from Buyck 08.068). (*d–e*) *R. crassotunicata* (subg. *Crassotunicata*). (*d*) Gloeocystidia from the ECM outer mantle; (*e*) Short emanating hyphal tips from the rhizomorph surface (from Buyck 13.195). Scale bar = 10 µm

subg. *Brevipes* as suggested by phylogenetic analysis of ITS sequence data (Buyck et al. 2017).

Clade IV: Subg. *Heterophyllidia* Romagnesi, Doc Mycol 18(69): 39. 1987, emend. Buyck & V. Hofst.

= subg. *Amoenula* Sarnari, Monografia Illustrata del Genere Russula in Europa 1: 98. 1998 = subg. *Ingratula* Romagn., Doc Mycol 18(69): 39. 1987

= sect. *Pellicullariae* R. Heim, Candollea 7. 1938

Mostly medium to large, rarely very small species that are thick- to extremely thin-fleshed, coming in almost all possible colors. Stipe occasionally with a well-developed annulus, often developing few to many internal cavities. Gills equal; lamellulae—when present—never frequent. Context unchanging or turning yellowish to rusty brown, often with distinct smell, tasting mild to strongly acrid, sometimes nauseous. Spore print mostly white or various shades of cream, rarely up to ochre. Secotioid and gasteroid representatives limited to a few species groups, e.g. *Amoeninae* (see observations under *R. pseudoamoenicolor* A. Ghosh, Buyck, K. Das, A. Baghela & R.P. Bhatt in Hyde et al. 2016).

Spores with inamyloid or partly amyloid suprahilar spot. Primordial hyphae absent. Gloeocystidia mostly abundant, although restricted or not to certain parts of the fruiting body, exceptionally absent, mucronate to obtuse-rounded, one-celled. Hyphal extremities of cap surface inflated or not.

Ectomycorrhizal mantle with a plectenchymatic outer layer, producing emergent, one-celled gloeocystidia that are generally minutely capitate with one or rarely two, central knobs, only exceptionally absent; aculeate, sometimes forking, thick-walled 'hairs' present in some species groups. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae limited to a few species complexes (Kong et al. 2015).

Type species: *R. grisea* Pers. ex Fr., Epicrisis: 361. 1838

Distribution: Europe (Sarnari 1998), North and South America (Barbosa 2016), Asia, New Zealand-New Caledonia-Australia (Lebel and Tonkin 2007, Cooper and Leonard 2014), Africa (Buyck 1994, this study)

Subdivision: Our phylogeny suggests at least the recognition of several sections in this subgenus for which already available names include *Subvelatae* Sing, *Ingratae* Quel., *Aureotactae* Buyck & V. Hofst., and *Heterophyllae* Fr. Probably subsect. *Oleiferinae* Buyck, *Cyanoxanthinae* and *Ilicinae* Buyck also merit upgrading.

Notes: This subgenus is probably one of the most complex entities of the whole genus. The traditional concept of subg. *Heterophyllidia* (Romagnesi 1967) has already strongly been impacted by descriptions of tropical African taxa when Buyck (1994) transferred here many extremely thin, often unusually bright-colored and sometimes extremely small tropical species (i.e. part of Heim's "*Pellicullariae*") several of which were annulate. At the same time, Buyck demonstrated the morphological continuum between tropical species of subsect. *Cyanoxanthinae* and those in subg. *Ingratula* subsect. *Foetentinae* (see Buyck 1994), sharing amongst others the same metachromatic reaction in Cresyl blue (Buyck 1989c). In later years, Buyck (2003) also discussed shared similarities between subsections *Ilicinae*, *Cyanoxanthinae* and sect. *Metachromaticae* Sing., the latter being probably part of subg. *Brevipes* as suggested by preliminary analyses of ITS sequence data (Barbosa 2016).

Some of the—clearly ancient—species groups that are here placed in subg. *Heterophyllidia*, e.g. subsect. *Aureotactinae* R. Heim had previously been placed (Buyck 1994) in sect. *Crassotunicatae* (Sing.) Sing. The latter species group is here now indeed resolved (although with some reservations because of possible long branch attraction, see above) as sister to subg. *Heterophyllidia* with strong support, but is clearly genetically too different to be included in the same subgenus. One of the species originally described in *Aureotactinae* (*R. oleifera* Buyck) has since become the type species of subsect. *Oleiferinae* Buyck (in Sanon et al. 2014), which is here recovered with full support as sister to northern temperate *Ingratae*. Subsections *Oleiferinae*, *Aureotactinae* as well as *Cyanoxanthinae* and *Ilicinae*, taken together, probably represent most of the ancient lineages within *Heterophyllidia.* All of these species groups are characterized by abundant gloeoplerous elements in all of their tissues (including in the below ground structures). Considering the fact that also the sister-group of subg. *Heterophyllidia* (i.e. subg. *Crassotunicata*) shares this abundance of gloeoplerous elements in all of its tissues, this therefore strongly suggests that their common ancestor is equally a species with abundant gloeoplerous elements.

Opposed to these species-groups having abundant gloeoplerous elements, *Heterophyllidia* also harbors several species groups having limited or—an exceptional feature in the genus—no gloeoplerous elements at all. This concerns particularly subsect. *Amoeninae* Buyck, a species group which differs from all other species in Romagnesi's *Heterophyllidia* by the complete absence of typical gloeoplerous elements. This, together with other features, were the reason for Sarnari (1998) to consider these species as a separate subgenus, subg. *Amoenula* Sarnari. *Amoeninae* is so far also the only subsection in *Heterophyllidia* that is known to harbor secotioid species (for descriptions see Lebel and Tonkin 2007) as recently suggested by ITS sequence data (see Buyck in Hyde et al. 2016).

Clade V: Subg. *Malodora* Buyck & V. Hofst., Cryptog. Mycol. 36: 373. 2015

Medium to large species, often firm and compact, never extremely thin-fleshed. Cap dull coloured, yellow brown, grey to almost black or whitish. Annulus never present. Gills regularly unequal to frequently and almost regularly forking. Context greying or browning, mostly developing rapidly a strongly disagreeable smell and taste. White spore print. Secotioid and gasteroid representatives unknown.

Spores with inamyloid suprahilar spot. Gloeocystidia moderately numerous to numerous on gill surface, mucronate and inconspicuous to absent elsewhere from the fruiting body. Hyphal extremities of cap surface typically with inflated, often voluminous cells, and strongly septate.

Ectomycorrhizal mantle with a plectenchymatic outer layer, with dispersed to rare gloeocystidia that are emergent, one-celled, flask-shaped, minutely capitate with one or rarely two knobs, sometimes accompanied by emergent, apically tapering to cylindrical, thick-walled hyphal extremities. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae rare (Kong et al. 2015).

Type species: *R. compacta* Frost in Peck, New York St. Mus. Ann. Rept. 32: 32. 1879.

Distribution: North America, South America (Barbosa 2016), Asia (Das et al. 2017c), Africa (Buyck 1993), New Caledonia, New Zealand, Australia (Lebel and Tonkin 2007; Buyck, this study).

Subdivision: Our phylogeny suggests at least the recognition of two, or more probably, three sections: *Pseudocompactae* Buyck & V. Hofst., *Edules* Buyck & V. Hofst. (see Das et al. 2017c) and perhaps also the species group around the still undescribed *R. cappilaris* sp. ined. merits sectional status as a species group having unequal but not forking gills.

Notes: Apart from the type species, this subgenus contains some of the species that were previously placed in the tropical African subsections *Meleagrinae* Buyck and *Brunneodermatinae* Buyck, both previously placed in section *Fistulosae* (Heim ex Singer) Buyck (see Buyck 1993; Sanon et al. 2014). With the exception of typical *R. compacta* itself and two still undescribed African species, nearly all of the other species in this subgenus have frequently forking gills.

Some of the below ground structures are here illustrated (see Fig. 4a–c for *R. blennia* sp. ined.) and show gloeocystidia with a single knob or, more rarely, two or more knobs, that are emerging from the plectenchymatic ECM outer mantle layer. These gloeoplerous cells are widely dispersed and difficult to find, particularly in *R. compacta,* thereby mirroring their being equally rare in the pileipellis of the fruiting bodies (see Adamcík and Jancovicová 2018).

Clade VI: Subg. *Brevipes* Buyck & V. Hofst., Cryptog Mycol 36: 372. 2015.

Mostly medium to very large species that are very thick-fleshed, only exceptionally also small and thin-fleshed. Cap whitish, often rapidly developing yellowish brown to reddish brown stains. Well-developed annulus never present. Gills regularly unequal. Context turning yellowish to rusty brown, mostly with distinct smell, acrid to strongly acrid, (rarely mild?). Spore print whitish to yellow. Secotioid and gasteroid representatives known only from species in subsect. *Lactarioideae* Maire.

Spores with inamyloid or amyloid suprahilar spot. Primordial hyphae absent. Gloeocystidia mucronate to obtuse-rounded, in all parts of the fruiting body. Hyphal extremities of cap surface inflated or not.

Ectomycorrhizal mantle with a plectenchymatic outer layer, covered with emergent, one-celled to secondarily septate, short, flask-shaped, mostly thick-walled gloeocystidia that are generally minutely capitate with one or rarely two, central knobs. Rhizomorphs common.

Associations with mycoheterotrophic Orchidaceae and Ericaceae documented only for species in subsect. *Lactarioideae* Maire (Kong et al. 2015).

Type species: *R. brevipes* Peck, Rep. (Annual) New York State Mus Nat Hist 43: 20. 1890.

Distribution: Europe (Sarnari 1998), North and South America (Barbosa 2016; Buyck et al., this study), Asia, New Zealand, Australia (Cooper and Leonard 2014; Lebel and Tonkin 2007), Africa (Buyck and Adamčík 2013; Buyck et al. 2017).

Subdivision: Our phylogeny suggests at least the recognition of sect. *Lactarioides* (Bataille) Konrad & Joss. for the *R. delica* group, but tropical taxa are here largely underrepresented. Available names for sections containing tropical taxa include *Metachromaticae* Singer and *Delicoarchaeae* Singer (considered taxonomic synonyms by Buyck and Ovrebo 2002). At subsectional level, northern temperate *Pallidosporinae* Bon and probably also the Central African *Pallidorimosinae* Buyck are composed of species that are phylogenetically more ancient than the *R. delica* group (as suggested by the inamyloid suprahilar spot on their spores—see Buyck et al. 2017).

Notes: The precise circumscription of this subgenus needs more sequencing of morphologically similar, tropical species (see Hongsanan et al. 2015; Buyck and Adamčík 2013) as it is clear that this subgenus has a more cosmopolitan distribution than is here reflected in the sampling. This study provides first observations on ECM structures for the American *R. vesicatoria* Burl. (see Fig. 3a–b), whose features correspond to the definition of *Pallidosporinae*, a subsection for which no data on ECM structures had been published so far.

Clades VII & VIII: Subg. *Russula*, emend. Buyck & V. Hofst.

= subg. *Coccinula* Romagn., Doc Mycol 18(69): 40. 1987.

= subg. *Incrustatula* Romagn., Doc Mycol 18(69): 39. 1987.

= subg. *Insidiosula* Romagn., Doc Mycol 18(69): 40. 1987.

= subg. *Polychromidia* Romagn., Doc Mycol 18(69): 40. 1987.

= subg. *Tenellula* Romagn., Doc Mycol 18(69): 39. 1987.

Very small to very large species that are thick- to extremely thin-fleshed, coming in all possible colors. Stipe exceptionally annulate, only sometimes developing few to many internal cavities. Gills equal or lamellulae—when present—almost always rare. Context unchanging, yellowing, browning, reddening, graying or blackening, sometimes with distinct agreeable to disagreeable smell, tasting mild to strongly acrid, sometimes nauseous. Spore print white to yellow. Secotioid and gasteroid representatives limited to a few species groups in clade VIII.

Spores with amyloid suprahilar spot. Primordial hyphae absent or present. Gloeocystidia rare to very abundant, sometimes restricted to certain parts of the fruiting body, exceptionally absent, generally obtuse-rounded, one- to multicelled. Hyphal extremities of cap surface usually narrow, rarely irregularly inflated.

Ectomycorrhiza reduced to intramatrical tissue in one, small terminal group within clade VII (see Online Resource 2); other species producing a smooth, ECM parenchymatic outer mantle layer with (sometimes many) embedded gloeoplerous elements in a surface otherwise composed of (1) either angular-shaped cells with external, isolated or grouped, individual cells, or (2) puzzle-shaped cells with an overlaying hyphal network that is embedded or not in a glutinous sheath that may in a few cases also contain gloeoplerous cells. Rhizomorphs rare and, if present, often lacking ladder-like hyphae or with untypical ladder-like hyphae.

Associations with mycoheterotrophic Orchidaceae and Ericaceae documented for several species complexes (Kong et al. 2015).

Type species: *R. emetica* (Schaeff.: Fr.) Pers., Observ mycol 1: 100. 1796.

Distribution: Europe, North and South America, Asia, Oceania, Africa (Buyck 1994; Sarnari 1998; Looney et al. 2016; Bazzicalupo et al. 2017; Barbosa 2016).

Subdivision: Our phylogeny presents here clades VII and VIII as a single large subgenus for which a number of clades are already clearly ancient enough to merit an upgrade to sectional level: *Sardoninae* Singer, *Felleinae* (Mlz & Zv.) Sarnari, *Echinospermatinae* Buyck, as is already the case for sect. *Ochroleucinae* Romagn. or *Flavisiccantes* Buyck & V. Hofst. [= subsect. *Lepidinae* (Melzer & Zvára) Singer,].

Notes: The present concept of this enormous clade that represents here subg. *Russula* is very similar to the concept of Sarnari (1998) with the sole difference that subg. *Incrustatula* has here also become part of this subgenus. Sarnari defined his subg. *Russula* as the 'rest group' that remained after exclusion of all relatively well-characterized species groups, a viewpoint to which we can adhere when considering only the European *Russula* mycota.

One might be surprised to find in the above definition that gills in this large subgenus are "almost" always nearly all equal in length (i.e. lamellulae absent or nearly so). Recently, Das et al. (2017b) described *R. aureorubra* K. Das, A. Ghosh, A. Baghela & Buyck as a very particular species in subsect. *Russula* characterized by unusual colors and near-unequal gills because of the presence of many shorter lamellulae.

We have long hesitated to adapt the views of Looney et al. (2016) and to consider Clades VII and VIII, both clades being also here significantly supported, as separate subgenera. Clade VII is composed of species that were part of Romagnesi's *Piperinae* (Romagnesi 1967), later becoming his subg. *Russula* (Romagnesi 1987). In Sarnari's monograph (1998), species of Clade VII represent three of the six subsections composing his subg. *Russula*, sect. *Russula*. However, we have several reasons for not creating two separate subgenera:

(1) the short branch lengths at the base of Clades VII & VIII compared to the much longer branch leading to their common ancestor;

(2) the basal placement of most tropical African species and in particular the very basal position of the tropical *Echinospermatinae* Buyck in Clade VIII as sister to the rest of this huge clade suggests, based on the collecting experience of the first author, that more sequencing of these tropical taxa might severely impact the topology.

(3) Clades VIII and VII form a very distinctive group of species that all share the synapomorphy of a pseudoparenchymatic ECM outer mantle layer. Differences among the below-ground features of both clades are principally quantitative, i.e. certain features are more widespread in one of these two clades, while characterizing also some species in the other clade. We predict that the study of below-ground features of more tropical species in these clades will add to the morphological similarity of the below-ground features of both clades (as did our first observations on ECM of the African *R. discopus* R. Heim).

(4) finally, lack of support and almost non-existant branches for most of the backbone of Clade VIII, which represents here most of the northern hemisphere taxa, suggest a very rapid radiation possibly at the time of

migration into the northern hemisphere, the dominant distribution area for both Clade VII and VIII.

Our phylogeny places the tropical subsect. *Echinospermatinae* as sister to the rest of clade VIII with high support. This subsection was erected (Buyck 1990) for three tropical species that share near identical features with subsections *Amoeninae/Virescentinae* in subg. *Heterophyllidia*, but differ principally in the spinulose spore ornamentation, partial amyloid suprahilar spot and—as opposed to *Amoeninae*—presence of hymenial gloeomacrocystidia. As a consequence, Buyck (1994) had placed subsect. *Echinospermatinae* in subg. *Heterophyllidia*, and the true affinities revealed in this phylogeny are quite unexpected. Although data on the below-ground structures of *Echinospermatinae* are as yet unavailable, their phylogenetic position suggests that they should have a pseudoparenchymatic ECM outer layer.

Below-ground features and correspondence with the classification of Beenken (2004)

Compared to the morphological diversity of the often very complex fruiting bodies of Agaricomycetes, one logically expects a highly reduced amplitude for the anatomical-morphological variation of the 'below-ground' features, i.e. ECM structures and rhizomorphs, even more so when comparing species that are part of the same genus. Nevertheless, some of the considerable anatomical diversity found in the fruiting bodies of *Russula* is also mirrored in their below-ground structures, although the homology between above- and below-ground features remains often problematic. Indeed, *Russula* is certainly the best documented genus in terms of detailed descriptions of ECM structures and rhizomorphs, but the different surface layers of ECM structures or the unique "ladder-like hyphae" for *Russula* (see below) are difficult to relate to specific corresponding parts of fruiting bodies.

The use of below-ground features in systematics of mushroom forming fungi has always been quite marginal. Even for the best studied genus in this respect, *Russula*, observations on below-ground structures are most often limited to those made on a single specimen for the few taxa that have been studied. In the absence of any appreciation of eventual variability within individual species, the extrapolation of observed features should therefore be taken with a certain amount of reservation. Available data nevertheless strongly suggest that features of below-ground structures allow to characterize the main clades better than those of their corresponding fruiting bodies, even if, also here, attenuations such as 'generally' or 'mostly' are often needed to include the existing exceptions to the general rule. Below-ground features are certainly not more useful (and certainly not easier to use) when considering identification of individual taxa, except in exceptional cases where presence of particular pigments allow for immediate species identification already by eye (e.g. *R. ochroleuca* in Europe, *R. discopus* in Africa).

Example given: one collects a beautiful bright red *Russula*. Such colored species can be found in clades VIII, VII and IV in our phylogeny. A simple check with Melzer's reagent of the amyloidity of the suprahilar spot of spores can already narrow down the identification to species in either Clades VIII + VII (distinctly amyloid) or in Clade IV (inamyloid or only very partially amyloid). The same result can also be obtained with a rapid observation of the surface of ECM or rhizomorphs: a pseudoparenchymatic ECM mantle type is typical of Clades VIII + VII and will exclude Clade IV. Beyond this stage, however, identification becomes impossible or at least highly problematic and technically difficult for below-ground structures, whereas characters of fruiting bodies are more easy to observe and allow certainly for a more precise and reliable identification.

On the other hand, when looking at all of the species in each of these three clades (together comprising most of the northern temperate *Russula* mycota), fruiting body morphology is so variable that it is impossible to find mutually exclusive delimitations or definitions for each of these clades taken as a whole. This means for example that it is impossible to make an identification key to the different subgenera using fruiting body features, except perhaps on a geographically (very) restricted scale.

The study by Beenken (2004) for the first time suggested that below-ground structures will allow to characterize most infrageneric clades in hardly two lines based on the presence of rhizomorphs and the aspect of gloeoplerous cells and other features of the ECM mantle layers. Considering the strong impact of the present phylogeny on the traditional subdivision of the genus in major clades, it was interesting to be able to compare this new subdivision with the classification of Beenken that introduced some systematic changes based on the anatomy of below-ground structures. A summary of the evolution of different types of below-ground features for each subgenus is shown in Fig. 5 (see Online Resource 6 for data on below ground features of the individual subgenera).

Before commenting on Beenken's classification, some explanation on the various below-ground features might be needed. Indeed, when looking at Online Resource 2, which more or less summarizes differences of below-ground structures of *Russula* species, one might be surprised to find a category corresponding to the trait 'ECM formation' which, at first sight, seems a superfluous or unnecessary consideration for obligatory ECM fungi. Nevertheless, *Russula* represents so far the only genus among basidiomycete ECM genera for which examples of species have

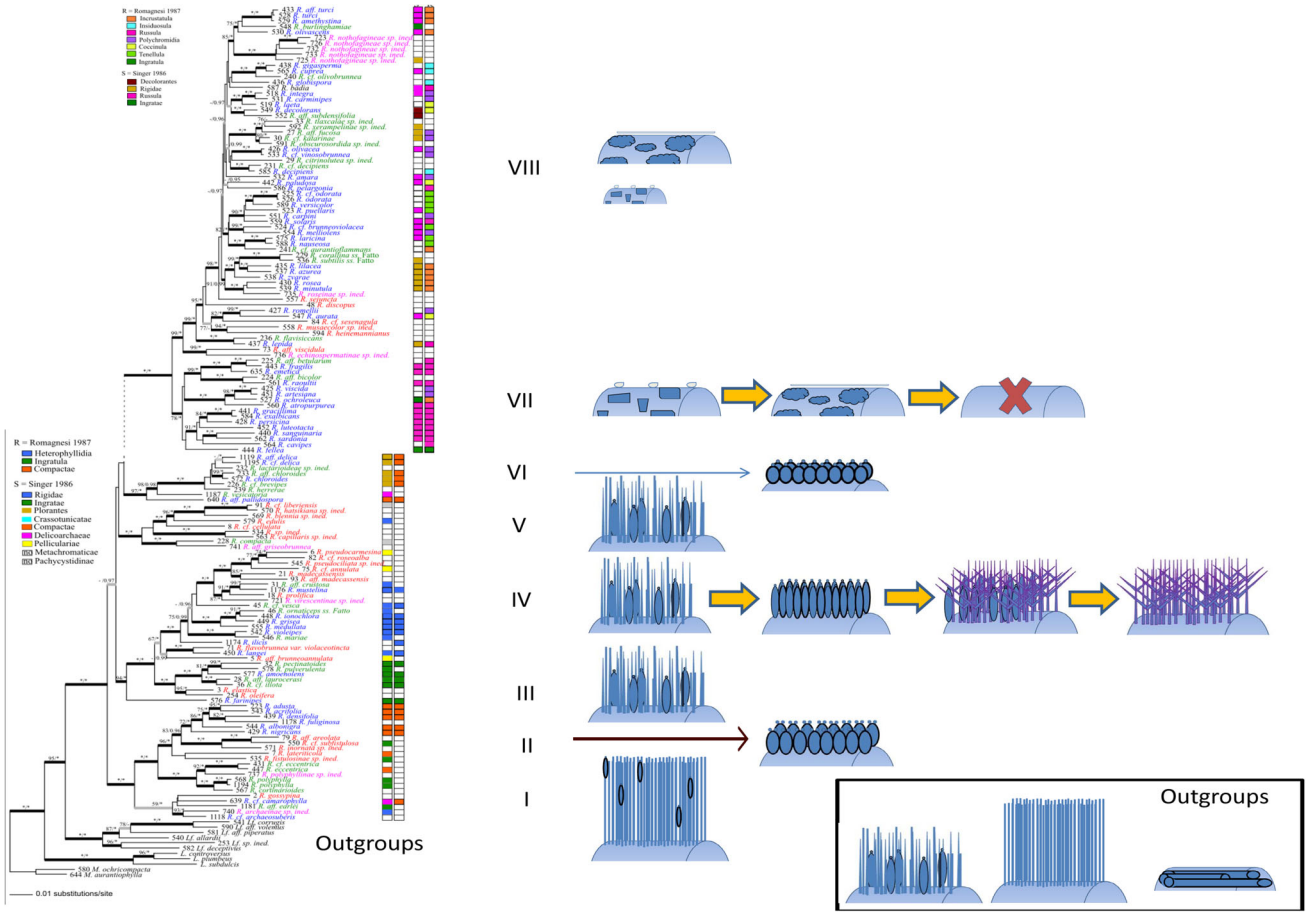


Fig. 5 Schematic comparison of ectomycorrhizal outer mantle layer anatomy among the various subgenera and between in- and outgroup retrieved in our phylogeny. For a summary description of the ECM mantle anatomy within each clade see Online Resource 6

been documented that do not form visible ECM because they only invest in Hartig net formation, not in formation of an ECM sheath around the root hairs (Beenken 2004). So far, this exception concerns two closely related species (*R. exalbicans* and *R. gracillima*) composing a terminal subclade in subg. *Russula* (Fig. 1), each forming a highly host specific association with one particular *Betula* species. To enter the host tree roots, each of these two species instead exploits the ECM structures formed by an equally host specific *Lactarius* species that is strictly associated with the same host.

Outer mantle layer anatomy: The ECM outer mantle is a very important structure for the fungus as it is the interface between the soil and the fungal tissues. The composition and structure of this ECM surface determine its hydrophilic or hydrophobic nature and thus its capacity for uptake of nutrients. Summarizing Beenken (2004), the ECM mantle anatomy in northern temperate *Russula,* can be described as follows: "ectomycorrhizal mantles of *Russula* are three-layered. The inner layer, which is in close contact with the root tissue, is typically a plectenchyma, but middle and outer layers can be either plectenchymatic or pseudoparenchymatic. The plectenchymatic type produces a 'rough' mantle surface with individual cells or multicellular extremities with or without gloeoplerous contents emanating from a dense plectenchyma, while the pseudoparenchymatic type produces a smooth mantle surface that is either composed of angular cells or of irregular, puzzle-shaped cells and that may sometimes be covered with dispersed individual cells or by a poorly developed network composed of thin- to thick-walled, branching hyphae lying on top of it".

Our phylogeny now demonstrates that the ECM outer mantle anatomy divides the genus in two clear-cut groups: subclades VII and VIII (subg. *Russula*) form together a strongly supported monophyletic clade for all species having a smooth pseudoparenchymatic mantle, whereas all other subgenera are composed of species with a plectenchymatic outer mantle. The former is therefore clearly the derived condition. Beenken (2004) suggested that the form of the cells that compose this outer pseudoparenchymatic mantle characterize different large systematic species groups. However, our phylogeny suggests that

this distinction does not oppose large species groups, but reflects rather similar evolutions within each of these two clades (VII and VIII), although our Online Resource 2 clearly suggests that the puzzle cell form of the outer ECM mantle cells correlates very well with the presence of an overlying hyphal net.

Gloeoplerous elements: Recognizable by their contents that react to varying degrees with sulfoaldehydes, gloeoplerous elements are considered the synapomorphy for the russuloid clade. Both existing data as well as our own observations demonstrate that gloeoplerous cells are present on the below-ground structures (both on the ECM outer mantle and the rhizomorph surface) of all examined *Russula* species so far, irrespective of whether gloeoplerous elements can be found in any, in part or in all of their fruiting body tissues. *R. amoena* var *acystidiata* (subg. *Heterophyllidia*), and also *R. griseocarnosa* X.H. Wang, Z.L. Yang & Knudsen (Clade VIII—see Wang et al. 2009), constitute the only species for which gloeoplerous elements are lacking in both the fruiting bodies and the below-ground structures.

To avoid any confusion, we want to point out that the term "gloeoplerous elements" applies here to cystidia, as well as to cystidioid hyphae and lactifers. Lactifers are always absent from the below-ground structures of *Russula* (as opposed to *Lactarius*), but only the plectenchymatic ECM outer mantle type is considered by most authors to be 'cystidiate', while the pseudoparenchymatic type is described as "acystidiate" (Beenken 2004). In our opinion, the choice of the term 'acystidiate' is here rather unfortunate as we do not see why these embedded clusters of gloeoplerous cells could not be the equivalent of (or homologous with) the often multiseptate, blunt dermatogloeocystidia that characterize the fruiting body surfaces of these same species? As a consequence, we prefer to avoid in this paper the opposition between ambiguous attributes such as "acystidiate" and "cystidiate" because all *Russula* ECM mantle types (apart from the two exceptions just mentioned) do have gloeoplerous elements on their below-ground surfaces.

Precise data on the abundance of gloeoplerous cells on the below-ground structures are not available, but our own observations suggest that there is not always a direct relation with their abundance on the fruiting bodies of the same species. Example given, *R. cyanoxantha* and other *Cyanoxanthinae* have many gloeoplerous elements (incl. cystidioid hyphae) in the tissues of their fruiting bodies, while gloeoplerous elements are few on their below-ground structures. Inversely, some species of subg. *Compactae* have few gloeoplerous elements in their fruiting bodies and sometimes no gloeoplerous elements at all inside their context, but gloeoplerous cells are mostly conspicuous and abundant on the below-ground structures (in *R. polyphylla* we counted more than 20'000 gloeocystidia/mm^2 on the ECM surface, i.e. 2 to 5 times superior to the number of gloeocystidia on the hymenial surfaces of the same species, but this abundance may compensate for their often much smaller size).

In general terms, we can state that below-ground structures in subgenera *Malodora* and *Archaea* have widely dispersed gloeoplerous elements, as opposed to most species of subgenera *Compactae* and *Brevipes* where gloeoplerous cells are found all over the surface of the ECM outer mantle. Clade IV (*Heterophyllidia* + *Ingratula*) is the most heterogeneous in this respect, with species belonging to sect. *Ingratae* having crowded gloeocystidia and those in sect. *Heterophyllae* showing a gradual reduction in gloeoplerous elements coinciding with increasing numbers of needle-shaped terminations (see below and also Fig. 5).

Needle-shaped cells: Needle shaped, sometimes repeatedly forking or star-shaped, thick-walled cells have been described from fruiting bodies in several genera in Russulales, particularly in crust-forming species, e.g. *Asterostroma* or *Vararia,* and are generally referred to as astero- or dichohyphidia (see Clémençon 2004). Among agaricoid Russulaceae needle-shaped cells are commonly found in *Lactifluus* (see e.g. Morozova et al. 2013), while similar structures are unknown from the genera *Lactarius* and *Multifurca*. In *Russula*, needle-shaped cells were first described as characteristic elements of the cap cuticle of the two European species that compose subsect. *Heterophyllinae,* i.e. *R. vesca* and *R. heterophylla* (subg. *Heterophyllidia*). Since such cells occur in these European species among the normal terminations of the pileipellis in a widely dispersed manner and are principally limited to the center of the pileus, they were easily overlooked. Much later, needle- as well as star-shaped cells were also described from surface tissues of the pileus, ring or stipe base for several tropical African species in subg. *Heterophyllidia* (Buyck 1994). Nevertheless, this type of cell never received any particular attention in systematic treatments because of the very few species that possess such cells on their fruiting bodies, and because of the difficulty of their observation. It was only when the below-ground structures of several species in subg. *Heterophyllidia* were studied (Beenken 2004) that similar needle-shaped cells were found to be much more common—as well as much more abundant and conspicuous—on the below-ground structures, forming on the outer ECM mantle and rhizomorphs sometimes a 'forest' of closely packed, rigid, sharp and often forked hairs pointing outward like spears.

However, one could raise the question as to whether these "needle-shaped" cells really merit to be considered as a separate cell-type. Indeed, in as far as ECM surfaces have very similar characters compared to surfaces of fruiting bodies (similarities in pigment, gloeoplerous elements etc.…), it is useful to point out that such needle-shaped cells are only found in those *Russula* species that have terminal cells on their fruiting bodies that are always long and tapering towards their apex. As below ground structures have nearly always thicker cell walls compared to above ground structures, would these needle-shaped cells not just be the thick-walled equivalent of similar terminal cells found on all surfaces? Although we are inclined to adhere to Beenken's viewpoint, at least for those species with very sharp and bifurcating to sometimes star-shaped "needle"-cells, we admit that the distinction is often very vague. For instance, Beenken (2004) mentions their presence on ECM surfaces of *R. insignis* Quél. (in our phylogeny, this would be the sister-species of *R. pulverulenta* Peck, sect. *Ingratae*, subg. *Heterophyllidia*) in the form of long tapering cells that are not thick-walled and have yellow incrustations, thus reminding strongly of the incrusted terminations (in particular the gloeocystidia) that compose the marginal 'veil' on the pileus of this species (see e.g. Sarnari 1998, p. 477). In this case, we consider that these cells are not homologous with the needle-shaped cells. Nor do we think that the aculeate endings of the ECM outer mantle in *R. blennia* (sp. ined., subg. *Malodora*—see our Fig. 4a) can be considered to be 'needle'shaped cells. Needle-shaped cells (Online Resource 6) in their most typical expression are characteristic for one terminal subclade of species in Clade IV (*Heterophyllidia*), appearing in the phylogeny at least from the point of the common ancestor shared by *R. vesca* and species of *Virescentinae* and higher up (mostly tropical, often annulate African species). Whether these appear already earlier, i.e. starting at the common ancestor shared by *Amoeninae*, *Griseinae* and all the other species groups just mentioned, is less obvious as examined *Amoeninae* and *Griseinae* lack typical thick-walled needle-shaped cells on fruiting bodies, while the needle-shaped cells on their below-ground structures are also less characteristic.

Rhizomorphs: Generally considered to represent the more sophisticated soil exploration structures of the fungal mycelium, their presence is principally limited to more basal clades in the genus. Moreover, highly differentiated rhizomorphs of *Russula* are typically those in connection with the ECM and have rarely been observed connected to the base of fruiting bodies as here illustrated for example for *Multifurca* (see Fig. 6). *Russula* is particular among ECM fungi in that its rhizomorphs extend from the ECM structures running alongside—and remaining attached to—

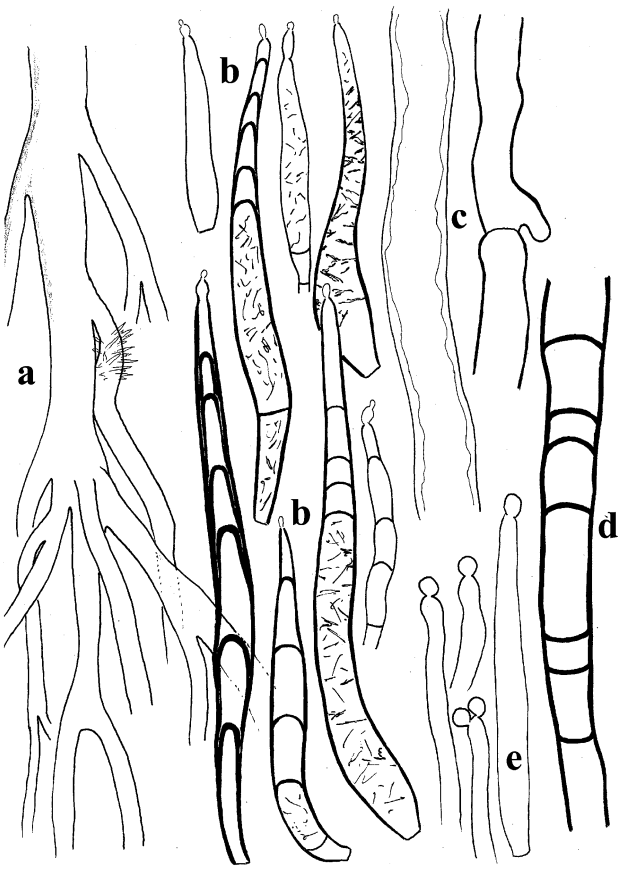


Fig. 6 Below-ground features. *Multifurca aurantiophylla*. (*a*) Partial view of rhizomorph habitus with detail indicating emergent gloeocystidia that are present on the entire surface. (*b*) "ladderlike" gloeocystidia of the rhizomorph surface; (*c*) fragments of vessel-like hyphae; (*d*) detail of ladder-like hyphae with arched septa of the inner parts of the rhizomorph. (e) Apical parts of gloeocystidia near the stipe base. Drawings from Buyck 09.345 (New Caledonia). Scale bar: approximately 0.5 mm for rhizomorph habitus; 10 µm for other elements

the root-tip of the host plant. Agerer (1999) pinned the term "russuloid rhizomorphs" for these highly differentiated *Russula* rhizomorphs as the agaricoid genera in Russulaceae are unique among ECM fungi in possessing both vessel-like and ladder-like hyphae in their inner below-ground tissues (see Fig. 6c–d). It is tempting to relate the ladder-like hyphae with the development of sphaerocytes, a unique cell-type found in fruiting bodies of these same genera, but the absence of ladder-like hyphae in some *Russula* species or species groups (e.g. in Clade IV—see Online Resource 6) seems to contradict this hypothesis.

Russula rhizomorphs are considered to be of the "short to medium distance" type, never of the "long distance" exploration type commonly observed in boletes for example (Agerer 2001); they typically remain in close contact with the host root surface and thus hardly explore the surrounding soil volume. The question has been raised

whether such russuloid rhizomorphs might be involved with fungal reticulation or spreading on—and repeated infection of—the host tree root rather than with soil exploration and nutrient transport as in most other ECM fungi (Beenken 2004, p. 346).

Regardless of the function of these rhizomorphs, the confrontation between existing data and our phylogeny suggests that the formation of russuloid rhizomorphs is a plesiomorphic feature in the genus and rhizomorphs are indeed mostly restricted to *Russula* species with plectenchymatic outer ECM mantles (Beenken 2004). So far, only six species with a pseudoparenchymatic outer ECM mantle in Clade VIII are known to form rhizomorphs, and in these cases with atypical ladderized hyphae (Agerer 1986; see Online Resource 5). So far, features of rhizomorphs, other than simple presence or absence, seem to be uninformative for the characterization of certain species-groups within individual clades, with the exception of clade IV, where russuloid rhizomorphs are not formed in all examined species of sect. *Ingratae*. Indeed, our phylogeny now confirms that species traditionally placed in subsections *Pectinatinae* (inclusive species of *Subvelatae*) and *Foetentinae* correspond to monophyletic clades. All examined species in *Foetentinae* (*R. laurocerasi* Mlz., *R. illota* Romagn., *R. foetens* Pers.) form less differentiated rhizomorphs lacking the typical ladder-like hyphae, while *Pectinatinae/Subvelatae* lack rhizomorph formation (*R.pectinatoides* Peck, *R. pulverulenta*), thereby resembling *Cyanoxanthinae* and *Aureotactinae*.

Rhizomorph formation in *Russula* has been related to the nature of the ECM surfaces. In general, it is assumed that rhizomorphs only develop in species with hydrophobic ECM surfaces incapable of (or at least less efficient in) absorbing soil nutrients at these sites. In *Russula*, this concerns in particular all subgenera composed of species with a plectenchymatic outer mantle where the emerging cells are suggested to create a film of air surrounding the surface (Beenken 2004). However, this explanation seems not very convincing for three reasons: (1) the rhizomorph surface in *Russula* has very similar characteristics as the ECM surface, (2) typical *Russula* rhizomorphs are hardly exploring the surrounding soil volume, and (3) the plectenchymatic outer mantle layers mostly have abundant gelatinous matrix in between the emergent cells.

Published ECM studies demonstrated that *R. claroflava* Grove and *R. vinosa* Lindbl. (both not in our sampling) stand out from other *Russula* species in Clade VIII in having straight septa in the ladder-like hyphae, a character also shared with *R. alnetorum* [in this case probably misidentified and probably = *R. leprosa* (Bres.) Crawshay?], a species belonging in Clade VII of subg. *Russula* (see Beenken 2004). If the assumption about the identity of this "*R. alnetorum*" is correct, than this species, and also *R. ochroleuca* Pers., both belong in sect. *Ochroleucinae* of subg. *Russula* and have been observed to form rhizomorphs, although in the case of *R. ochroleuca* the described rhizomorphs were again not typical (on www.deemy.de *R. ochroleuca* is now mentioned as not forming [russuloid] rhizomorphs). Rhizomorphs have also been observed in *R. xerampelina* (Agerer 1986), but equally lack the ladder-like hyphae.

Another intriguing fact is that in nearly all of the more ancient lineages of *Russula* (subg. *Archaea, Brevipes, Compactae, Malodora*) certain species have a stipe that is not narrowing downward and typically becomes irregularly and strongly furrowed near the base. When sectioning such a stipe base (see Fig. 7), it is easily observed that these species do not have an obtusely rounded stipe base as in most of the species in more terminal clades, but that the stipe base is 'spreading out' into the soil. This spreading is of a different nature than what is usually referred to as 'basidiomal rhizomorphs' (Clémençon 2004) and it suggests rather that individual mycelial strands participate together in the elaboration of a single fruiting body. Such multiple arrivals of mycelial strands converging together to participate in the elaboration of a single fruiting body can also be observed in some *Lactifluus* species (Buyck unpubl.). While this does not explain why typical russuloid rhizomorphs are running along the roots and not exploring the surrounding soil, it might explain why presence of rhizomorphs represent the plesiomorphic state in our phylogeny.

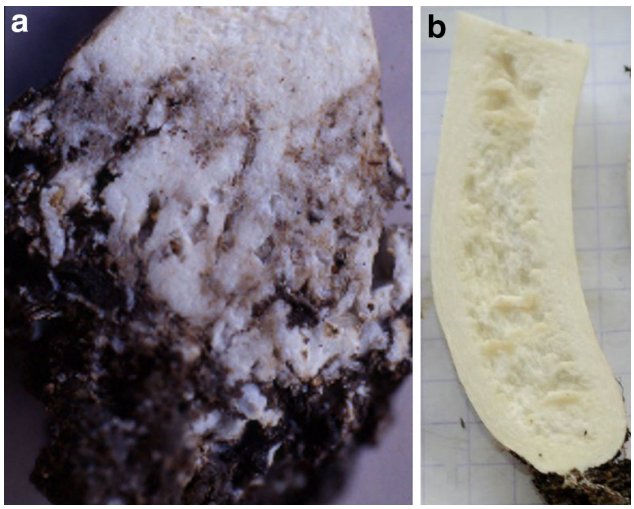


Fig. 7 Form of the stipe base. **a** Undescribed Malagasy relative of *R. fistulosa* (Buyck 00.1324) showing the fusion of several individual mycelium strands into a single stipe, a feature typical of some species in the phylogenetically more ancient clades of the genus (as well as those in *Lactifluus*). **b** Typical stipe base of species in more derived clades

Data on 'russuloid' rhizomorphs are quasi inexistent for *Lactifluus* and seem quite more rare in *Lactarius*. We present here first data on a more exploring type of rhizomorphs in the supposedly ancient genus *Multifurca*.

Comparison with Beenken's classification: The (never formalized) subdivision proposed by Beenken (2004) as the result of his study of below-ground structures in *Russula* was—not unexpectedly—unable to adhere to the subgeneric division proposed by Romagnesi (1967, 1985) and divided the genus in 19 sections, mainly the consequence of oversplitting our Clade VIII. Species of our Clades III (subg. *Crassotunicata*) and V (subg. *Malodora*) were not studied by Beenken.

When considering species with unequal gills, traditionally placed in subg. *Compactae*, Beenken argumented that the below-ground differences were such that they could not be considered as constituting a single systematic group and his classification recognized five independent sections: *Gossypinae* and *Compactae* (corresponding to our Clade 1 and II respectively) and three sections corresponding to our clade VI (subg. *Brevipes*) for which Beenken studied three closely related species, all in the *R. delica* species complex (his *Lactarioides*), as well as two species for which there were no available sequence data at the time, i.e. *R. fuegiana* and *R. aucarum* (type species of *Delicoarchaeae*) which he both placed in a section of their own. Recent phylogenies based mainly on ITS sequence data (e.g. Kong et al. 2015; Buyck et al. 2017) place both these species basal to *Lactarioides.* For *R. fuegiana* this means probably that it is closer to *Pallidosporinae* Bon (see Online Resource 4) because of the inamyloid suprahilar spot. All in all, it can be said that Beenken's proposal closely mirrors our phylogeny.

When taking into account a few systematic updates as a result of recent sequence data, Beenken also correctly placed all species of our Clade IV by distinguishing four main groups. Indeed, recent molecular results suggest that *R. aeruginea* is not a member of *Ilicinae* as suggested by Beenken (who followed here Sarnari 1998) but belongs in *Griseinae* (fide F. Hampe & coll.). Secondly, the tropical *R. cf. radicans* R. Heim and *R. acriannulata* Buyck are unrelated to *Crassotunicatae*, although they remain good members of *Aureotactae* (this study) which Beenken considers to constitute a separate section close to *Ingratae*. As a result, *Ilicinae* and *Crassotunicatinae* are not sampled by Beenken, but the remaining groups of species with plectenchymatic ECM mantles are correctly divided over three remaining sections. Beenken's sect. *Heterophyllae* is correctly delimited and includes, for ex., *Amoeninae*, but not *Cyanoxanthinae*, which he (correctly) considers to belong to a section of its own. Finally, sect. *Ingratae* is divided on the basis of below-ground features in three subsections (*Foetentinae*, *Pectinatinae* and *Subvelatae*). The good performance of below-ground features in subg. *Heterophyllidia* is due to the higher heterogeneity of features among the species groups that compose this subgenus and allowed Beenken to take advantage of middle mantle structure, as well as emanating hyphal or needle-shaped endings on the ECM surface to define species groups.

When looking at the subdivision of all species with a pseudoparenchymatic outer ECM mantle layer (i.e. those considered as 'acystidiate" ECM by Beenken), the poor variation of the various below-ground features clearly promoted the adherence to the traditional fruiting body classifications, although clearly influenced by the first published ITS phylogenies on the genus (Eberhardt 2002; Miller and Buyck 2002), as clearly stated by Beenken himself (2004, p. 314). As a consequence, below-ground structures are treated as secondary criteria for the recognition of systematic groups. For example, if ECM features were to be determinate for the grouping of species, then *Sardoninae* and *Persicinae* should have been classified (which is not the case) with the other species having an ECM outer mantle layer with irregularly shaped cells and overlying hyphal network (i.e. species in our Clade VIII). The placement of *R. fellea* as a monospecific subsection in his sect. *Russula*, contrary to its traditional placement in subg. *Ingratula* (accepted in all previous monographs, except for Bon 1988), was a transfer clearly suggested by the first published phylogenies but, in this case, fitted also perfectly with ECM features. As a result of our phylogenetic analysis, we have to agree with Beenken that below-ground features do not allow to distinguish between our Clades VII and VIII. At least within our Clade VIII (see Online Resource 2), the distribution of below-ground features is not suggesting the existence of some morphologically well-characterized species-groups. Part of the explanation resides probably in the very fast radiation of this clade as suggested by the unresolved backbone for most of this clade. The study of more extra-European species will eventually introduce a higher variability of below-ground features within these clades as it did already with the inclusion of *R. discopus* as the only species in Clade VIII so far with a pseudoparenchymatic outer mantle layer aspect that is typical of species in Clade VII.

Below-ground features of *Russula* versus other agaricoid Russulaceae

In conclusion of the above, it can be stated that below-ground features, not surprisingly, are more conserved than features of their above-ground fruiting bodies. Below-ground features could, therefore, also be very important toward a better understanding and delimitation of the different ECM genera in Russulaceae. As explained above,

the simple presence/absence of gloeoplerous cells on the surface of the ECM outer mantle in *Russula* species appears uninformative with respect to the phylogenetic subdivision of the genus, but what about the presence/absence of gloeoplerous elements when opposing *Russula* to the other agaricoid genera in Russulaceae? All previous publications state that *Russula* differs from *Lactarius* in the absence of lactifers inside the ECM and rhizomorph tissues. However, all of these publications date from before the reinstatement of *Lactifluus* and *Multifurca* as separate genera (Buyck et al. 2008, 2010). When checking all published accounts on ectomycorrhizal features of milk caps, we were struck by the fact that below-ground features of species that have since been transferred to *Lactifluus* are almost non-existent. Indeed, Beenken (2004) described in detail the ECM structures of *Lf. piperatus* (as *Lactarius*), noting their similarity to those of *R. gossypina* (subg. *Archaea*), while a very recent paper by Leonardi et al. (2016) described the ECM of *Lf. rugatus* (Kuhner & Romagn.) Verbeken. These two papers, together with some short notes on ECM anatomy of two South-American *Lactifluus*, including the pleurotoid *Lf. panuoides* Singer (Henkel et al. 2000) and one *Lf.* cf *venezuelianus* (Haug et al. 2005, as identified here by 99% BLAST similarity) represent the short list of available data on ECM of *Lactifluus* and all of these were molecularly verified. More importantly, however, all these descriptions mention that lactifers were not found inside the ECM, nor in the rhizomorph tissues of these *Lactifluus* species. This absence is further confirmed by our own examination (Buyck, unpubl.) of ECM structures of our outgroup species and some other American and African species in *Lf.* subg. *Lactifluus* and *Lf.* subg. *Lactariopsis*, as well as in subg. *Pseudogymnocarpi*, i.e. for species belonging to three of the four presently recognized subgenera of the genus (De Crop et al. 2017). It therefore appears that *Russula* shares the absence of lactifers with all molecularly identified ECM structures of *Lactifluus* so far, contrary to *Lactarius*.

In order to study also below-ground features for the fourth genus, *Multifurca*, we have been searching repeatedly for ECM structures in the soil when collecting species over the past years, but we were as yet unable to find them. However, we did examine rhizomorphs of two lactarioid *Multifurca* [*M. stenophylla* (Berk.) T. Lebel, C.W. Dunk & T.W. May and *M. furcata* (Coker) Buyck & V. Hofst.], both having typical lactifers, and also of two russuloid species [*M. aurantiophylla* (Buyck & Ducousso) Buyck & V. Hofst.—see Fig. 6, and *M. ochricompacta* (Bills & O.K. Miller) Buyck & V. Hofst.] both of which lack distinct lactifers. The studied rhizomorphs of *M. aurantiophylla* do not correspond to the typical russuloid rhizomorphs, i.e. they do not run alongside the root-system of the host emanating from ECM structures (as we did not find these), but are freely ramifying in the soil in connection with the base of the fruiting bodies. Yet, they do possess the anatomical attributes of typical russuloid rhizomorphs. In the case of *M. aurantiophylla* Buyck but not the other species, the rhizomorph surface is covered by multicelled gloeocystidia, a morphotype so far unknown in below ground structures of Russulaceae (see Fig. 6b), although a single secondary septum is often forming two-celled gloeoplerous cystidia on the below-ground structures in subg. *Brevipes* (see Fig. 3a).

Major diversification events for *Russula*

As the result of our phylogenetic analyses, there appear to have been at least two major events that have triggered a rapid and profound diversification for Russulaceae in general, and for *Russula* in particular: the first one concerns the context composition of fruiting bodies, the second one concerns the below ground structures.

When considering the context composition of *Russula*, one can easily make a parallel with a similar evolution recently described for the saprotrophic Psathyrellaceae, for which the phenomenon of "coprinoidization" has been interpreted as a unique means of protection from desiccation, as the faster ontogeny allows for more successful spore production through fast expansion of the mushroom being achieved by cellular uptake of water without need for further cell division or additional stages of elongation (Nagy et al. 2012). Indeed, Russulaceae have achieved a very similar advantage by building a heteromerous context through the formation of sphaerocytes: voluminous, globose cells that constitute part or all of the context tissue and allow for a very rapid expansion of the fruiting body through cellular uptake of water with minimal demand on carbon from the host. The formation of sphaerocytes as major constituent of stipe and cap tissues can therefore be considered to have been a first key innovation leading to a very rapid diversification for all main clades in *Russula* and even for the agaricoid Russulaceae as a whole. The success of this particular type of anatomy, which reached its culmination in *Russula*, can be deduced from a simple comparison of published names in the russsuloid clade: halve of the ca. 6000 published names (www.mycobank.org, accessed in 2012) in the gigantic russuloid clade are represented by the ectomycorrhizal, sphaerocyte producing Russulaceae, among which *Russula* represents more than halve of the available names. Buyck et al. (2008, 2010) observed that, technically speaking, the very short branches leading to the four agaricoid genera in Russulaceae would easily argue for the recognition of a single, large genus. The here presented five-gene phylogeny now shows that all of the main clades in *Russula* diverged in an even much shorter time period.

The second major diversification event in *Russula* concerns most of the northern hemisphere diversity in the genus and was triggered by the below-ground transition from a plectenchymatic to a pseudoparenchymatic ECM mantle type. It went hand in hand with the the near-suppression of the formation of rhizomorphs and, in one small monophyletic subclade of subg. *Russula* core clade VII, resulted even in the complete suppression of ECM outer sheath formation, leaving only an investment in Hartig net formation while exploiting the ECM structures of equally host-specific *Lactarius* species to associate with and then penetrate the roots of the shared tree host. Our phylogeny suggests that this below-ground transition likely happened already in the tropics as suggested by the ECM of *R. discopus* and the preponderance of tropical species groups closer to the base of clade VIII (see Fig. 1). The pseudoparenchymatic ECM outer mantle clearly should have represented a major advantage for these species as suggested by the 'explosion' of taxa in subgenus *Russula* in the northern hemisphere. One might even hypothesize that this changed anatomy facilitated host shifts when migrating into the northern hemisphere.

Which geographic origin for Russula?

We would finally like to make some concluding remarks on the origin for *Russula* as hypothesized by Looney et al. (2016). These authors suggested that *Russula* was most likely of post-Gondwanan, northern temperate origin with/delica,/farinipes,/archaea and/russula clades all significantly supported as having a northern temperate origin, most likely in association with Fagaceae. The present study includes now for the first time a considerable number of Central American, Central African/Malagasy, as well as New Caledonian species in our multigene analysis (see geographic origin in Online Resource 3 or Fig. 1). Our data, combined with those available on *Russula* in Australia (Lebel and Tonkin 2007), New Zealand (Cooper and Leonard 2014), lowland tropical South (Barbosa 2016) as well as Central America (Buyck 1988a, b, 1989a, b; Buyck and Ovrebo 2002; Singer et al. 1983), allow some skepticism concerning these conclusions once a more representative sampling will be analyzed in a multigene approach.

Given the near cosmopolitan distribution patterns described above for most of the subgenera, we predict that many tropical and southern hemisphere species will occupy most of the ancient lineages in each of these clades as already predicted and discussed previously (Buyck 1995; Buyck et al. 1996). In our phylogeny, this is already quite evident for the predominantly tropical subg. *Malodora* (clade III), while it is here also suggested for subg. *Heterophyllidia* (clade IV), *Compactae* (clade II) and part of subg. *Russula* (clade VIII), although for these three subgenera tropical species are certainly still largely underrepresented in our phylogeny. For subg. *Brevipes*, only northern hemisphere taxa have here been retained due to too many missing data for the few tropical specimens we disposed of. Yet, the few published ITS phylogenies containing some of the tropical relatives of this subgenus (e.g. Barbosa 2016, or the phylogeny presented for *R. pseudoaurantiophylla* in Buyck et al. 2017) clearly suggest that tropical species will occupy in subg. *Brevipes* lineages that are more ancient than the northern temperate *R. delica*-group.

The most notable exceptions to this near cosmopolitan pattern observed for the abovementioned clades concerns subgenera *Crassotunicata* (clade III) and the *Russula* core clade (clade VII) for which good tropical candidates have not yet been revealed, although Buyck (1994) described several, as yet unsequenced species in subsect. *Sardoninae* from tropical Africa (Clade VII). Possible links between Clade VII and tropical species have also been suggested by Buyck and Mitchell (2003) for sect. *Ochroleucinae*. (= *Viscidinae* Sarnari). Subg. *Crassotunicata* is presently limited to North America (both West and East coast) and Europe, but still undescribed representatives have recently also been collected by the first author in subtropical parts of China (Buyck, unpubl.).

Another troubling aspect (at least to the first author) is the absence of subg. *Maladora* and subg. *Compactae* sect. *Polyphyllae* from Europe. These clades are not only well represented in the paleotropics and Australia, but equally present in temperate North America and Asia (contrary to other ancient lineages that are common in the tropics but absent from the entire northern hemisphere—e.g. some species groups in Clades VIII, VI). In such a case, it is tempting to attribute this absence to recent glaciations in Europe that wiped out most of the then existing mycota, but in such a scenario all other lineages that are potentially at least equally ancient, should also be absent from Europe (e.g. subg. *Archaea, Crassotunicata*) which is not the case. Compared to other continents, these small subgenera are actually well represented in Europe (with at least two species each). Yet, we have to admit that unstudied collections demonstrate already that a considerable part of the diversity of *Archaea* on other continents remains to be described (Buyck unpubl.).

Few tropical or southern hemisphere lineages occupy isolated terminal positions in otherwise mainly northern temperate assemblages. This is here, for example, the case for subsect. *Tricholomopsidae*, a species group that was initially described from *Nothofagus* forests in temperate southern America (Singer 1950) and which accompanies *Nothofagus* throughout its distribution area in South America (Singer 1969). More recently, this same species group has been demonstrated to be much more diverse and

common under *Nothofagus* (sensu lato—see Heenan and Smissen 2013) in New Zealand (Cooper and Leonard 2014) and Australia (Lebel and Tonkin 2007). It is equally diverse in New Caledonia as here demonstrated by our first collections for this island (Online Resource 4). Such a scenario of an isolated southern hemisphere lineage in an otherwise temperate species assemblage might suggest a host shift as probable cause of the re-invasion of this southern hemisphere habitat. Pirozynski (1983) might thus have been correct when describing *Russula* as part of an originally warmth-adapted mycota that only much more recently invaded the ancient *Nothofagus* forests of the temperate southern hemisphere. The possibility of this host shift seems supported by the observation that several *Russula* species (incl. *Tricholomopsidae*) are also associated with *Leptospermum* and other Myrtaceae in New Zealand and Australia. Apart from *Tricholomopsidae*, subg. *Russula* (clades VII & VIII) is hardly represented in Oceania and this appears not to be an artefact from undercollecting. The first author has also not encountered species of the *Russula* core clade (clade VII) during his collecting trip in New Caledonia. The *Russula* core clade has at least one representative (apparently close to subsect. *Emeticinae*) reported from New Zealand as the result of many years of intensive collecting (Cooper and Leonard 2014) and a second one is known from Australia (Lebel and Tonkin 2007). As far as the largest subclade of subg. *Russula* (clade VIII) is concerned, there are—apart from the very diverse *Tricholomopsidae*—equally few species reported from Oceania. Again, New Zealand and Australia share closely related taxa and both are equally poor in species composition with each region having a single species that is apparently not so distantly related to *R. caerulea*, another one close to *R. adulterina*, and further also a few species that are at the very base of Clade VIII, apparently belonging to similar groups as those we collected in New Caledonia (see Online Resource 4, i.e. members of subsect. *Roseinae*, *Echinospermatinae* or perhaps sect. *Flavisiccantes*). Most of these Oceanian species outside subsect. *Tricholomopsidae* are often associated with *Nothofagus* (s.l.) and nearly all of these Oceanian species in Clade VIII are poor in gloeoplerous elements (i.e. having similarities, possibly affinities, with species of subg. *Malodora*, although BLAST top scores for ITS sequences from species of the latter subgenus are mainly corresponding to species of the *Russula* core clade (clade VII). It is tempting to hypothesize from this strange pattern and performance of subg. *Russula* in Oceania that *Russula* species with a pseudoparenchymatic ECM outer mantle layer perform only well with Fagaceous hosts in the southern hemisphere where the genus has known—at least in *Tricholomopsidae*—a similar 'explosion' of species as the whole of the *Russula* crown clade has experienced in the northern hemisphere. *Russula* cannot be considered to be particularly diverse in Oceania and many species (also?) associate with Myrtaceae, including representatives of all of the here recognized subgenera with the exception of subg. *Crassotunicata* which is for the moment the only subgenus not yet recorded from the southern hemisphere.

Finally, subg. *Heterophyllidia* is the only subgenus where tropical taxa are so intricately mixed with temperate taxa across the entire clade, occupying both terminal and basal clades in all lineages, although our sampling is too strongly biased toward northern hemisphere taxa to reflect this correctly. From our collecting expeditions around the world, it is obvious that this is a tremendously diverse clade, also in neo- and paleotropics. One element that suggests that this clade achieved its worldwide distribution already long time ago is the fact that morphologically similar species that are geographically separated constitute distinct lineages. (e.g. subsect. *Oleiferinae* and northern temperate sect. *Ingratae* produce morphological twins but are sister lineages sitting on long branches).

Based on the abovementioned observations, we think it is unlikely that *Russula* originated in the temperate northern hemisphere, although the answer to this question will depend strongly on the position of subg. *Crassotunicata*, which has previously been recovered as a very ancient lineage (e.g. Bazzicalupo et al. 2017). Additionally, it is the only lineage among the other ancient lineages where conifers cannot be excluded as original hosts. Yet, the high support recovered here to place *Crassotunicata* now as sister to *Heterophyllidia*, suggests that it is not a good candidate for the oldest lineage in the genus, and this is also supported by its more evolved morphological features compared to species in subg. *Archaea*.

In this age of rapidly disappearing tropical habitats, we need not only to sequence as much as possible of the already discovered diversity, but also to intensify our search for missing links that can complete the overall picture before these will go extinct.

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References

Adamčík S, Jančovičová BB (2018) The Russulas described by Charles Horton Peck. Cryptog Mycol 39(1):3–110

Agerer R (1986) Studies on ectomycorrhizas II. Introducing remarks on characterization and identification. Mycotaxon 26:473–492

Agerer R (ed) (1987–2012) Colour atlas of Ectomycorrhizae. 15 parts. Schwäbisch Gmünd. Einhorn-Verlag

Agerer R (1995) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Berlin, pp 685–734

Agerer R (1999) Anatomical characteristics of Identified Ectomycorrhizas: An attempt towards a natural classification. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Münich, pp 633–682

Agerer R (2001) Exploration types of ectomycorrhizae—a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11:107–114

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5:67–107

Alfaro M, Zoller S, Lutzoni F (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol Biol Evol 20:255–266

Barbosa JAD (2016) Analise filogenetica de *Russula* Pers. (Russulaceae, Russulaes: Agaricomyctes). PhD dissertation, Florianopolis, pp 1–94. https://repositorio.ufsc.br/handle/123456789/167945

Bazzicalupo AL, Buyck B, Saar I, Vauras J, Carmean D, Berbee ML (2017) Troubles with mycorrhizal mushroom identification where morphological identification lags behin barcode sequence divergence. Taxon 66(4):791–810

Beenken L (2004) Die Gattung *Russula*. Untersuchungen zu ihrer Systematik anhand von Ektomykorrhizen. Dissertation. Ludwig-Maximilians Universität München; https://edoc.ub.uni-muenchen.de/3175/1/Beenken_Ludwig.pdf

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

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understnding global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77

Buyck B (1988a) Etude microscopique de spécimens-types de Russules tropicales de la sous-section *Diversicolores*. Mycotaxon 33:57–70

Buyck B (1988b) Etude microscopique de spécimens-types de Russules tropicales de la sous-section *Pluviales*. Mycotaxon 33:71–80

Buyck B (1989a) Quelques Russules à lamprocystides. Mycotaxon 35:45–54

Buyck B (1989b) Etudes microscopiques de Russules tropicales: *Mimeticinae* subsect. nov. Mycotaxon 35:55–63

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

Buyck B (1990) Nouveaux taxons infragénériques dans le genre *Russula* Persoon en Afrique centrale. Bull Jard Bot Nat Belg 60:191–211

Buyck B (1993) *Russula* I (Russulaceae). In: Rammeloo J, Heinemann P (eds) Flore illustrée des Champignons d'Afrique Centrale, vol 15, pp 335–408

Buyck B (1994) *Russula* II (Russulaceae). In: Rammeloo J, Heinemann P (eds) Flore illustrée des Champignons d'Afrique Centrale, vol 16, pp 411–542, pl 69–87

Buyck B (1995) A global and integrated approach on the taxonomy of Russulales. Russulales News 3:2–17

Buyck B (1998) Une révision critique de sect. *Archaeinae* (*Russula*, Russulales). Belg J Bot 131(2):116–126

Buyck B (1999) Two remarkable new African *Russula* species with reddening context. Kew Bull 54:663–673

Buyck B (2003) Notes on the central American *Russula cremeolilacina* var. *coccolobicola* (=*R. littoralis* Pegler). Cryptog Mycol 24(2):117–124

Buyck B, Adamčík S (2013) Type studies in *Russula* subsection *Lactarioideae* from North America and a tentative key to North American species. Cryptog Mycol 34(3):259–279

Buyck B, Halling R (2004) Two new *Quercus*-associated *Russula*'s from Costa Rica and their relation to some very rare North American species. Cryptog Mycol 25(1):3–13

Buyck B, Mitchell D (2003) *Russula lentiginosa* spec. nov. from West Virginia, USA, a probable link between tropical and temperate Russula-groups. Cryptog Mycol 24(4):317–325

Buyck B, Ovrebo C (2002) New or interesting *Russula* from Panama. Mycologia 94(5):888–901

Buyck B, Thoen D, Watling R (1996) Ectomycorrhizal fungi of the Guinea-Congo Region. In: Alexander IJ, Swaine MD, Watling R (eds) Essays on the ecology of the Guinea-Congo rain forest, vol 104 of Proceedings B. The Royal Soc of Edinburgh, Edinburgh, pp 313–333

Buyck B, Halling R, Mueller G (2003a) The inventory of Russula in Costa Rica: discovery of two very rare North American species in montane oak forest. Boll Ass Mycol Bresadola (Nuova Ser) 46:57–74

Buyck B, Heriveau P, Martin P (2003b) Quelques récoltes récentes de *Russula camarophylla* Romagnesi. Bull Soc Mycol France 119:217–229

Buyck B, Hofstetter V, Eberhardt U, Verbeken A, Kauff F (2008) Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect *Ochricompactae*. Fungal Divers 28:15–40

Buyck B, Hofstetter V, Verbeken A, Walleyn R (2010) Proposal to conserve *Lactarius* nom. cons. (Basidiomycota) with a conserved type. Taxon 59(1):295–296

Buyck B, Kauff F, Eyssartier G, Couloux A, Hofstetter V (2014) A multigene world phylogeny for *Cantharellus*. Fungal Divers 64:101–121

Buyck B, De Crop E, Verbeken A, Hofstetter V (2016) Untangling the Central African *Cantharellus* sect. *Tenues*: *Cantharellus minutissimus* sp. nov. and epitypification of *Cantharellus alboroseus*. Cryptog Mycol 37(3):329–344

Buyck B, Duhem B, Das K, Jayawardena RS, Niveiro N et al (2017) Fungal biodiversity Profiles 21–30. Cryptog Mycol 38(1):101–146

Cabón 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 syapomorphic characters. Mycol Progress 16(9):877–892

Clémençon H (2004) Cytology and plectology of the hymenomycetes. Bibl Mycol 199:1–488

Comandini O, Rinaldi AC, Kuyper T (2012) Measuring and estimating ectomycorrhizal fungal diversity: a continuous

challenge. In: Pagano M (ed) Mycorrhiza: occurrence in natural and restored environments. Nova Science Publishers, Hauppauge, pp 165–200

Cooper J, Leonard P (2014) Notes on *Russula* in New Zealand. https://www.funnz.org.nz/sites/default/files/RussulaForBlog.pdf

Das K, Ghosh A, Baghela A, Avchar R, Bhatt B, Buyck B (2017a) Morphological and phylogenetic evidence for *Russula butyroindica* sp. nov. (Russulaceae) from the Indian Himalaya. Nov Hedw 105:3–4

Das K, Ghosh A, Chakraborty D, Li EJ, Qiu L et al (2017b) Fungal biodiversity profiles 31–40. Cryptog Mycol 38(3):353–406

Das K, Ghosh A, Bhatt RP, Chakraborty D, Hofstetter V, Buyck B (2017c) Fungal biodiversity profiles 41–50. Cryptog Mycol 38(4):527–547

De Crop E, Nuytinck J, Van de Putte K, Wisitrassameewong K, Hackel J et al (2017) A multigene phylogeny of *Lactifluus* (Basidiomycota, Russulales) translated into a new infrageneric classification of the genus. Persoonia 38:58–80

De Kesel A, Amalfi M, Kasongo B, Yorou NS, Raspé O, Degreef J, Buyck B (2016) New and interesting *Cantharellus* from tropical Africa. Cryptog Mycol 37(3):283–328

Eberhardt U (2002) Molecular kinship analyses of the agaricoid Russulaceaae: correspondence with mycorrhizal anatomy and sporocarp features in the genus *Russula*. Mycol Progress 1(2):201–223

Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. Syst Biol 27(4):401–410

Haug I, Weiss M, Homeier J, Oberwinkler F, Kottke I (2005) Russulaceae and Thelephoraceae form ectomycorrhizas with members of the Nyctaginaceae (Caryophyllales) in the tropical mountain rain forest of southern Ecuador. New Phytol 165:923–936

Heenan PB, Smissen RD (2013) Revised circumscription of *Nothofagus* and recognition of the segregate genera *Fuscospora*, *Lophozonia*, and *Trisyngyne* (Nothofagaceae). Phytotaxa 146(1):1–31

Heim R (1938) Les Lactario–russulés du domaine oriental de Madagascar, essai sur la classification et la phylogénie des Astérosporales. Prodome à une flore mycologique de Madagascar et dépendances I: 196 pp, 4 pl

Heim R (1970) Particularites remarquables des Russules tropicales Pellicullariae lilliputiennes: les complexes annulata et radicans. Bull Soc Mycol France 86:59–77

Henkel TW, Aime MC, Miller SL (2000) Systematics of pleurotoid Russulaceae from Guyana and Japan, with notes on their ectomycorrhizal state. Mycologia 92:1119–1132

Hofstetter V, Clémençon H, Vilgalys R, Moncalvo JM (2002) Phylogenetic analyses of the Lyophylleae (Agaricales, Basidiomycota) based on nuclear and mitochondrial rDNA sequences. Mycol Res 106:1043–1059

Hongsanan S, Hyde KD, Bahkali AH et al (2015) Fungal biodiversity profiles 11–20. Cryptog Mycol 36(3):355–380

Hyde KD, Hongsanan S, Jeewon R, Bhat DJ et al (2016) Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal species. Fungal Divers 80:1–270

Koide RT, Fernandez C, Malcolm G (2014) Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. New Phytol 201:403–439

Kong A, Cifuentes J, Estrada-Torres A, Guzman-Davalos L, Garibay-Oriel R, Buyck B (2015) Russulaceae associatedd with mycoheterotroph *Monotropa uniflora* (Ericaceae) in Tlaxcala, Mexico: a phylogenetic apporach. Cryptog Mycol 36(4):479–512

Larsson L, Larsson K-H (2003) Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. Mycologia 95:1037–1065

Lebel T, Tonkin JE (2007) Australasian species of *Macowanites* are sequestrate species of *Russula* (Russulaceae, Basidiomycota). Aust Syst Bot 20:355–381

Leonardi M, Comandini O, Rinali AC (2016) Peering into the Mediterranean black box: *Lactifluus rugatus* ectomycorrhizas on *Cistus*. IMA Fungus 7(2):275–284

Lilleskov EA, Hobbie EA, Horton TR (2011) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecol 2:174–183

Liu NG, Ariyawansa HA, Hyde KD, Maharachchikumbura SSN et al (2015) Fungal diversity notes 1–110: taxonomic and phylogenetic contributions to fungal species. Fungal Divers 72:1–197

Liu YJ, Hall BD (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II, phylogeny. Proc Natl Acad Sci USA 101:4507–4512

Looney BP, Ryberg M, Hampe F, Sánchez-García M, Matheny PB (2016) Into and out of the tropics: global diversification patterns in a hyper-diverse clade of ectomycorrhizal fungi. Mol Ecol 25:630–647

Machnicki N, Wright LL, Allen A, Robertson CP, Meyer C, Birkebak JM, Ammirati JF (2006) *Russula crassotunicata* identified as host for *Dendrocollybia racemosa*. N Am Fungi 1:1–7

Maddison DR, Maddison WP (2002) MacClade: analysis of phylogeny and character evolution, version 4.05. Sinauer Associates Inc., Sunderland, Massachusetts, USA

Mason-Gamer R, Kellog E (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). Syst Biol 45:524–545

Matheny PB, Aime MC, Bougher NL, Buyck B, Desjardin D et al (2009) Out of the palaeotropics? Historical biogeography and diversification in the cosmopolitan ectomycorrhizal mushroom family Inocybaceae. J Biogeogr 36:577–592

McNabb RFR (1973) Russulaceae of New Zealand 2. *Russula* Pers. Ex SF Gray. N Z J Bot 11:673–730

Miller SL, Buyck B (2002) Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classification. Mycol Res 106(3):259–276

Morehouse EA, James TY, Ganley ARD, Vilgalys R, Berger L, Murphy PJ, Longcore JE (2003) Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. Mol Ecol 12:395–403

Morozova OV, Popov ES, Kovalenko AE (2013) Studies on mycobiota of Vietnam. II. Two new species of *Lactifluus* (Russulaceae) with pleurotoid basidiomata. Mikol Fitopat 47:92–102

Nagy LG, Házi J, Szappanos B, Kocsubé S, Bálint B et al (2012) The evolution of defense mechanisms correlate with the explosive diversification of autodigesting *Coprinellus* mushrooms (Agaricales) fungi. Syst Biol 61:595–607

Palfner G, Godnoy R (1996) *Russula fuegiana* Singer + *Nothofagus pumilio* (Poepp. Et Endl.) Krasser. Descr Ectomyc 1:119–145

Park MS, Lee H, Oh SY, Jung PE, Seok SJ, Fong JJ, Lim YW (2014) Species delimitation of three species within the *Russula* subgenus *Compacta* in Korea: *R. eccentrica, R. nigricans,* and *R. subnigricans*. J Microbiol 52(8):631–638

Pirozynski KA (1983) Pacific mycogeography: an appraisal. Aust J Bot 10:137–159

Redhead SA, Seifert KA (2001) *Asterophora* Ditmar ex Link 1809 versus *Nyctalis* Fries 1825, and the status of *Ugola* Adanson 1763. Taxon 50:243–268

Roberts C (2007) Russulas of Southern Vancouver island coastal forests. PhD dissertation, University of Victoria, Victoria, BC, Canada

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

Romagnesi H (1985) Les Russules d'Europe et d'Afrique du Nord. Reprint mit Online Resourcement. Cramer, Vaduz

Romagnesi H (1987) Novitates 3: status et Noms nouveaux pour les taxa infragénériques dans le Genre *Russula*. Doc Mycol Tome XVIII, Fascicule 69:39–40

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sanon E, Guissou KML, Yorou NS, Buyck B (2014) Le genre Russula au Burkina Faso (Afrique de l'Ouest): quelques espèces nouvelles de couleur brunâtre. Cryptog Mycol 35(4):377–397

Sarnari M (1998) Monographia illustrate des genere Russula in Europa. Associazioni Micologica Bresadola, Trento, Italy. Tomo primo

Sarnari M (2005) Monographia illustrate des genere Russula in Europa. Associazioni Micologica Bresadola, Trento, Italy. Tomo secundo

Shaffer RL (1970) Notes on the subsection *Crassotunicatinae* and other species of *Russula*. Lloydia 33(1):49–96

Singer R (1932) Monographie der Gattung *Russula*. Beih Bot Centralbl 49:205–380

Singer R (1950) Die höheren Pilze Argentiniens. Bull Suisse Mycol 28:181–196

Singer R (1969) Mycoflora australis. Beih Nova Hedw 29. 404 pp

Singer R (1975) The Agaricales in modern taxonomy. Cramer, Vaduz

Singer R (1986) The Agaricales in modern taxonomy. Koeltz, Koenigstein

Singer R, Araujo I, Ivory MH (1983) The ectotrophically mycorrhizal fungi of the neotropical lowlands, especially Central Amazonia. Beih Nova Hedw 77:1–352

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Syst Biol 57(5):758–771

Stiller JW, Hall BD (1997) The origins of red algae: implications for plastid evolution. Proc Natl Acad Sci USA 94:4520–4525

Taylor AFS, Alexander IJ (2005) The ectomycorrhizal symbiosis: life in the real world. New Phytol 159:102–112

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genet Biol 31(1):21–32

Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biol Rev 27:83-99

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Van de Putte K, Nuytinck J, Das K, Verbeken A (2012) Exposing hidden diversity by concordant genealogies and morphology a study of the *Lactifluus volemus* (Russulales) species complex in Sikkim Himalaya (India). Fungal Divers 55(1):171–194

Wang XH, Yang ZL, Li YC, Knudsen H, Liu PG (2009) *Russula griseocarnosa* sp. nov. (Russulaceae, Russulales), a commercially important edible mushroom in tropical China: mycorrhiza, phylogenetic position, and taxonomy. Nov Hedw 88:269–282

White TJ, Bruns TD, 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, San Diego, pp 315–322