Molecular kinship analyses of the agaricoid Russulaceae: correspondence with mycorrhizal anatomy and sporocarp features in the genus *Russula*

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A data set of LSU DNA sequences of mainly European *Russula* and *Lactarius* species was subjected to molecular phylogenetic analysis. Species could be allocated to six clades, with an unresolved phylogeny. One of these clades represents the genus *Lactarius*. The only analysed species of the section *Archaeinae* (*Russula*) was placed basal to both genera. Thus *Lactarius* appears to be derived from *Russula*. *Russula* was divided into four clusters, corresponding to the sections *Plorantinae* and *Nigricantinae*, subgenus *Heterophyllidia* including the section *Foetentinae*, and a cluster representing the remaining subgenera of the "Genuinae". Even though the resulting groups can be considered as valid classificatory groups, species associations resulting from molecular analyses neither support the division of *Russula* into the subgenus *Compacta* (including the sections *Nigricantinae, Plorantinae*, and *Archaeinae*) and the "Genuinae" (including all remaining taxa), nor do they support previously proposed evolutionary lineages within the "Genuinae". Ribosomal ITS DNA sequences of *Russula* species were analysed to achieve better infrageneric resolution. The results are discussed in relation to current classification systems and to what is known about the mycorrhizae formed by *Russula* species. While the systematic value attached to many macroscopic and microscopic sporocarp features was not supported by sequence data, mycorrhizal anatomy is in good correspondence with many of the results from the phylogenetic analysis.

The agaricoid genera *Russula* Pers. and *Lactarius* Pers. (Russulaceae Lotsy) are cosmopolitan members of the Hymenomycetes that have been found to form different types of mycorrhizae with a wide variety of plant groups. Both taxa are of great importance as mycorrhizal partners of all major groups of ectomycorrhizal host trees such as the Fagales, Dipterocarpaceae, Caesalpinoideae and Myrtaceae (BUYCK, THOEN & WATLING 1996). Moreover, it has been shown that russulaceous species form arbutoid and monotropoid mycorrhizae and endomycorrhizae with an achlorophyllous orchid species (MOLINA & TRAPPE 1982, HORTON, BRUNS & PARKER 1999, CULLINGS, SZARO & BRUNS 1996, TAYLOR & BRUNS 1997, 1999).

Currently, the ectomycorrhizae of almost 80 *Russula* and *Lactarius* species are known, with the majority of these having been described in detail. Although ectomycorrhizae are morphologically and anatomically highly complex structures, so far, mycorrhizal features have not been considered for the definition of species and higher taxa. This is the first of two articles in which it is demonstrated that the structure of the hyphal mantle is a suitable character for the delimitation of certain taxa within the genera *Russula* and *Lactarius* (the latter will be dealt with in the second article). In support of this contention, DNA sequence data of the 5'-terminus of the large subunit (LSU) and the internal transcribed spacer (ITS) of the nuclear coded ribosomal RNA genes of *Russula* and *Lactarius* species were obtained and subjected to phylogenetic analysis. The results of the analyses of the LSU of *Russula* and *Lactarius* and of the ITS of *Russula* sequence data are discussed in relation to current classification systems. Unless mentioned otherwise, the classification of ROMAGNESI (1985, 1987) was followed for *Russula*. A summary of his classification and authority names of subgenera are given in Tab. 1.

Within a wider taxonomic context, it is well recognised that *Russula*, *Lactarius*, and several genera of secotiod and gasteroid fungi form the Russulales (KREISEL 1969). The Russulales ss. Kreisel are defined by the possession of a lactiferous system, spores with amyloid ornamentation, and a heteromerous hyphal system consisting of hyphae (lacking clamp connections) and globular thin-walled cells called sphaerocytes. The family Russulaceae in its strict sense is limited to the agaricoid genera. Within the agaricoid fungi, the Rus-

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Tab. 1. Overview of the classification systems of *Lactarius* and *Russula* referred to in this paper unless indicated otherwise. Abbreviations refer to Fig. 1.

Abbreviation	Subgenus
Lactarius Pers. (following HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT 1998)	
Pip	*Piperites* (Fr.) Kaufman
Rul	*Russularia* (Fr.) Kaufman
Pli	*Plinthogalus* (Burl.) Hesler & A.H.Sm.
Laf	*Lactifluus* (Burl.) Hesler & A.H.Sm.
Lap	*Lactariopsis* (Henn.) R.Heim
Lac	*Lactarius*
Russula Pers. (following ROMAGNESI 1987)	
Com	*Compacta* (Fr.) Bon
Het	*Heterophyllidia* Romagn.
Ing	*Ingratula* Romagn.
Rus	*Russula* (Pers.) Fr. emend. Romagn.
Inc	*Incrustatula* Romagn.
Ten	*Tenellula* Romagn.
Pol	*Polychromidia* Romagn.
Coc	*Coccinula* Romagn.
Ins	*Insidiosula* Romagn.
not classified by ROMAGNESI (1987)	
Cra	sect. *Crassitunicatae* (Singer) Singer

sulaceae are generally considered an isolated group (KÜHNER 1980, but see REIJNDERS 1991). For about 50 years, a number of authors have pointed out that fungi with a variety of sporocarp types share the most important of the Russulales' characters, the possession of a lactiferous system and amyloid ornamentation of the spores (see OBERWINKLER 1977, KÜHNER 1980). Molecular phylogenetic analyses based on various DNA regions support a common origin of *Russula* and *Lactarius* (BRUNS et al. 1998, MONCALVO et al. 2000), and of the Russulales *sensu lato* (HIBBETT & DONOGHUE 1995, HIBBETT et al. 1997, HIBBETT, GILBERT & DONOGHUE 2000). However, the integrity of *Russula* and *Lactarius* within their long-established agaricoid bounds has been questioned by results from DNA sequence analyses (MARTÍN, HÖGBERG & LLISTOSELLA 1999, CALONGE & MARTÍN 2000, HENKEL, AIME & MILLER 2000, MILLER et al. 2001). These results suggest that secotioid, gasteroid or pleurotoid species might have arisen several times independently within *Russula* and *Lactarius*.

In spite of the diminishing distinction between the two genera (BUYCK 1991a, 1993, VERBEKEN 1997), *Russula* and *Lactarius* are usually classified independently. At present, only one character separates *Russula* from *Lactarius*: In the latter genus, the lactiferous system is developed more distinctly. *Lactarius* sporocarps exude milk when broken. BUYCK (1991a, 1993) observed that, in contrast to *Russula*, the lactiferous system of *Lactarius* is branched in the lamellar trama and the tips of the lactifers extend into the hymenium where they form pseudocystidia. It remains questionable as to whether this is a qualitative or a quantitative character. Its separating strength has been further diminished by an observation, cited in BUYCK & HORAK (1999a), of sporocarps formed by the same mycelium some of which showed pseudocystidia while others did not. To this may be added that, in contrast to *Russula*, the mycorrhizae of *Lactarius* species possess lactifers, usually located in the middle to inner mantle layer and of generally greater diameter than the surrounding hyphae. Though mycorrhizae of *Russula* species are not devoid of latex containing cells, lactifers have so far never been encountered in them (AGERER 1995, 1999b, EBERHARDT 2000).

The European species of *Russula* and *Lactarius* are generally considered to have been thoroughly investigated. Although largely agreeing on the composition of many systematic groups, the more recent attempts at their classification (e.g. ROMAGNESI 1967, 1985, 1987, SINGER 1986, BON 1988, SARNARI 1998 for *Russula*; HESLER & SMITH 1979, BON 1980, 1983, HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT 1998 for *Lactarius*) show quite distinct differences concerning underlying evolutionary assumptions, the transitions between different infrageneric groupings, and the placement of certain species.

The core of ROMAGNESI's classification of the genus *Russula* (ROMAGNESI 1967, 1985, 1987) and of that of his successors (e.g. BON 1988, SARNARI 1998, but not SINGER 1986) is the division of the genus *Russula* into (a) the subg. (subgenus) *Compacta* including the sections *Nigricantinae* Bataille, *Archaeinae* R.Heim ex Romagn., and *Plorantinae* Bataille,

and (b) the "Genuinae"[2] encompassing the (according to ROMAGNESI 1967, 1985, 1987) remaining eight subgenera. The species of the subg. *Compacta* are united by their stature (compact, with cap rim rolled in, and lamellae regularly interspersed by lamellulae). These characters are considered primitive within the genus (SCHAEFFER 1935, ROMAGNESI 1967, 1985, SARNARI 1998). The classification of the eight remaining subgenera is dependent on the colour of the spore print, taste, the presence and shape of specialised cells in the pileipellis (termed dermatocystidia and encrusted primordial hyphae or fuchsinophile hyphae), localisation of pigments at the cellular level, micro- and macro-chemical reactions, discoloration of the broken flesh, and pileus colours. Differences in the systems of ROMAGNESI (1967, 1985, 1987), BON (1988), and SARNARI (1998) are largely due to the differing degrees of significance they attach to each of the above characters. By contrast, in SINGER's (1986) system, macroscopic features (pileipellis, rim of cap, lamellae, colour of spore print, discoloration of flesh, colour reactions with certain chemicals) take first priority. According to SINGER (1986), the sections of the subg. *Compacta* sensu ROMAGNESI form independent taxa; the remaining species are distributed over four sections (sections form the highest subgeneric taxonomic units in SINGER 1986) that are not easily described in terms of ROMAGNESI's classification.

Tab. 2 lists published descriptions of *Russula* species mycorrhizae. The surface of the mycorrhizal mantles of *Russula* species of the sections *Plorantinae*, *Nigricantinae*, and *Foetentinae* Melzer & Zvára and of the subg. *Heterophyllidia* is more or less densely covered with cystidia. The cystidia are in many species fusiform to bottle-shaped with apical knobs (0-1(2)) that tend to break off, leaving an apical opening. The contents of the cystidia react positively with sulfovanillin in many species. In the mycorrhizae of the subg. *Heterophyllidia*, a second type of cystidia was observed without sulfovanillin-reactive contents. Most of these cystidia were described as awl-shaped, in *R. vesca* as branched to form star-like structures, and in *R. cyanoxantha* as formed like hyphal ends. The identified mycorrhizae of the *Russula* species of the remaining subgenera, *Russula*, *Incrustatula*, *Tenellula*, *Polychromidia*, as well as *Insidiosula* and of the sect. (section) *Felleinae* Melzer & Zvára, are acystidiate. This listing includes *R. ochroleuca* and *R. fellea* that were classified alongside the sect. *Foetentinae* in the subg. *Ingratula* by ROMAGNESI (1967). The outer mantles of these species are pseudoparenchymatous with either angular (many species of the subg. *Russula, R. ochroleuca*, and *R. fellea*) or irregularly shaped, often interlocking cells (for the remaining species). For a more concise summary of mycorrhizal features of *Russula* species, see EBERHARDT (2000) and BEENKEN (2001b, 2001e, 2001k, 2001l). Descriptions of mycorrhizae of species from the subg. *Coccinula* have not been published; however, Beenken (1999, abstr.) reported that *R. decolorans* and additional species of other subgenera correspond in their mycorrhizal structures to the summary given above. Accounts of cystidia on the mycorrhizae of *R. violascens* or *R. xerampelina* can be considered as doubtful. The overwhelming majority of *Lactarius* mycorrhizae are acystidiate, possibly with the exception of *L. panuoides* Singer (HENKEL, AIME & MILLER 2000, EBERHARDT 2000).

In addition to mantle features, BEENKEN (2001b) analysed characters of rhizomorphs of the genus *Russula*. AGERER (1999a) defined a russuloid type of rhizomorph, distinguished by its appression to a substrate, often to thicker roots, and differentiated into a peripheral layer with or without cystidia and a central layer with vessel-like hyphae. Many species frequently form septate hyphae, termed ladder-like hyphae by BEENKEN (2001b), in the central layer alongside with vessel-like hyphae. The rhizomorphs of most species of *Russula*, subgenera *Compacta* and *Heterophyllidia,* and of the sect. *Foetentinae* display cystidia while the rhizomorphs of *Russula* species with acystidiate mantles do not. According to BEENKEN (2001b), the septae of the ladder-like hyphae of species of the subgenera *Compacta* and *Heterophyllidia* are arched, without visible pores and regularly spaced. In contrast, the septae of the ladder-like hyphae of the rhizomorphs of *R. alnetorum* (subg. *Russula*) and of *R. vinosa* (subg. *Incrustatula*) are straight, dissolved or with enlarged pores, and occur in clusters. Ladder-like hyphae have not so far been encountered in the rhizomorphs of the sect. *Foetentinae*.

Materials and methods

Specimens investigated are listed in Tab. 3. Most of the specimens, sporocarps and mycorrhizae of the genera *Russula* and *Lactarius*, were specifically collected for this study, principally in southwest Germany. Additional sporocarp material was contributed by M. Guttenberger, I. Kottke, F. Oberwinkler (all Tübingen, Germany), N. Luschka (Schwäbisch Gmünd, Germany), and U. Sittig (Göttingen, Germany). Voucher specimens are deposited at TUB.

Species identification of sporocarps was carried out with ROMAGNESI (1967), supplemented by EINHELLINGER (1985, 1990) and GRÖGER (1996), or with HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT (1998), supplemented by SCHWÖBEL (1979) and BON (1980). Species and authority names follow HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT (1998) or NOORDELOOS & KUYPER (1999), supplemented by WALLEYN, VERBEKEN & NOORDELOOS (1996) and EINHELLINGER (1990).

[2] Originally, ROMAGNESI's (1967) classification used varying numbers of hierarchical levels in different groups and did not apply the taxonomic system of subgenus, section, subsection etc. ROMAGNESI (1967, 1985) defined a sister group of the *Compactae (*termed subg. *Compacta* by ROMAGNESI 1987), called *Russula* (also referred to as Genuinae) and comprising eight sub-groups that later became subgenera (ROMAGNESI 1987). In the publication of 1987, this super-subgeneric level was dropped. For the sake of simplicity, the term „Genuinae" is used here in lieu of *Russula*. For authority names of subgenera see Tab. 1.

Tab. 2. Descriptions of *Russula* spp. mycorrhizae. Classification follows ROMAGNESI (1985, 1987). *R.* = *Russula*. * Mycorrhizae not described in detail. ** Identification of mycorrhizae not reliable (mycorrhizae assumed to belong to sporocarps of the given species growing in the vicinity)

Species and classification	Authors
Subgenus *Compacta*	
R. acrifolia Romagn.	AGERER, FRANZ & ACKER 1994
R. brevipes Peck	KERNAGHAN, CURRAH & BAYER 1997
R. chloroides Krombh.	Eberhardt, unpublished results
R. delica Fr.	YAMADA 1998a, YAMADA & KATSUYA 1996, BEENKEN 2001a
R. densifolia Secr.	CERUTI, BENVENUTI & LUPPI MOSCA 1988, EBERHART & LUOMA 2000, BEENKEN 2001b, 2001c
R. fuegiana Singer	PALFNER & GODOY 1996
R. nigricans Fr.	YAMADA 1998b, YAMADA & KATSUYA 1995, YAMADA & KATSUYA 1996
Subgenus *Heterophyllidia*	
R. aeruginea Lindbl. ap. Fr.	TAYLOR & ALEXANDER 1989, BEENKEN 2001d
R. atroglauca Einhellinger	BEENKEN 2001e
R. cyanoxantha Bull. ex Fr.	BEENKEN 2001f
R. grisea Gill.	CERUTI & BUSSETTI 1962**
R. mariae Peck	YAMADA & KATSUYA 1995
R. medullata Romagn.	BEENKEN 2001g
R. vesca Fr.	BEENKEN 2001h
R. virescens Schaeff. ex Fr.	BEENKEN 2001i
Subgenus *Ingratula*	
R. amoenolens Romagn.	JAKUCS & BEENKEN 1999
R. fellea Fr.	BRAND & AGERER 1987 (as Fagirhiza granulosa), BRAND 1991 (identification)
R. foetens Pers. ex Fr.	BRAND 1991*,**, BEENKEN 2001j
R. illota Romagn.	BRAND 1991
Subgenus *Russula*	
R. alnetorum Romagn.	BEENKEN 2001k
R. emetica Bull. ex Fr.	BRAND 1991*
R. exalbicans (Pers.) Melzer & Zvára	Beenken 1997, abstr.*
R. fragilis Pers. ex Fr.	MELIN 1924
R. gracillima J. Schaeff.	Beenken 1997, abstr.*
R. mairei Singer	BRAND 1991
R. nana Killerm.	BRAND 1991*
R. pumila Rouz. & Mass.	PRITSCH, BUSCOT & MUNCH 1997
R. sanguinea Bull. ex Fr.	DUÑABEITIA et al. 1996
R. silvicola Shaffer	KERNAGHAN, CURRAH & BAYER 1997
R. solaris Ferd. & Winge	Eberhardt, unpublished results
R. violascens (Secr.) Sacc.	LUPPI & GAUTERO 1967**
Subgenus *Incrustatula*	
R. ochroleuca (H.C.Hall) Pers.	AGERER 1986, HAUG 1987, GRONBACH 1988, BERG 1989, PILLUKAT & AGERER 1992
R. vinosa Lindblad.	BEENKEN 2001l
Subgenus *Tenellula*	
R. laricina Velen.	TREU 1990
R. versicolor J. Schaeff.	BEENKEN 2001m
Subgenus *Polychromidia*	
R. xerampelina Fr.	AGERER 1986
R. integra L. ex Fr.	KERNAGHAN 2001
Subgenus *Insidiosula*	
R. firmula J. Schaeff.	TREU 1990
R. veternosa Fr.	Eberhardt, unpublished results
Not classified	
R. nothofaginea Singer	BEENKEN 2001n

The identification of many sporocarps was verified by A. Verbeken (*Lactarius*) and R. Walleyn (*Russula*) (both Gent, Belgium).

Identification of mycorrhizae was achieved by comparing ITS DNA sequences (for *Lactarius camphoratus*: LSU DNA sequences) of sporocarps and mycorrhizae. Sequence variation between sporocarps and mycorrhizae that I assumed to be conspecific amounted to no more than 2 bp of 500-600 bp (for *L. camphoratus*, 0 bp of 607 bp), including ambiguously read base pairs in at least one of the sequences. In addition, macroscopic and microscopic features of the mycorrhizae were compared to published descriptions, if available (EBERHARDT 2000). For each species, the sequence of the greatest length and with the least number of ambiguous basepair callings was chosen for phylogenetic analysis, notwithstanding its source.

Two collections of mycorrhizae (*Russula* sp. mycorrhizal type 1 and 2) could not be identified. Their macroscopic and microscopic features corresponded to mycorrhizae of *Russula* species from the "Genuinae" group excluding the sect. *Foetentinae* and the subg. *Heterophyllidia* (classification according to ROMAGNESI 1967, 1985, 1987). The mycorrhizae displayed translucent, smooth mantles lacking cystidia, with few and inconspicuous emanating hyphae; the outer mantle was formed by an irregular pseudoparenchyma of interlocking cells, some of which displayed needle-like aggregated contents (EBERHARDT 2000).

DNA was extracted from dried and frozen (-20 °C) sporocarp fragments (circa 1 mm^3) and from frozen mycorrhizal tips (1-3 tips from the same system). Isolation of DNA was carried out as given in EBERHARDT, WALTER & KOTTKE (1999) and EBERHARDT et al. (2000). PCR and sequencing primers used were NL1 and NL4 (O'DONNELL 1992) for the LSU and ITS1 or ITS1F and ITS4 (WHITE et al. 1990, GARDES & BRUNS 1993) for the ITS region. PCR and sequencing were performed as in EBERHARDT, WALTER & KOTTKE (1999) or EBERHARDT et al. (2000).

Sequences were aligned using the clustal algorithm of Megalign from the Lasergene Package (version 3.08, DNASTAR, Madison, Wisconsin) and edited by hand using the sequence editor Se-Al (version 1.0a1, RAMBAUT 1996). The alignments are available from TreeBase (acc.no. M1115-M1117). Phylogenetic analyses were conducted using PAUP* (version 4.0b2, SWOFFORD 1999; version 4.0b8, SWOFFORD 2001 for Shimodaira Hasegawa tests). Unweighted maximum parsimony (MP; FITCH 1971), maximum likelihood (ML; FELSENSTEIN 1981) and neighbor joining (NJ; SAITOU & NEI 1987) analyses were carried out. Gaps were always considered as missing data. ML and MP analyses were performed as heuristic searches from starting trees obtained by random addition of species, with as many replicates as computing time allowed. When using parameter rich substitution models, the number of replicates and the intensity of the search was often severely restricted. Therefore, additional searches with simpler models and more replicates were performed (refer to the results section for details; explanation of technical terms is provided by SWOFFORD et al. 1996). Maximum likelihood scores of a neighbour joining tree were calculated to evaluate the fit of increasingly complex substitution models, variants of the general time reversible model (GTR; LANAVE et al. 1984, TAVARÉ 1986, RODRÍGUEZ et al. 1990), via log-likelihood ratio tests (GOLDMAN 1993, see also MONCALVO et al. 2000). Bootstrap support (FELSENSTEIN 1985) was calculated for each topology. In ML and MP bootstrap analyses, one replicate of heuristic search was calculated for each bootstrap replicate. In addition, simpler substitution models were applied in some of the ML and NJ analyses, the Jukes Cantor model (JC; JUKES & CANTOR 1969), the Kimura two parameter model (K2P; KIMURA 1980), and the Hasegawa Kishino Yano model (HKY85; HASEGAWA, KISHINO & YANO 1985). Likelihood scores of different tree topologies were manually compared in one-tailed Shimodaira Hasegawa tests ($P < 0.05$) using RELL bootstrap with 1000 replicates (SHIMODAIRA & HASEGAWA 1999, GOLDMAN, ANDERSON & RODRIGO 2000) under the model with the best fit for the respective align-ment. Constraint topologies were constructed using MacClade (version 3.05, MADDISON & MADDISON 1992).

Results

Analysis of LSU sequences

The sequences of the 5'-terminus of the nuclear LSU (approx. 620-650 bp) from 63 isolates of 61 *Russula* and *Lactarius* species were aligned. The isolate labelled *Lactarius* sp. was from a morphologically unidentifiable specimen with an ITS sequence identical to that of *L. lacunarum* (EBERHARDT 2000). Six sequences were taken from GenBank (see Tab. 3).

A number of sequences of additional species of *Russula* and *Lactarius* was published by T. W. Henkel, M. C. Aime and S. L. Miller and by S. L. Miller, B. Buyck, J. W. Walker and R. Vilgalys after the completion of the phylogenetic analysis presented here. By including these sequences in provisional phylogenetic analyses (unpublished), the results presented here were confirmed.

Heterobasidion annosum (Fr.) Bref. and *Tylospora asterophora* (Bon.) Donk, together or separately, were considered as potential outgroup species. Neither species belonged to the ingroup (E. Larsson, personal communication), but their sequences were still fairly similar to the ingroup sequences. *T. asterophora* was eventually chosen as outgroup because, though its evolutionary distance to the Russulaceae was slightly higher than that of *H. annosum*, the resolution of the phylogenetic results was clearer with *T. asterophora*. This was most obvious in the MP results, where the number of most parsimonious trees was many times smaller than with *H. annosum* as outgroup (unpublished results).

The alignment contained 675 positions, including primer sequences. Of these, 93 bp were removed to account for sequences with a short reading span. On the whole, the sequences were fairly well alignable. Positions where alignment was

Tab. 3. List of investigated specimens and sequences. Acc. no. = GenBank accession number. * sequence obtained from GenBank. S = sporocarp, M = mycorrhizae.

Species	Voucher	Source	Acc. no. LSU	Voucher	Source	Acc. no. ITS
Lactarius badiosanguineus Kühner & Romagn.	fo46862	S	AF325268			
Lactarius blennius (Fr.: Fr.) Fr.	ue98	M	AF325269			
Lactarius camphoratus (Bull.: Fr.) Fr.	ue140	M	AF325270			
Lactarius circellatus Fr.	fo46812	S	AF325271			
Lactarius corrugis Peck*		S	U11919			
Lactarius deterrimus Gröger	lw104	S	AF325272			
Lactarius fuliginosus (Fr.: Fr.) Fr.	fo46889	S	AF325273			
Lactarius helvus (Fr.: Fr.) Fr.	lw78	S	AF325274			
Lactarius lignyotus Fr.	lw98	S	AF325275			
Lactarius lilacinus (Lasch.: Fr.) Fr.	fo46891	S	AF325276			
Lactarius necator (Bull.: Fr.) Pers.	hue205	S	AF325277			
Lactarius pallidus Pers.: Fr.,	ue134	M	AF325278			
Lactarius piperatus (L.: Fr.) Pers.	hue93	S	AF325279			
Lactarius piperatus (L.: Fr.) Pers.*		S	AF042573			
Lactarius porninis Rolland	fo46805	S	AF325280			
Lactarius pubescens Fr.	hue135	S	AF325281			
Lactarius quieticolor Romagn.	hue141	S	AF325282			
Lactarius ruginosus Romagn.	hue95	S	AF325283			
Lactarius salmonicolor R.Heim & Leclair	fo46879	S	AF325284			
Lactarius scrobiculatus (Scop.: Fr.) Fr.	fo46774	S	AF325285			
Lactarius sp.	fo46778	M	AF325286			
Lactarius sphagneti (Fr.) Neuhoff	lw83	S	AF325287			
Lactarius spinosulus Quél.	nl1486	S	AF325288			
Lactarius subdulcis (Pers.: Fr.) Gray	hue33	S	AF325289			
Lactarius subsericatus (Kühner & Romagn.) ex Bon	fo46777	S	AF325290			
Lactarius tabidus Fr.	fo46676	S	AF325291			
Lactarius torminosus (Schaeff. ex Fr.) Pers.	hue201	S	AF325292			
Lactarius uvidus (Fr.: Fr.) Fr.	mh0963	S	AF325293			
Lactarius vellereus (Fr.: Fr.) Fr.	hue57	S	AF325294			
Lactarius volemus (Fr.: Fr.) Fr*.		S	AF042574			
Russula aeruginea Lindblad ap. Fr.				nl1292	S	AF418612
Russula amethystina Quél.				hue215	S	AF418640
Russula amoenolens Romagn.	nl27.9.95.6	S	AF325295	nl27.9.95.6	S	AF418615
Russula atropurpurea Krombh.	hue178	S	AF325296	hue178	S	AF418618
Russula caerulea Fr. ss Cooke	hue146	S	AF325297	hue146	S	AF418633
Russula cavipes Britzelm. ss. Heim	hue163	S	AF325298	hue163	S	AF418623
Russula chloroides Krombh.	ue68	M	AF325300	ue68	M	AF418604
Russula compacta ˝Frost*		S	AF287888			
Russula cyanoxantha Bull. ex Fr.	hue34	S	AF325301	ue92	M	AF418608
Russula decolorans Fr.	hue39	S	AF325302	hue39	S	AF418637
Russula delica Fr.	hue22	S	AF325303	hue22	S	AF418605
Russula densifolia Secr.	hue105	S	AF325304	ue116	M	AF418606
Russula earlei Peck*		S	AF042571		S	
Russula emetica Bull. ex Fr.	lw81	S	AF325305	lw81	S	AF418619
Russula exalbicans Secr. ss. Melzer-Zvára	nl79/93	S	AF325306	nl79/93	S	AF418622
Russula fellea Fr	ue114	M	AF325307	hue177	S	AF418616
Russula firmula J. Schaeff.	hue184	S	AF325308	hue173	S	AF418631
Russula foetens Pers. ex Fr.	hue124	S	AF325299	hue124	S	AF418613
Russula fuscorubroides Bon		S		hue168	S	AF418624
Russula heterophylla Fr.	hue103	S	AF325309	hue103	S	AF418609
Russula integra L. ex Fr. ss. Maire		S		nl1346	S	AF418636
Russula laurocerasi Melzer		S		nl1348	S	AF418614
Russula lepida Fr.	hue208	S	AF325310	hue208	S	AF418641
Russula mairei Singer	hue54	S	AF325311	lw113	S	AF418620
Russula mykorrhizal type ue73	ue73	M	AF325322	ue73	M	AF418639
Russula mykorrhizal type ue53				ue53	M	AF418629
Russula nigricans Fr.	fo46761	S	AF325312	fo46792	S	AF418607
Russula ochroleuca (H.C.Hall.) Pers.	ue39	M	AF325313	ue39	M	AF418617
Russula olivacea Fr.				hue138	S	AF418635
Russula olivacea Fr.	hue85	S	AF325314	hue85	S	AF418634
Russula parazurea J. Schaeff.				nl1370	S	AF418611
Russula puellaris Fr.	hue83	S	AF325315	nl1372	S	AF418628
Russula queletii Fr.	fo46861	S	AF325316	hue183	S	AF418625
Russula raoultii Quél.	hue94	S	AF325317	hue94	S	AF418621

Tab. 3. continued

Species	Voucher	Source	Acc. no. LSU	Voucher	Source	Acc. no. ITS
Russula sardonia Fr. ss. Melzer & Zvára	hue155	S	AF325318	hue41	S	AF418626
Russula solaris Ferd. & Winge	hue219	S	AF325319	hue219	S	AF418627
Russula vesca Fr.	fo46762	S	AF325320	hue122	S	AF418610
Russula veternosa Fr.	ue137	M	AF325321	hue212	S	AF418630
Russula vinosa Lindblad		S		nl1386	S	AF418638
Russula virescens Fr.*		S	AF041548			
Russula xerampelina Fr.				fo46888	S	AF418632
Tylospora asterophora (Bon.) Donk	lw79	M	AF325323			

slightly doubtful remained in the analysis because the affinity of related sequences was often based on these sequence stretches. When removing these positions, the number of optimal or almost optimal solutions increased considerably, leading to a considerable reduction in resolution of the consensus. As this paper is centred around the detection of valid sub-groups of the Russulaceae rather than on a valid reconstruction of phylogenetic relationships between these groups, the inclusion of all remaining 582 positions of the alignment was considered justified. Approximately two thirds of the alignment (391 positions) was constant.

Maximum likelihood (ML)

For the above alignment, tests of model fit (P = 0.05) indicated that the most adequate variant of the general time reversible substitution model differentiated between three classes of substitutions, estimated base frequencies, substitution rate matrix and the number of invariable sites from the data, and assumed that substitution rates across different positions of the alignment to follow a discrete gamma distribution with shape parameter $\alpha = 0.5$ (GTR3+G+I model). Conducting ML analysis under the above GTR3+G+I substitution model (setting the parameters according to previous assumptions), the number of replicates was severely restricted. Thirteen replicates were completed with NNI (nearest neighbour interchange) branch swapping and random addition of sequences, resulting in 16 solutions of differing log-likelihoods. Owing to the small number of replicates and the necessary omission of TBR branch swapping, it was not possible to confirm the best result by repetition.

A ML analysis under the simpler K2P model was performed with NNI branch swapping in 78 replicates and random addition of sequences. All resulting 90 trees were saved. The best trees from this analysis, two trees from the MP analysis, and two trees from NJ analysis under different substitution models were entered into NNI branch swapping under the ML criterion and the GTR3+G+I model with the above settings. The 112 resulting trees of all of the different ML analysis schemes were evaluated using Shimodaira Hasegawa tests. One of the trees calculated under the above GTR3+G+I model scored highest and is shown in Fig. 1. None of the solutions from ML analysis was significantly ($P < 0.05$) worse than the best solution. Bootstrap analysis was performed as fast bootstrap analysis with 1000 replicates under the K2P model.

Maximum parsimony (MP)

Of the 191 variable positions in the alignment, 121 were parsimony-informative. MP analysis was performed as a heuristic search with 100 replicates, random addition of sequences, and TBR (tree bisection reconnection) branch swapping on no more than 1000 trees of length greater than 636 steps per replicate. These restrictions in tree number and length had to be used in order to reduce computation time because unrestricted calculations gave rise to ten thousands of sub-optimal trees in single replicates. Twenty-four replicates resulted in a tree-island of 207 trees of 635 steps length. The strict consensus is shown in Fig. 2. This consensus was used as a topological constraint for further searches to find out whether additional solutions existed that were not represented by the consensus. Additional searches were performed, with 100 to 1000 replicates, with and without this topological constraint, with the number of saved trees undergoing branch swapping limited to one, 100, or 500 trees per replicate. No other tree-islands of 635 steps or less were found. Bootstrap analysis was performed with 1000 replicates, random addition of sequences, TBR branch swapping, and the "MulTrees" option switched off.

Neighbor joining (NJ)

The results from NJ analyses under different substitution models were calculated. The resulting trees differed slightly in the internal topologies of the clusters I-V. The NJ results were compared in Shimodaira Hasegawa tests under the ML criterion and the GTR3+G+I model with the above settings. None of the trees were significantly better than any other ($P < 0.05$), but all were significantly worse than the best ML tree. The NJ tree shown in Fig. 3 was calculated under the JC model. Bootstrap analysis was performed with 1000 replicates.

Testing phylogenetic hypotheses

The topologies shown in Figs. 1-3 represent to some extent contradictory hypotheses of the phylogeny of the Russulaceae.

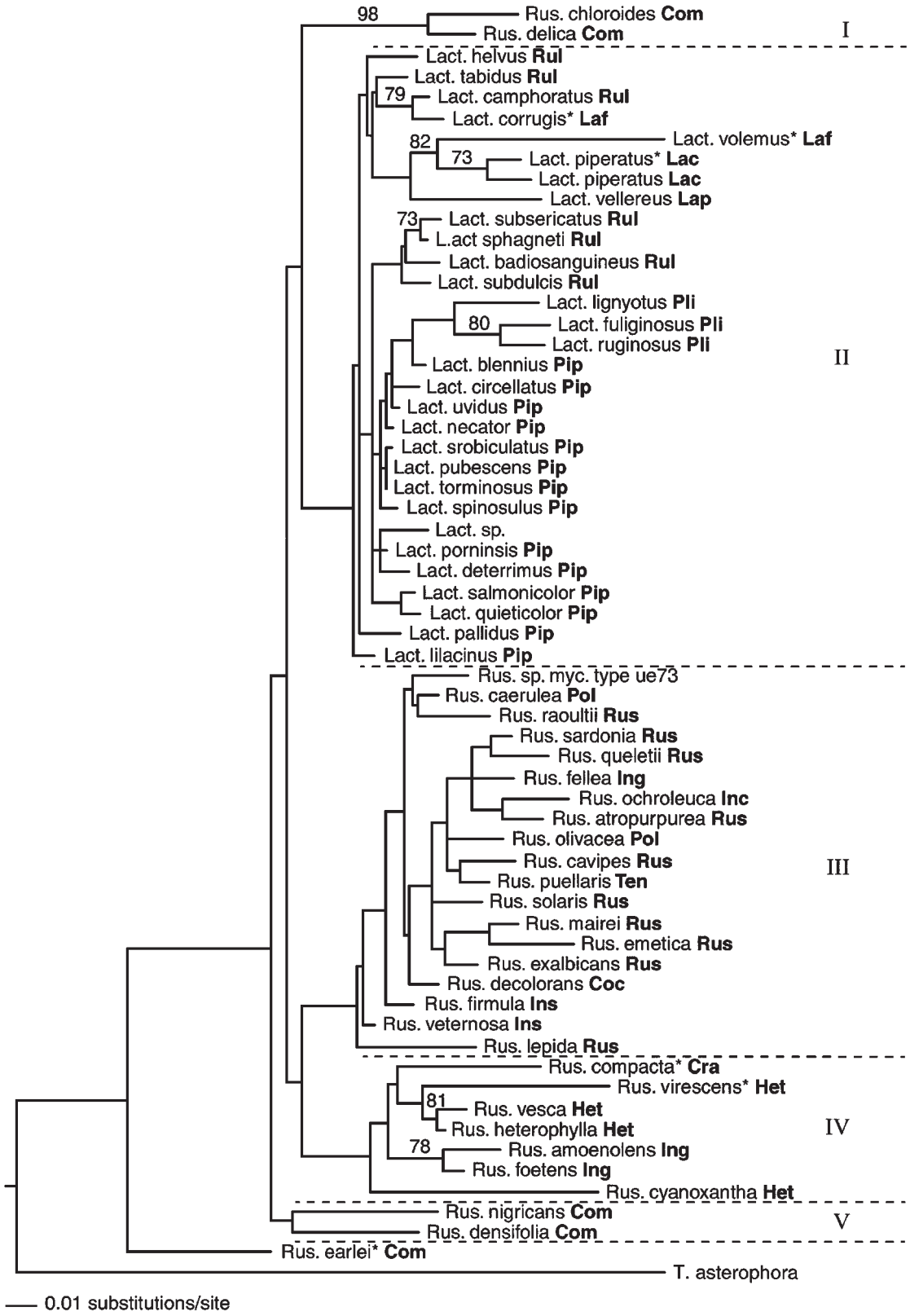


Fig. 1. Maximum likelihood topology calculated under a GTR3+I+G substitution model (for details, see text) from LSU DNA sequence data. The topology is rooted with *Tylospora asterophora*. Only bootstrap values ≥ 70 % are given. Lact. – *Lactarius*, Rus. – *Russula*. * indicates sequences obtained from GenBank. The identity of the *Lactarius corrugis* sequences and some other GenBank sequences is considered in the discussion. I *Russula*, subg. *Compacta*, sect. *Plorantinae*, II *Lactarius*, IV *Russula*, subg. *Heterophyllidia* and *Russula,* sect. *Foetentinae*, and *R. compacta* (sect. *Crassitunicatae*), V *Russula*, subg. *Compacta*, sect. *Nigricantinae*, III *Russula*, remaining "Genuinae" (subgenera *Coccinula*, *Incrustatula*, *Insidiosula*, *Russula*, *Polychromidia*, *Tenellula*, and *Ingratula*, and section *Felleinae*). For each species, the abbreviation in bold refers to its classification to subgenus level, as explained in Tab. 1.

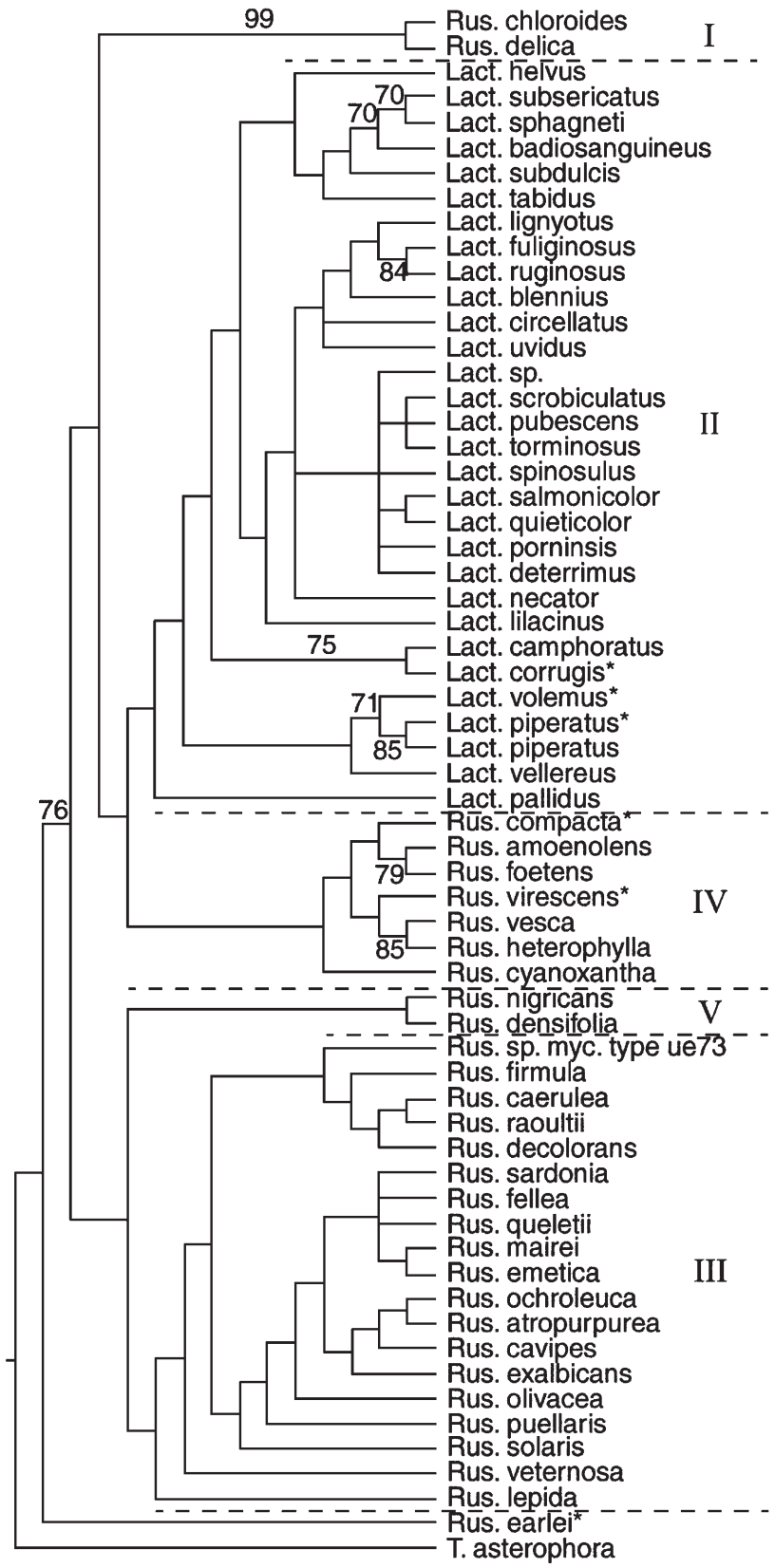


Fig. 2. Strict consensus of the 207 most parsimonious trees, calculated from the LSU DNA sequence data. The topology is rooted with *Tylospora asterophora*. Only bootstrap values ≥ 70 % are given. Lact. – *Lactarius*, Rus. – *Russula*. * indicates sequences obtained from GenBank. The identity of the *Lactarius corrugis* sequences and some other GenBank sequences is considered in the discussion. I *Russula*, subg. *Compacta*, sect. *Plorantinae*, II *Lactarius*, IV *Russula*, subg. *Heterophyllidia* and *Russula,* sect. *Foetentinae*, and *R. compacta* (sect. *Crassitunicatae*), V *Russula*, subg. *Compacta*, sect. *Nigricantinae*, III *Russula*, remaining "Genuinae" (subgenera *Coccinula*, *Incrustatula*, *Insidiosula*, *Russula*, *Polychromidia*, *Tenellula*, and *Ingratula*, and section *Felleinae*).

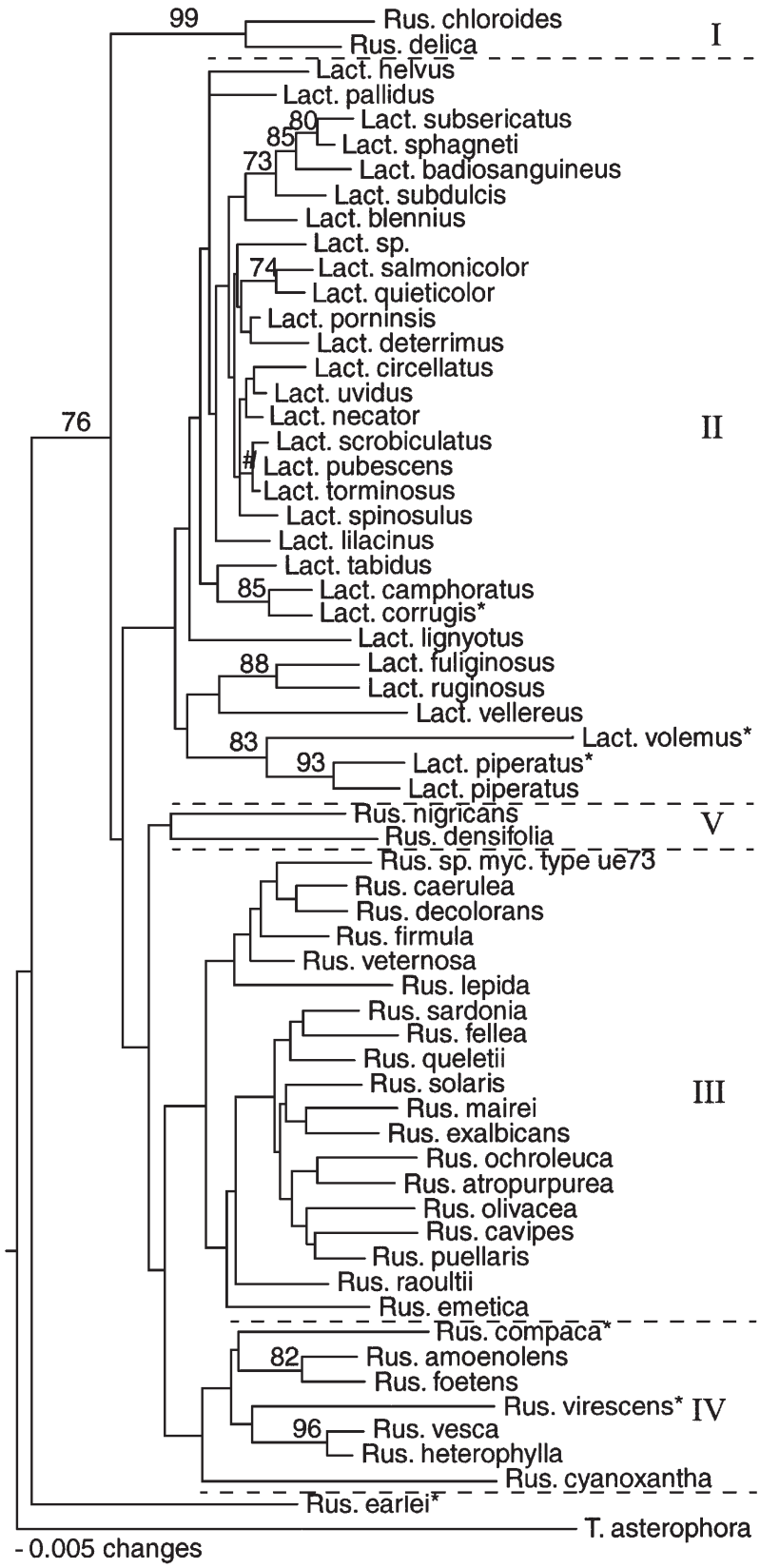


Fig. 3. Neighbor joining topology calculated under the JC substitution model from LSU DNA sequence data. The topology is rooted with *Tylospora asterophora*. Only bootstrap values ≥ 70 % are given. Lact. – *Lactarius*, Rus. – *Russula*. * indicates sequences obtained from GenBank. The identity of the *Lactarius corrugis* sequences and some other GenBank sequences is considered in the discussion. I *Russula*, subg. *Compacta*, sect. *Plorantinae*, II *Lactarius*, IV *Russula*, subg. *Heterophyllidia* and *Russula,* sect. *Foetentinae*, and *R. compacta* (sect. *Crassitunicatae*), V *Russula*, subg. *Compacta*, sect. *Nigricantinae*, III *Russula*, remaining "Genuinae" (subgenera *Coccinula*, *Incrustatula*, *Insidiosula*, *Russula*, *Polychromidia*, *Tenellula*, and *Ingratula*, and section *Felleinae*).

Therefore, trees depicting different phylogenetic hypotheses were compared with the optimal results of the ML analysis to find out whether specific hypotheses relating to aspects of the phylogeny of the Russulaceae could be rejected. For this purpose, trees were constructed representing a given phylogenetic hypothesis (e.g. *Russula* and *Lactarius* are separate monophyletic groups). These trees were used as topological constraints. Simplified MP analyses were performed (1000 replicates, random addition of sequences, using TBR branch swapping, but with the MulTrees-option off). One of the most parsimonious trees was entered into ML analysis with NNI branch swapping. If necessary, further, more extensive MP analyses were performed (e.g. more replicates with TBR branch swapping on higher numbers of saved trees). In all procedures, only those solutions were accepted that were consistent with the topological constraint. The aim was not necessarily to find the best tree according to the respective criterion and constraint but to see whether, under the given constraint, results were to be found that were not significantly worse in comparison to the unconstraint best tree in Shimodaira Hasegawa tests ($P < 0.05$).

Many hypotheses concerning the relationship of clusters I-V were tested. Topologies in which clusters I-V remained intact could not be rejected in comparison to the best ML solution. Those topologies generally corresponding to the NJ trees (in the proposed phylogeny of clusters I-V and *R. earlei* at the base, not considering the internal topology of clusters I-V) were not necessarily significantly worse. Topologies could not be rejected in which *Russula* (including *R. earlei*) and *Lactarius* were separate monophyletic groups. In addition, topologies could be found that were not significantly worse in which *Lactarius* was depicted paraphyletic, either by separation of a clade containing *L. volemus*, *L. piperatus* and *L. vellereus* with or without the subg. *Plinthogalus,* or by splitting up the subg. *Piperites* (classification sensu HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT 1998). However, I failed to find a topology depicting *Russula*, subg. *Ingratula*, in which the sections *Foetentinae* and *Felleinae* were on the same branch. With subg. *Ingratula* intact, the best solution found was significantly worse than the best ML topology.

Analyses of *Russula* ITS sequences

Sequence variation in the internal transcribed spacers (ITS1 and ITS2) of the ribosomal RNA genes was so great among the Russulaceae that alignment of all sequences was not possible without removing long sequence stretches. In contrast, the coding regions included in the sequenced DNA fragment were very similar. Even within *Russula*, ITS sequence variation was very high. However, the sequences could be allocated to four groups within *Russula* that aligned reasonably well: a) sect. *Plorantinae*, b) sect. *Nigricantinae*, c) subg. *Heterophyllidia* including sect. *Foetentinae* and d) the remaining species of six subgenera including the sect. *Felleinae*. Even within these groups, sequence alignment was partially hampered by the variability of some stretches within the spacer regions. Removal of the variable regions diminished severely the resolution of the analyses. Several independent attempts to crop the alignment resulted in varying, badly resolved topologies. The main problem arose from the difficulty in defining criteria concerning which positions to exclude from the alignment. In the end, all sites were included because topologies derived from different alignments including all positions did not vary as distinctly as topologies derived from alignments with differing exclusion sets. Only the frayed ends of the alignments were removed along with a 250 bp insert in the ITS1 of the *R. olivacea* sequences. Likewise, owing to high sequence variation, the inclusion of outgroup species in the alignment was impossible. Consequently, the ITS topologies were not rooted.

Russula, subg. *Compacta*, subg. *Heterophyllidia* and sect. *Foetentinae*

As the data set did not contain many sequences of *Russula*, subg. *Compacta*, subg. *Heterophyllidia* and sect. *Foetentinae*, a combined alignment was created, inserting gaps (treated as missing data in all analyses) where an alignment among sequence stretches of different groups was impossible (see HERSHKOWITZ & LEIPE 1998). The alignment contained sequences of 12 species and spanned 830 bp, of which 740 bp were entered in the analyses. As an appropriate substitution model, a GTR3+G model (one class of transversions, two classes of transitions) was determined by log-likelihood ratio tests (base frequencies and the substitution rate matrix estimated from the data, substitution rates across the alignment assumed to follow a gamma distribution with shape parameter $\alpha = 0.5$).

The alignment included 232 variable sites, of which 130 sites were parsimony informative. All of 100 replicates from a MP analysis (random addition of sequences, TBR branch swapping) gave the same topology (Fig. 4), as did a ML analysis under the HKY85 substitution model (100 replicates, TBR branch swapping). The log-likelihood of this topology was almost the same as the log-likelihood of another topology (not shown) resulting from a ML analysis under the above GTR3+G model (10 replicates, NNI branch swapping) and from NJ analyses under the JC and HKY model. In the topology of Fig. 4, the branch of *R. cyanoxantha* is inserted basal to species of *Russula*, sect. *Foetentinae*. In this second topology, the branch of *R. cyanoxantha* is inserted basal to the other species of *Russula*, subg. *Heterophyllidia*. None of the variants was supported by bootstrap values ≥ 50 %.

Russula, remaining "Genuinae"

The remaining *Russula* species consisted of 26 sequences of 25 species of the subgenera *Russula*, *Incrustatula*, *Tenellula*, *Polychromidia*, *Coccinula*, *Insidiosula*, and *R. fellea*. This latter species was classified with the sect. *Foetentinae* in the subg. *Ingratula* by ROMAGNESI (1967, 1985, 1987). ITS and LSU sequence analysis show that *R. fellea* is more closely re-

lated to the *Russula* taxa that are united into group III (the remaining "Genuinae") than to the species of the sect. *Foetentinae*. Owing to the great intraspecific variability among *R. olivacea* isolates (EBERHARDT 2000), two sequences of this species were considered. The alignment consisted of 1053 sites of which 310 bp were omitted from the analysis.

ML analysis was performed as heuristic search with 100 replicates and random addition of species under the HKY85 substitution model, using TBR branch swapping. Forty replicates resulted in the topology given in Fig. 5 which had the best likelihood value. Bootstrap analysis was conducted in the fast bootstrap mode with 1000 replicates. MP analysis was performed with 1000 replicates of heuristic search. Of 262 variable sites, 172 sites were parsimony-informative. Two tree-islands with, in total, eight most parsimonious trees were found. Bootstrap analysis was carried out using 1000 replicates, random addition of sequences and TBR branch swapping. NJ analysis was performed under the K2P and the HKY substitution model, leading to identical results. Bootstrapping was again performed using 1000 replicates. The NJ tree and the strict consensus of the most parsimonious trees varied in only minor points from the ML topology shown (Fig. 5). The cluster consisting of species of *Russula*, subg. *Russula* including *R. ochroleuca* and *R. fellea* ("Russula" in Fig. 5) was distinguished by bootstrap values of at least 99 % in all analyses. The inner topology of the "Russula" cluster varied among the best solutions of the different analyses. Only the clade *R. mairei* and *R. emetica* and the cluster *R. queletii*, *R. fuscorubroides*, *R. sardonia*, *R. cavipes*, and *R. exalbicans* were constant in their species composition, though not in topology. In the second cluster of Fig. 5, the positions of *R. xerampelina*, *R. caerulea*, and *R. amethystina* were not constant among methods of analysis or were not resolved by MP. The remaining clades in this cluster correspond across methods with similar bootstrap support.

In log-likelihood ratio tests, best results were obtained by the substitution model with the following parameters: two classes of transitions and two classes of transversions (GTR4+G+I), base frequencies and the number of invariable sites estimated from the data, substitution rates assumed to follow a gamma distribution, and with the shape parameter of the gamma distribution estimated from the data. Under this model, a ML analysis was performed with NNI branch swapping in 10 replicates, resulting in five solutions, including two nearly identical topologies with the best likelihood values. In Shimodaira Hasegawa tests, none of the solutions apart from the NJ trees was significantly worse than these topologies.

Discussion

Identity of the GenBank sequences (LSU) for *R. mairei*, *R. compacta*, and *L. corrugis*

The LSU sequence of *Russula compacta* (Tab. 3) was identical to another *Russula* sp. LSU sequence from GenBank (U89999), named as *R. mairei*. Comparison of my own LSU sequences of *R. mairei* and the GenBank sequence of that species revealed numerous differences, thus suggesting that both sequences cannot stem from the same species. In all analyses carried out (see also HENKEL, AIME & MILLER 2000, MILLER et al. 2001), *R. mairei* (GenBank) clustered with *Russula* species of the subg. *Heterophyllidia*. My own sequences of *R. mairei* are similar to the sequences of *R. emetica* or *R. silvicola* Shaffer (GenBank AF218549) that are both morphologically and anatomically closely related to *R. mairei*. This confirms the suspicion that the sequence U89999 of GenBank is likely to be incorrectly named, and *Russula compacta* is considered the likely source of this sequence.

The *Lactarius corrugis* (subg. *Lactifluus*) sequence from GenBank (Tab. 3) clustered very closely in all analyses with *L. camphoratus* (subg. *Russularia*). This branch was commonly confirmed by fairly high bootstrap values. Yet *Lactarius corrugis* is morphologically similar to *L. volemus* in many respects (HESLER & SMITH 1979), including a number of characters very uncommon in temperate *Lactarius* species, such as its browning context, its compact stature in combination with its orange-brownish colour, the occurrence of thick walled cystidia in the hymenium, and its pileipellis which consists of thick walled cells forming a palisade-like structure. The sequence of *L. volemus* is highly divergent from the majority of *Lactarius* sequences, including those of the *Lactarius* subg. *Russularia* species (note the length of the terminal branch of *L. volemus* in Figs. 1 and 3). Therefore, I consider it unlikely that *L. corrugis* is closely related to *L. camphoratus* and only distantly related to *L. volemus*, as the sequence data would suggest. However, *Lactarius corrugis*, *L. camphoratus* and some allied species share a macroscopic similarity in sporocarp and milk coloration (vinaceous-brown and white/whitish, respectively) and in the low specificity of their association with host trees (HESLER & SMITH 1979). Species distributional areas overlap in North America. The sequence variation between *L. corrugis* and *L. camphoratus* amounts to 8 bp. This is smaller than the sequence variation between the supposedly conspecific isolates of European and American *L. subdulcis* (own data and GenBank AF218552), *L. scrobiculatus* (own data and GenBank AF218558) or *L. piperatus* (own data and GenBank AF218556 and AF042573). Therefore, the sequence assigned to *L. corrugis* is probably also incorrectly named. It probably stems from a species belonging to the *Russularia* group of *Lactarius*. R. Vilgalys (personal communication) confirmed that the name of *L. corrugis* was only later and possibly erroneously attached to the voucher specimen (collection number RV88/61) that was initially collected as *Lactarius*

sp. The GenBank sequences of *L. corrugis* and *R. compacta*/*R. mairei* were included in the analysis to facilitate comparison with other studies.

Relationships among subgroups of the Russulaceae

Three tree-building methods were applied to the LSU data set, resulting in different hypotheses concerning the phylogeny of the agaricoid Russulaceae. However, clusters I-V (Figs. 1-3) were of the same species composition in all three approaches. Following ROMAGNESI's (1967, 1985, 1987) classification of the genus *Russula*, cluster I represents sect. *Plorantinae*, cluster V sect. *Nigricantinae*, cluster IV subg. *Heterophyllidia*, sect. *Foetentinae* and *R. compacta* (a member of the *R. crassitunicata* Singer -group), and cluster III the remaining members of the genus except for *R. earlei*. Cluster II consists entirely of *Lactarius* species. *R. earlei*, a species of the sect. *Archaeinae*, appears in all topologies at the very base of the Russulaceae, supported by a bootstrap value of 76 % in the MP and NJ analysis. Similarity among ITS sequences supports the hypothesis that these five clusters correspond to natural kinship groups. The isolated position of *R. earlei* may indicate that the sect. *Archaeinae* forms a sixth group. *Russula* appears as paraphyletic, even though analyses incorporating topological constraints depicted *Russula* as monophyletic, and were not significantly worse than the best solutions obtained. *Lactarius* appears to be derived from *Russula*. However, the results of the various methods of analysis and the (only marginally worse) solutions produced under topological constraints do not agree as to which of the *Russula* clusters forms the sister group to *Lactarius*. In addition to the analyses presented here, HENKEL, AIME & MILLER (2000) and MILLER et al. (2001) published phylograms representing different phylogenetic hypotheses of the Russulaceae. Thus, molecular phylogenetic analysis cannot yet provide a definitive resolution to the discussion of the infrageneric phylogeny of the agaricoid Russulaceae.

Owing to the homogeneity of LSU sequences within the Russulaceae, the resolution of LSU analyses among closely related species could not be expected to be high. Internal topologies of clusters II and III did not correspond across methods of analysis and will therefore not be discussed. Within clusters, bootstrap values of 70 % or higher usually support species pairs or triplets that are morphologically similar, such as *R. vesca* and *R. heterophylla*, *L.fuliginosus* and *L. ruginosus*, *L. badiosanguineus*, *L. sphagneti* and *L. subsericatus* (MP, NJ). The comparatively high bootstrap support of 75-85 % for the branch of *L. corrugis* and *L. camphoratus* reinforces the assumption that *L. corrugis* may not be correctly named.

Lactarius

So far, the results of the molecular analyses (Figs. 1-3, all *Lactarius* sequences included in the analysis assembled in cluster II) support the classical distinction between *Russula* and *Lactarius*. This corroborates the value attached to the lactiferous system and its extension into the hymenium and mycorrhizae in *Lactarius* as a character distinguishing the two taxa.

The LSU sequences of the majority of the included *Lactarius* species, belonging to the subgenera *Piperites*, *Russularia*, and (to a somewhat lower degree) *Plinthogalus* (classification sensu HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT 1998), are similar, the only exceptions being *L. volemus*, *L. vellereus*, and *L. piperatus*. The latter two species were traditionally considered to form the bridge between *Russula* and *Lactarius* (e.g. HEIM 1938, 1948, SINGER 1986) owing to their conspicuous habitual resemblance to *Russula* species of the sect. *Plorantinae* that occur as a sister clade to *Lactarius* in one (Fig. 1) out of three topologies resulting from the LSU data set.

Though rather contradictory in their hypotheses of the phylogeny of the Russulaceae, the topologies presented by HENKEL, AIME & MILLER (2000) and MILLER et al. (2001) both contain branches combining unpigmented *Lactarius* species (*L. piperatus* and *L. deceptivus* Peck) with members of *Russula*, subg. *Compacta* sensu ROMAGNESI (1967, 1985, 1987). Again, the respective clades stand out in both analyses due to their branch lengths. In both studies (HENKEL, AIME & MILLER 2000, MILLER et al. 2001), only one species of each of the sections of *Russula*, subg. *Compacta*, the *L. piperatus*-group and *Lactarius* species of the *Lactariopsis*-group were considered, making these species especially prone to long branch attraction (FELSENSTEIN 1978).

In spite of superficial morphological similarity, the *Russula* species of the sect. *Plorantinae*, *L. piperatus*, *L. vellereus* and *L. deceptivus* show distinct differences in spore characters (ornamentation, amyloidity of the plage), shape of the basidia, and features of the pileipellis (cf. ROMAGNESI 1967, HESLER & SMITH 1979, HEILMANN-CLAUSEN, VERBEKEN & VESTERHOLT 1998). Neither LSU nor ITS sequences (ITS sequences of the respective *Lactarius* species are not published) contain sequence motives common to species of *Russula*, sect. *Plorantinae*, *L. piperatus* and *L. vellereus*. On the whole, the molecular results rather confirm the opinion of BUYCK and VERBEKEN (BUYCK 1993, 1994, 1997, BUYCK, THOEN, & WATLING 1996, VERBEKEN 1997) that, in the face of microscopic differences, macroscopic similarity is insufficient proof of a close relationship between the unpigmented *Russula* and *Lactarius* species. Likewise, what little is known about the mycorrhizae of the respective *Lactarius* species argues against their similarity to the cystidiate mycorrhizae of the sect. *Plorantinae* (*Russula*) (EBERHARDT 2000).

Russula, subg. *Compacta*

It is generally agreed that the sections *Archaeinae* (represented by *R. earlei* in Figs. 1-3), *Plorantinae* (Figs 1-3, cluster I) and *Nigricantinae* (Figs. 1-3, cluster V), comprising *Russula*, subg. *Compacta* sensu ROMAGNESI (1967, 1985, 1987), are among the ancient groups of the genus, with the *Archaeinae* being the most ancient. A remarkable history of differing definitions has

been used to define the sect. *Archaeinae*, summarised and augmented by SHAFFER (1990) and BUYCK (1998). According to the latter, the sect. *Archaeinae* is mainly characterised by their small, white or off-white spores with only fine, predominantly isolated ornamentation. According to BUYCK (1998), within the genus *Russula*, it is only in some species of the sect. *Nigricantinae* and some tropical species of the *R. cyanoxantha*-group (sect. *Indolentinae* Melzer & Zvára according to ROMAGNESI's (1967, 1985) classification) that similar spores occur.

BON (1988) and SARNARI (1998) placed the sect. *Archaeinae* at the base of the genus *Russula*, thereby following SCHAEFFER (1935) and ROMAGNESI (1967, 1985) who considered white spores, inamyloid plages, and little differentiated dermatocystidia reacting only slightly with sulfovanillin as conserved characters in the genus *Russula*. SARNARI (1998) interpreted the *Archaeinae* as *Nigricantinae* (sensu ROMAGNESI 1967, 1985 = sect. *Compactae* Fr. sensu SARNARI 1998) that do not turn grey or blackish. Other authors stressed similarities between the sections *Archaeinae* and *Plorantinae* (ROMAGNESI 1985, BUYCK 1994). BUYCK (1998) pointed to affinities between the sect. *Archaeinae* and several sections of *Russula*. He considered this as an indication of the age of the group. HEIM (1938, 1948) even regarded *R. archaea* R.Heim, the then only known species of the sect. *Archaeinae*, as a common ancestor of *Russula, Lactarius* and gasteroid Russulales. This interpretation was prompted by the lactarioid appearance of the sporocarps with decurrent, forked lamellae interleaved by lamellulae, enhanced by the lack of the strong colours (typical for many *Lactarius* species) and the uniform coloration of the stipe and pileus. Without accepting HEIM's (1938, 1948) line of reasoning based on macroscopic similarities, the current view is that a possible link exists between some infrageneric groups of *Russula* and *Lactarius* that have fewer ancient characters (BUYCK 1993, 1994, 1997, BUYCK, THOEN & WATLING 1996, VERBEKEN 1997), thus implying that the sect. *Archaeinae* may be basal to both *Russula* and *Lactarius*.

The basal position of *R. earlei* in the topologies (Figs. 1-3) obtained by various methods is congruent with the notion that the *R. archaea*-group is the most ancient of the Russulaceae (but see Results, section "testing phylogenetic hypotheses", HENKEL, AIME & MILLER 2000, MILLER et al. 2001). However, against the background of today's knowledge of *Russula* and *Lactarius,* it is difficult to define features in the sect. *Archaeinae* of *Russula* that are typical of each of the genera. SHAFFER (1990) mentioned the lack of lactifers in trama (and pileipellis) in his definition of the sect. *Archaeinae*, a feature not confirmed by BUYCK (1998, 1994). Pseudocystidia sensu BUYCK (1991a), usually present in *Lactarius* and lacking in *Russula*, do not occur in the sect. *Archaeinae*. BUYCK's (1998) definition of *Russula*, sect. *Archaeinae* stated that the trama of its species' lamellae is formed by strongly septate hyphae embedded in matrix material. The lack of sphaerocytes in the trama of the lamellae, if this is the implicit meaning of BUYCK's (1998) description, is more typical for *Lactarius* (if only in a European context) than for *Russula*. The presence of numerous dermatocystidia in species of the *R. archaea*-group (BUYCK 1998) is more typical for *Russula* and is considered to be a derived feature in *Lactarius* (Verbeken 1998). Owing to the lack of phylogenetic resolution in the molecular analyses, the evolutionary polarisation of the characters mentioned above could only be supported by the basal position of *R. earlei* in the topologies of Figs. 1-3. The placement of the other sections of *Russula*, subg. *Compacta*, if basal at all, is not sufficiently consistent among trees to allow conclusions to be drawn with respect to the polarisation of characters.

The LSU-topologies presented here (Figs. 1-3) do not support the monophyly of *Russula*, subg. *Compacta* sensu ROMAGNESI (1967, 1985, 1987). Neither the topology presented by HENKEL, AIME & MILLER (2000) nor that from MILLER et al. (2001) argue for the preservation of *Russula* subg. *Compacta* sensu ROMAGNESI (1967, 1985, 1987). However, the molecular results (Figs. 1-3: *R. earlei*, species of *Russula*, sections *Plorantinae* and *Nigricantinae* form three separate branches) confirm that *Russula*, sect. *Nigricantinae* and *Russula*, sect. *Plorantinae* (and *R. earlei)* are in fact distinct from the remaining species in the analysis. Provided the monophyly of the sections of *Russula*, subg. *Compacta,* will be confirmed with more extensive analyses, it can be considered likely that the species of *Russula*, sect. *Nigricantinae*, sect. *Plorantinae*, and sect. *Archaeinae* form valid subgroups within the genus *Russula* or the Russulaceae.

The compact *Russula* species, sections *Nigricantinae* and *Plorantinae*, form a joint cluster in Fig. 4. However, a close relationship of the species of the two sections disagrees with structural features of their ITS sequences. The sequences of species of *Russula*, sect. *Plorantinae* differ from those of other species by inserts of several basepairs in the ITS (and LSU, see alignments in TreeBase). The topology presented in Fig. 4 was calculated from an alignment achieved by introducing gaps where sequence alignment was not possible (see Results, "Analyses of *Russula* ITS sequences", section "*Russula,* subg. *Compacta*, subg. *Heterophyllidia* and sect. *Foetentinae*"). This procedure is likely to have produced artefacts in those inner branches of the topology in Fig. 4 that link groups of sequences that align well among each other but not with the remaining species of the database. An example of these is the branch joining the clades representing two sections of *Russula,* subg. *Compacta*, the sections *Nigricantinae* and *Plorantinae*.

The molecular phylogenetic results support SINGER's (1986) and BUYCK's (1995) position who stressed the importance of distinct differences that exist among the sections of *Russula*, subg. *Compacta*, in pigmentation, discoloration of the flesh, shape of basidia, amyloidity of the plage (compare SARNARI 1998) and geographical distribution. As BUYCK (1995) pointed out, among non-European species, the macroscopic characters defining the subg. *Compacta* sensu ROMAGNESI are not restricted to this group. A predominantly northern hemisphere distribution, amyloidity of the plage, voluminous

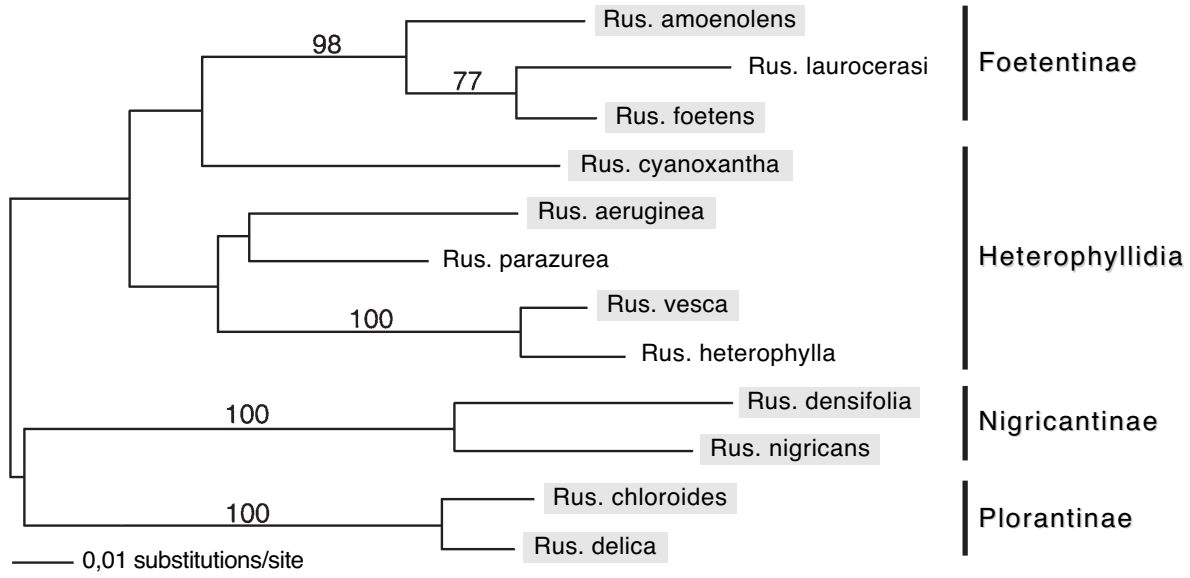


Fig. 4. Unrooted toplogy of a maximum likelihood analysis calculated from ITS sequences of *Russula* species from the subgenera *Compacta* (sections *Plorantinae* and *Nigricantinae*) and *Heterophylidia* as well as sect. *Foetentinae* (subg. *Ingratula*) under the HKY85 DNA substitution model. Bootstrap values from 250 replicates of fast bootstrap. Percentage values < 70 % are not shown. Names of species with identified mycorrhizae are shaded.

basidia, and pronounced spore ornamentation are considered derived characters of the sect. *Plorantinae* (BUYCK 1991b, 1995).

If one accepts that the *Russula* species of the sections *Nigricantinae* and *Plorantinae* are fairly ancient groups, the formation of cystidia on the surface of mycorrhizal mantles has to be considered an ancestral character. Distinct differences in the microscopic characters separating the mycorrhizae formed by these two sections do not exist (EBERHARDT 2000, for references to mycorrhizal descriptions see Tab. 2).

Russula, subg. *Heterophyllidia*, *Russula compacta* and *Foetentinae*

Russula species of the subg. *Heterophyllidia* and the sect. *Foetentinae* as well as *R. compacta* from the *R. crassitunicata*-group (cluster IV, Figs. 1-3) share many traits, namely they all possess a white or at most lightly coloured spore print and inamyloidity of the plage (compare SARNARI 1998). The available information on *R. compacta* and the *R. crassitunicata*-group (SHAFFER 1970, SINGER 1975, 1986, BUYCK 1994, PHILLIPS 1991, SARNARI 1998) is too incomplete and contradictory to warrant inclusion of these species in further discussion. *Russula* species of the subg. *Heterophyllidia* and the sect. *Foetentinae* also have unicellular dermatocystidia that do not show a pronounced reaction with sulfoaldehydes in many species. The mycorrhizae of both groups (for references see Tab. 2) form cystidia. This latter character and the lack of amyloidity of the plage discriminate the species of cluster IV, Figs 1-3, from the *Russula* species of Cluster III, Figs. 1-3 (SARNARI 1998). All of the characters named above, white spores, inamyloidity of the plage, and unicellular dermatocystidia, are considered ancestral (ROMAGNESI 1967, BON 1988, SARNARI 1998), implying that cluster IV lacks common derived characters.

In the European context, the species of *Russula*, subg. *Heterophyllidia* and sect. *Foetentinae* are easy to separate. Species of *Russula*, subg. *Heterophyllidia* excluding the *R. amoena* Quélet-group (not present in the analysis) are united and distinguished by the possession of certain ammonia-soluble pigments (ROMAGNESI 1967, 1985) and by the formation of a second type of cystidia on the surface of the mycorrhizae (for references, see Tab. 2). *Russula*, sect. *Foetentinae* (and *R. fellea*, see below) is set apart by its species' extracellular yellowish to brownish pigmentation (ROMAGNESI 1967, 1985). Further differences exist in the macroscopic appearance of their fruit bodies, coloration, taste (ROMAGNESI 1967, 1985), and, according to BEENKEN (2001b), with respect to the formation of ladder-like hyphae in rhizomorphs.

In a tropical African context, however, BUYCK (1994) found it difficult to separate *Russula*, subg. *Heterophyllidia* from *Russula*, sect. *Foetentinae* on the one hand and from *Russula,* sect. *Crassitunicatae* (Singer) Singer on the other (Buyck 1994, BUYCK, THOEN & WATLING 1996). According to BUYCK's (1994) definition, *Russula*, sect. *Indolentinae* is distinguished from the remaining species of *Russula*, subg. *Heterophyllidia* by almost all hyphae having a metachromatic reaction with cresil blue, most notable in the dermatocystidia and in the basis of the cystidia of the hymenium. The only

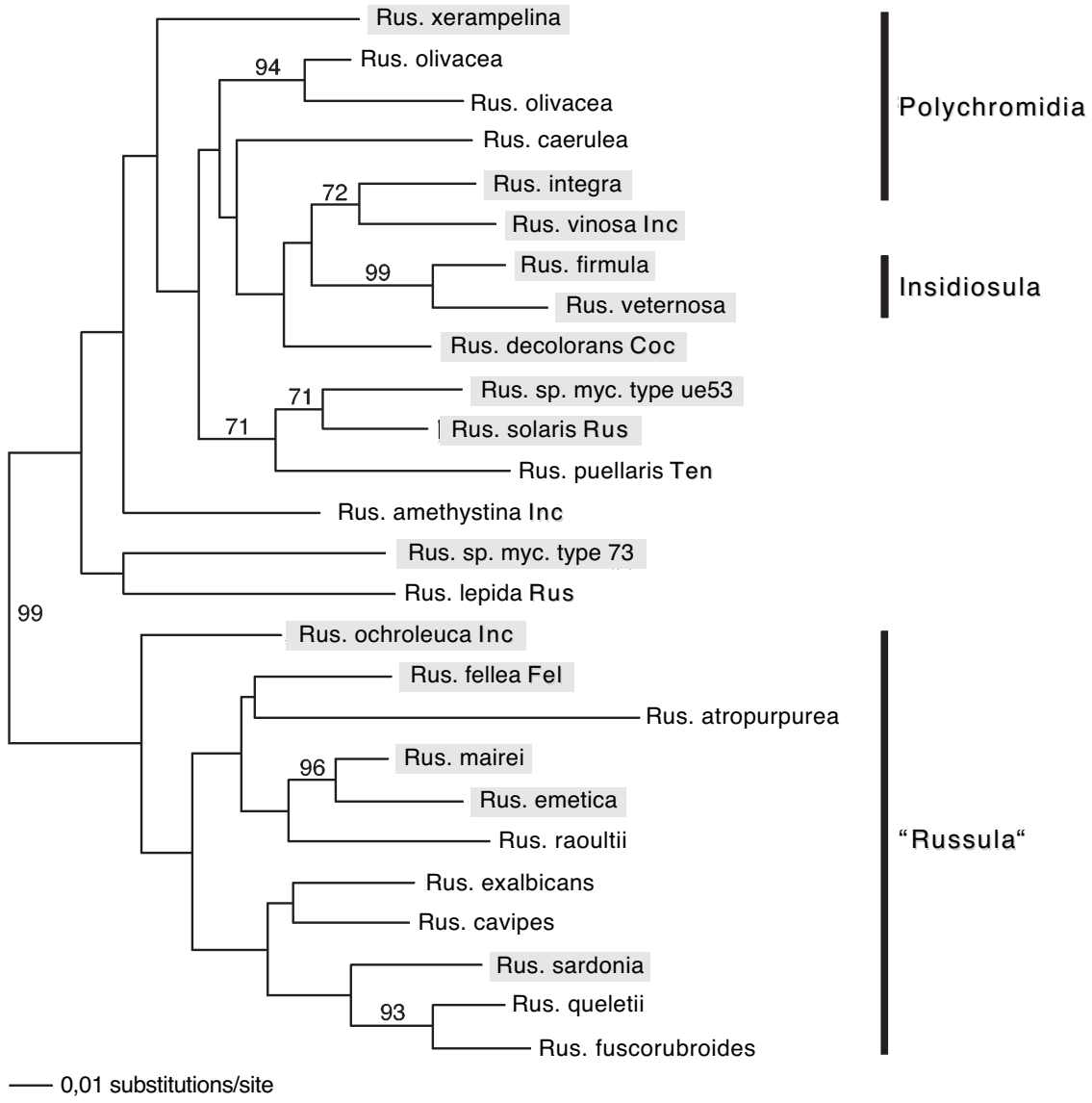


Fig. 5. Unrooted topology of a maximum likelihood analysis calculated from ITS sequences of *Russula* species from the subgenera *Russula* (Rus), *Incrustatula* (Inc), *Tenellula* (Ten), *Coccinula* (Coc), *Polychromidia*, *and Insidiosula* and from sect. *Felleinae* (Fel) of *Russula*, subg. *Ingratula* under the HKY85 DNA substitution model. Bootstrap values from 100 replicates of fast bootstrap, percentage values < 70 % not shown. Rus. myc. type – unidentified mycorrhizal type formed by *Russula* sp. Names of species with identified mycorrhizae are shaded.

European species of *Russula,* sect. *Indolentinae* is *R. cyanoxantha*. However, *R. laurocerasi* shows a similar reaction with cresil blue and, to a lesser extent, so do other species of *Russula*, sect. *Foetentinae* (BUYCK 1989b, BUYCK & HORAK 1999b). The separate placement of *R. cyanoxantha* in relation to the remaining species of *Russula*, subg. *Heterophyllidia* within the topologies presented in Figs.1-4 supports the above observations. In addition, among the mycorrhizae described for *Russula* species of the subg. *Heterophyllidia*, only *R. cyanoxantha* forms cystidia that are not awl-shaped but rather are formed like hyphal ends (for references see Tab. 2, reviewed by BEENKEN 2001f).

In spite of the value BUYCK (1989, 1994) attached to the cresil blue reaction, he nevertheless considered the extracellular localisation of the pigments of species of *Russula*, sect. *Foetentinae* more meaningful. Accordingly, the sect. *Foetentinae* appears as a subsection of *Russula*, sect. *Fistulosae* (R.Heim) Buyck that is considered a more ancient group than *Russula*, subg. *Heterophyllidia* (BUYCK, THOEN & WATLING 1996). This is in apparent contradiction to the molecular results (Figs.1-4) in which *Russula*, sect. *Foetentinae* appears to be derived from *Russula*, subg. *Heterophyllidia*. Considering the lack of sequences from *Russula* species of the sect. *Fistulosae* in the data set and the fact that neither of the se-

quenced DNA regions is particularly suited to resolve refined phylogenetic estimates, this contradiction should not be overemphasized.

Remaining "Genuinae" (*Russula*)

The association of *Russula*, subg. *Heterophyllidia* and *Russula*, sect. *Foetentinae* in one cluster (IV, Figs. 1-3) and the unification of the remaining six subgenera of the "Genuinae"-group of *Russula* into another (cluster III, Figs. 1-3) contrasts starkly with ROMAGNESI's (1967, 1985) view of the evolution of the subgenera forming the "Genuinae". He visualised several possible evolutionary lineages within the "Genuinae"-group of *Russula*, with different species of *Russula*, sect. *Foetentinae* and subg. *Heterophyllidia* occurring at the base of different lines. The most persistent of ROMAGNESI's evolutionary hypotheses linked the acrid tasting species of sect. *Foetentinae* via *R. fellea* with the subgenus *Russula* (see ROMAGNESI 1967, BON 1988, SARNARI 1998); the species of subg. *Heterophyllidia* were considered as the most conservative group among the mild tasting species. This view would have been supported by separate clades formed in the DNA sequence-based phylogenetic analyses, containing relatives of the proposed ancestral group (members of cluster IV, Figs. 1-3) alongside the respective derived members of the proposed lineages (species of cluster III, Figs. 1-3).

Cluster III (Figs. 1-3) and the ITS topology in Fig. 5 represent the majority of European *Russula* species that were allocated by ROMAGNESI (1967, 1985, 1987) to the subgenera *Russula*, *Tenellula*, *Incrustatula*, *Polychromidia*, *Coccinula* and *Insidiosula*. All species of cluster III have spores with an amyloid plage (SARNARI 1998) and mycorrhizae devoid of cystidia, the outer mantle layer displaying a pseudoparenchymatous structure (for references, refer to Tab. 2). Both features occur only in infrageneric groups that are considered derived in ROMAGNESI's system. DNA sequence differences among these species are far smaller than, for example, between *Russula* species of the sections *Nigricantinae* and *Plorantinae*. Therefore, according to the molecular phylogenetic results, the use of so many subgenera is not reasonable. However, the species composition of the ITS data set is not well balanced so only preliminary conclusions can be drawn regarding the relationships within this group. Fig. 6 provides a diagrammatic overview of the features of the species included in the analysis.

Though ROMAGNESI (1967, 1985, 1987), BON (1988) and SARNARI (1998) vary in the weighting they attach to characters used for classification, they generated infrageneric groups that were similar in species composition; infrageneric groups were, however, not necessarily similar in inner structure. The core species of each group (those consistently placed in the same group by all authors) had the following traits: *Russula*, subg. *Russula*, acrid tasting species with lightly coloured spores; *Russula*, subg. *Incrustatula*, mild tasting species with pale coloured or yellow spores and encrusted primordial hyphae in their pileipellis; *Russula*, subg. *Tenellula*, species with dermatocystidia formed in the pileipellis and with slender habit; and *Russula*, subg. *Insidiosula*, acrid tasting species with yellow spores (following the colour code of ROMAGNESI (1967, 1985) for spore colour). The remaining mild tasting species with more solid stature than those ascribed to *Russula*, subg. *Tenellula* and with differing composition of the pileipellis were allocated by ROMAGNESI to subgenera according to the discoloration of the flesh or cap colour, namely to *Russula*, subg. *Polychromidia* (yellowing or browning context, violet, green or brownish caps) or to *Russula*, subg. *Coccinula* (greying context, orange, reddish or coppery caps). BON (1988) and SARNARI (1998) favoured systems of classification in which the elements of the pileipellis, dermatocystidia, and encrusted primordial hyphae played a more important role. While BON (1988) still attached great significance to taste, SARNARI (1998) placed utmost significance to the elements of the pileipellis.

Considering only the results of the ITS analysis (Figs. 5-6), the species fall into two clusters. One cluster ("Russula") represents *Russula*, subg. *Russula* sensu ROMAGNESI (1967, 1985, 1987), consisting of light spored species with acrid taste and sulfovanillin reactive dermatocystidia, including *R. ochroleuca* and *R. fellea*, but excluding *R. solaris* and *R. lepida*. The other cluster is formed by species of six subgenera (*Tenellula*, *Incrustatula*, *Coccinula*, *Polychromidia*, *Insidiosula*, and *Russula*) and is not easily described in morphological terms.

The inclusion of *R. fellea* and *R. ochroleuca* in the "Russula"-cluster of Fig. 5 (otherwise corresponding to the core species *Russula* subg. *Russula*) is supported by the anatomy of their mycorrhizal mantles, featuring polygonal cells in the outer and middle mantle layers (EBERHARDT 2000, BEENKEN 2001k). A similar mantle anatomy has to date only been found in members of the *R. emetica*-group, *R. pumila,* and the closely related *R. alnetorum*, all members of the subgenus *Russula*. All other acystidiate mycorrhizae of *Russula* species form irregular pseudoparenchyma, often with interlocking cells (for references see Tab. 2). Sporocarp features of *Russula fellea* that are shared with species of the subg. *Russula* include dermatocystidia reacting positive with sulfovanillin, amyloidity of the plage, habit, spore ornamentation, and acrid taste (ROMAGNESI 1967, 1985, SARNARI 1998). Anatomical features of their mycorrhizae distinguish *R. fellea* from the remaining species of *Russula*, subg. *Ingratula*, but other differentiating features include differences in habit, amyloidity of the plage and the strong positive reaction of the dermatocystidia with sulfovanillin. Yet, the colouration and the extracellular localisation of the pigments of *R. fellea* are very similar to some *Russula* species of the sect. *Foetentinae*. Therefore, *R. fellea* has been considered by many (e.g. ROMAGNESI 1967, 1985, BON 1988, SARNARI 1998) as a link between *Russula*, sect. *Foetentinae* and *Russula*, subg. *Russula*, a role that is not supported by the results of the molecular analyses (Figs. 1-5).

Russula ochroleuca deviates from the other species in the "Russula" cluster (Fig. 5) by the absence of dermatocystidia in the pileipellis, and by the presence of encrusted hyphae.

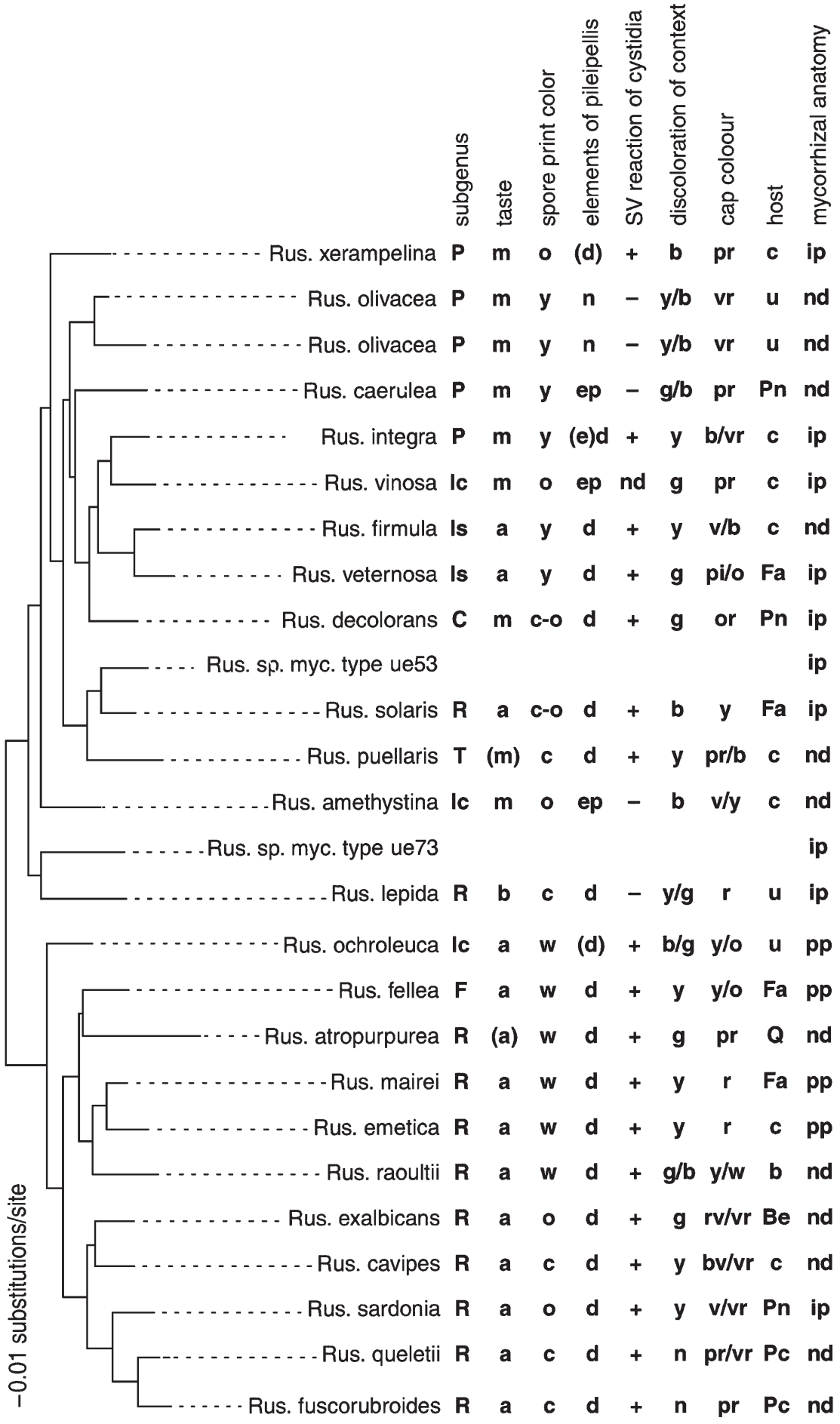
These incrustations were interpreted as extracellular pigmentation by ROMAGNESI (1967), who classified *R. ochroleuca* along with *R. fellea* in *Russula*, subg. *Ingratula*. Following chemical analysis, he later revised his classification and placed *R. ochroleuca* together with other species possessing encrusted hyphae, e.g. *R. vinosa*, in the newly erected and rather heterogeneous sect. *Ochroleuceinae* Romagn. in *Russula*, subg. *Incrustatula* (ROMAGNESI 1985). BON (1988) followed ROMAGNESI (1985) in suggesting that the encrusted hyphae of *R. ochroleuca* were homologous with encrusted primordial hyphae. This interpretation is not supported by the ITS results and the bootstrap analysis (Fig. 5). SARNARI (1991) defined a new section, *Russula*, sect. *Viscidinae* Sarnari for species with pale coloured spore prints, a more or less acrid taste, indistinctive dermatocystidia, and remains of a velum at the basis of the stipe that give a red reaction with KOH (SARNARI 1998). These species, among them *R. ochroleuca*, are considered by SARNARI (1998) as close to *Russula*, subg. *Russula* sensu ROMAGNESI (1967, 1985, 1987) and only separated from this by the possession of a velum. Though unable to confirm infrageneric delimitations at this stage, the molecular results as well as the mycorrhizal anatomy are in favour of SARNARI's (1998) proposal. In the DNA sequence analysis published by MILLER et al. (2001), the sequence of the annulate species *R. discopus* R.Heim clustered with that of *R. violacea* Quélet (*Russula*, subg. *Russula*) and in additional analyses (Eberhardt, unpublished) with other species of *Russula*, subg. *Russula*. This points to considerable plasticity of sporocarp form and possibly also development in the subg. *Russula* kinship group.

ROMAGNESI (1967, 1985) placed *R. lepida* in the subg. *Russula* owing to similarities in macroscopic appearance (coloration of sporocarps, colour of spore print). BON (1988) classified *R. lepida* alongside species possessing encrusted primordial hyphae whereas SARNARI (1998) established *Russula*, sect. *Paraincrustatae* Sarnari to hold *R. lepida*. This newly established section unites species that form both encrusted primordial hyphae and (sometimes encrusted) dermatocystidia. In contrast, species of *Russula*, subg. *Incrustatula* possess only the former. The only other member of *Russula,* sect. *Paraincrustatae* included in the analysis is *R. integra* (Fig. 5). The rather isolated position of *R. lepida* in the second cluster of the ITS analysis (Fig. 5, see also Fig. 6) supports neither BON (1988) nor SARNARI (1998).

Russula solaris stands out among members of subsect. *Citrinae* Romagn. (subg. *Russula*) because of its spore ornamentation (long isolated spines as opposed to interconnected warts) and the darker colour of its spore print (ROMAGNESI 1967). In the molecular analysis, the pairing of *R. solaris* and *R. puellaris* (*Russula*, subg. *Tenellula*) was supported by a bootstrap value of 71 % (Fig.5). *R. solaris* corresponds in many characters to the definition of *Russula*, subg. *Tenellula* (ROMAGNESI 1967, EINHELLINGER 1985), such as in habit, spore characters, and microscopic features of the pileipellis and of the basidia, as well as the presence of lactifers in the trama of the pileus (the stipe cortex could not be checked in the incompletely preserved specimen of *R. solaris*). *Russula solaris* deviates from the definition of *Russula*, subg. *Tenellula* by its acrid taste. Though species of *Russula*, subg. *Tenellula* are by definition of mild taste, an acrid taste was noted in some specimens of species of *Russula*, sect. *Puellarinae* Singer (*R. puellaris*, *R. versicolor* J. Schaeff., fide ROMAGNESI 1967, EINHELLINGER 1985). Overall, anatomical characters support the surprising association of *R. solaris* with *R. puellaris* that arose from the molecular analyses.

With respect to the infra-subgeneric classification of *Russula*, subg. *Russula*, ROMAGNESI's (1967, 1985) division of the subgenus into the sections *Russula* (Quélet) Romagn. (red and yellow cap colours) and *Atropurpureae* Romagn. (violet, brown or mixed cap colours) was neither shared by BON (1988) nor by SARNARI (1998), nor was it depicted in the molecular phylogenetic results. *Russula ochroleuca*, *R. fellea*, the *R. emetica*-group, *R. raoultii*, and *R. atropurpurea* have white spore prints; the remaining species of the "Russula"-cluster (Fig. 5) have coloured spores and more restricted host specificities: *R. exalbicans* for birch, the other species of the data set (all of the *Sardoninae* Singer emend. Romagn.) for conifers

Fig. 6. Table, summarizing classification and features of species (following ROMAGNESI 1967, EINHELLINGER 1985, GRÖGER 1996) appended to the same topology as in in Fig. 5. Rus. – *Russula*. Subgenus: *Russula* (R), *Incrustatula* (Ic), *Tenellula* (T), *Coccinula* (C), *Polychromidia*, *Insidiosula* (Is) and from *Ingratula*, sect. *Felleinae* (F). Taste: acrid (a), bitter (b), mild (m). Spore print colour: white (w), crème (c), ochre (o), yellow (y). Elements of the pileipellis: dermatocystidia (d), untypical dermatocystidia ((d)), dermatocystidia and encrusted dermatocystidia ((e)d), encrusted primordial hyphae (ep). Reaction of cystidia with sulfovanillin: positive (+), negative (-), not determined (nd). Discoloration of context: browning (b), greying (g), yellowing or yellowish (y); the extend of the discoloration of the context is rather different between species and in many species dependent on age and environmental conditions. Cap colour: white (w), yellow (y), ochre (o), orange (or), red (r), pink (pi), violet (v), brown (b), brown violet (bv), purple (pr), variable, including greenish, ochre, brownish, violet, vinaceous and other colours (vr); cap colours are generally very variable in *Russula* species and dependent on age and environmental conditions. Host (principal host): conifers (c), broadleaves (b), unspecific (u), *Betula* (Be), *Fagus* (Fa), *Quercus* (Q), *Picea* (Pc), *Pinus* (Pn). Mycorrhizal anatomy: outer mantle layers with irregular pseudoparenchyma (ip), pseudoparenchyma with polygonal cells (pp), not determined (nd).


subgenus
taste
spore print color
elements of pileipellis
SV reaction of cystidia
discoloration of context
cap colour
host
mycorrhizal anatomy
Rus. xerampelina P m o (d) + b pr c ip
Rus. olivacea P m y n – y/b vr u nd
Rus. olivacea P m y n – y/b vr u nd
Rus. caerulea P m y ep – g/b pr Pn nd
Rus. integra P m y (e)d + y b/vr c ip
Rus. vinosa Ic m o ep nd g pr c ip
Rus. firmula Is a y d + y v/b c nd
Rus. veternosa Is a y d + g pi/o Fa ip
Rus. decolorans C m c-o d + g or Pn ip
Rus. sp. myc. type ue53 ip
Rus. solaris R a c-o d + b y Fa ip
Rus. puellaris T (m) c d + y pr/b c nd
Rus. amethystina Ic m o ep – b v/y c nd
Rus. sp. myc. type ue73 ip
Rus. lepida R b c d – y/g r u ip
Rus. ochroleuca Ic a w (d) + b/g y/o u pp
Rus. fellea F a w d + y y/o Fa pp
Rus. atropurpurea R (a) w d + g pr Q nd
Rus. mairei R a w d + y r Fa pp
Rus. emetica R a w d + y r c pp
Rus. raoultii R a w d + g/b y/w b nd
Rus. exalbicans R a o d + g rv/vr Be nd
Rus. cavipes R a c d + y bv/vr c nd
Rus. sardonia R a o d + y v/vr Pn ip
Rus. queletii R a c d + n pr/vr Pc nd
Rus. fuscorubroides R a c d + n pr Pc nd
–0.01 substitutions/site

(see also Fig. 6). Considering the mycorrhizae described so far (see Tab. 2), all species of the former group display polygonal cells in the hyphal mantle. Apart from *R. ochroleuca*, they form a monophyletic branch in the ITS analysis (Fig. 5). *Russula sardonia* (L. Beenken, personal communication) and the closely related species *R. sanguinea* were reported to form irregularly pseudoparenchymatous mantle structures, as commonly found in species represented by the second cluster of the ITS analyses. *R. exalbicans* forms an exception in that this species was so far only detected in mycorrhizae by molecular methods as a second partner to *Lactarius pubescens* in *Lactarius* type mycorrhizae (Beenken 1997, abstr.).

The second cluster formed in the ITS topology (Fig. 5) consisted of species of several subgenera that are homogeneous in mycorrhizal mantle structures (acystidiate, with irregularly formed, often interlocking cells in the pseudoparenchymatous outer mantle layers; for references refer to Tab. 2), but rather heterogeneous concerning their sporocarp characters, such as elements of the pileipellis, spore print colour, taste, habit, colour, chemical reaction and discoloration of the context (see Fig. 6). Within the data set, only *Russula*, subg. *Insidiosula*, represented by two species, can be recognised in the topology. *Russula*, subg. *Polychromidia* (ROMAGNESI 1967, 1985, 1987) was neither accepted by BON (1988) nor SARNARI (1998). It contains mild tasting *Russula* species with variable (greenish, violet, brownish) cap colours and browning context that neither fit the definition of subg. *Heterophyllidia,* subg. *Incrustatula,* nor that of subg. *Tenellula* (ROMAGNESI 1967, 1985). The analysis (Fig. 5) supports the view of BON (1988) and SARNARI (1998) that *Russula*, subg. *Polychromidia* is an artificial grouping. However, BON's (1988) system of strictly sorting taxa according to a set character weighting system (acrid before mild species, elements of the pileipellis (dermatocystidia before encrusted primordial hyphae), reactivity of dermatocystidia with sulfobenzaldehydes (SBA- before SBA+), spore colour (light before dark)) was not supported, either. SARNARI'S (1998) classification was also not supported; he took a more differentiating view of the pileipellis elements of many species by introducing the *Paraincrustatae* and by homologising certain elements of the pileipellis of species of the *R. olivacea*-group with encrusted primordial hyphae.

The subgenera *Coccinula*, *Incrustatula*, *Tenellula*, possibly also the subgenera *Polychromidia* and *Insidiosula* of *Russula* were only poorly represented in the (ITS) data set (Fig. 5). As stated earlier, neither of the employed DNA regions is ideal in terms of alignment and phylogenetic resolution of the Russulaceae. The molecular results do not yet allow any conclusions to be drawn concerning the evolutionary sequence of developments within the Russulaceae. So far, the molecular kinship analyses suggest that many sporocarp characters (Fig. 6) traditionally used for classification of the genus *Russula*, such as taste, spore print colour or the elements of the pileipellis, are highly variable, at least within the "Genuinae" of the second cluster of Fig. 5, and might therefore be inappropriate at delineating species groups. In contrast, the molecular phylogenetic results suggest that the amyloidity of the plage and characters associated with the anatomy of mycorrhizae are less often subject to changes within infrageneric groups of the *Russula*.

Acknowledgements

This work was partially financed by a Ph.D. scholarship within the DFG-Graduiertenkolleg "Interaktionen in Waldökosystemen". I thank all those who provided additional specimens for this study. I am particularly obliged to Annemieke Verbeken and Ruben Walleyn who not only confirmed many of my identifications (and corrected others), but also helped me at a crucial point of my work by introducing me to their global view of the Russulaceae.

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Accepted: 18.1.2002