
Walking the thin line between *Russula* and *Lactarius*: the dilemma of *Russula* subsect. *Ochricompectae*

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

This paper questions the validity of the traditional features used to delimit genera in the order *Russulales*. Molecular phylogenetic analyses of ribosomal genes (ITS-nucLSU) and part of a protein-coding gene (*RBP2*) indicate that four phylogenetically distinct clades are identified within *Russulaceae*. In the light of molecular and morphological evidence, the authors demonstrate that one group of species, presently classified by several modern authors as subsection *Ochricompectae* within *Russula* subgenus *Compacta*, corresponds to a monophyletic entity that includes typical species of both *Russula* and *Lactarius*, and that the shared morphology between these *Russula* species and the very rare American *Lactarius furcatus* Coker is not a matter of convergence. Several of the species here discussed are remarkable for their outstanding hymenial features and reminiscent of resupinate taxa. *Multifurca* gen. nov. is described to accommodate *L. furcatus* and the species of *Russula* subsect. *Ochricompectae*, with the exception of *R. grossa*, which should be transferred to *Russula* sect. *Heterophyllae*. *Multifurca roxburghiae* sp. nov. is described from India for *R. grossa* sensu Bills & Pegler, a taxon that would traditionally have fitted in *Russula*.

Key words: *Multifurca* gen. nov., ITS-nucLSU, molecular systematics, *RBP2*, *Russulaceae*

Article Information

Received 14 November 2007

Accepted 13 December 2007

Published online 31 January 2008

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Introduction

The *Russulaceae* are a family of ectomycorrhizal basidiomycetes. Among the numerous genera described in this group (Miller *et al.* 2007), only 8-10 are still currently in use. The vast majority of the known species are agaricoid taxa and belong to the genera *Russula* or *Lactarius*. In addition to the well-developed agaricoid taxa that characterize both genera, the family *Russulaceae* comprises also a number of pleurotoid, secotioid and gasteroid species that share the same microscopical features. With one exception (Buyck and

Horak, 1999), these taxa were traditionally placed on the basis of their different morphology in a number of separate, much smaller genera with the choice of genus depending on overall morphology and on presence or absence of latex exudation, thereby usually disregarding the overwhelming microscopical similarities with some of the agaricoid taxa. Many of the molecular phylogenetic studies on the russuloid clade addressed the question of the monophyly of these pleurotoid, secotioid and gasteroid taxa (Calonge and Martin, 2000; Henkel *et al.*, 2000; Miller *et al.*, 2001), and resulted invariably in the same clear answer: fruit body

shapes deviating from the agaricoid form have evolved independently many times within the family. Following a phylogenetic taxonomic concept, therefore, genera based on fruit body shapes deviating from the agaricoid habit are redundant. As a consequence, modern authors have started to adhere to a much wider morphological concept of *Russula* and *Lactarius*, with both genera embracing the entire range from agaricoid, pleurotoid, secotioid to gasteroid forms (Henkel *et al.*, 2000; Miller *et al.*, 2002; Desjardin, 2003; Eberhardt and Verbeken, 2004; Nuytinck *et al.*, 2004, 2006; Shimono *et al.*, 2004; Le *et al.*, 2007a,b; Lebel and Tonkin, 2007). In the last years, focus in phylogenetic studies shifted mainly on reiterating or confirming the results of Larsson and Larsson (2003) in situating *Russulaceae* within the broader context of the russuloid clade (Lutzoni *et al.*, 2004; Binder *et al.*, 2005; Miller *et al.*, 2007). So far all phylogenetic studies recovered a monophyletic *Russulaceae* but internal relationships between *Lactarius* and *Russula* remained weakly supported or unresolved (Miller *et al.*, 2001; Larsson and Larsson, 2003; Eberhardt and Verbeken, 2004; Shimono *et al.*, 2004; Binder *et al.*, 2005; Lebel and Tonkin, 2007). Except for Eberhardt and Verbeken (2004), all of these studies were predominantly concerned with northern hemisphere taxa, and although the majority of the species fell reproducibly in the same subclades (many of which correspond to traditional systematic entities), the internal relationships within this family remained weakly supported. All of the thus far supported monophyletic clades were also homogeneous in the sense that they exclusively contained species from either *Russula* or *Lactarius*. This paper presents for the first time evidence for the existence of a fully supported monophyletic clade of a heterogeneous nature, i.e. comprising species assigned to both genera.

In this paper, we are mainly concerned with a small number of recently discovered and extremely rare, tropical or subtropical taxa that have never been included in phylogenetic studies. We will demonstrate that these species - which were classified in *Russula* subgenus *Compacta*, sect. *Compactae* subsection *Ochricompactae* - form a fully supported monophyletic group that includes the equally rare

Lactarius furcatus Coker and, together, present a morphological series that covers the entire gradient from a stereotype *Russula* (not exuding latex, no pseudocystidia) to a stereotype *Lactarius* (latex exudation, abundant pseudocystidia). Moreover, several of these species have evident microscopic affinities not only to the well-known agaricoid taxa from both genera, *Russula* and *Lactarius*, but also possess features reminiscent of *Corticiaceae* (Buyck, 1995). In this context, it is of particular interest that several resupinate taxa have been proven to be part of the Russuloid clade and very closely related to the traditional agaricoid genera of *Russulaceae* (Larsson and Larsson, 2003). In the light of new molecular and morphological evidence, we will discuss the difficulty of maintaining the species of subsection *Ochricompactae* within the genus *Russula* as well as the implications of their transfer to either a new genus or to the genus *Lactarius*.

Recapitulation of the history of Subsection Ochricompactae

This subsection was proposed by Bills and Miller (1984) in *Russula* sect. *Compactae* Fr. for the rare *Russula ochricompacta*, known at that time from only two collections from the mountains of south western Virginia, USA. Shortly before this publication, in 1982, Saini and Atri had described and illustrated a recent collection from India which in their opinion corresponded to *R. grossa* Berk. (1851). As Saini and Atri's recent collection of *R. grossa* presented strong similarities with the newly described *R. ochricompacta*, Bills and Pegler (1988) published a short note in which they compared both species and accepted *R. grossa* as a second species in *Ochricompactae*. About 15 years later, two additional species were described in this subsection that considerably widened its concept: *R. zonaria* from Thailand (Buyck and Desjardin, 2003) and *R. aurantiophylla* Buyck & Ducousso from New Caledonia (Buyck, 2004). The morphological characters of these new species and of a number of very recent collections of *R. ochricompacta* from the southeastern United States by Buyck and D. Lewis (Texas, USA) made it very clear that members of the subsection *Ochricompactae* possess a number

of unique features that question their assumed systematic position in the genus *Russula*.

The fact that *Ochricompectae* possess also a morphological twin in *Lactarius* remained unknown and is here reported for the first time. The species in question is *L. furcatus* Coker, a taxon that remained undocumented since its original description (Coker, 1918) because the type specimen seems lost. Additional collections have never been reported until it was recently rediscovered in Texas (by D. Lewis, unpubl.) and in Costa Rica (Montoya *et al.*, 2003). Montoya *et al.* suggested a very close relationship between *L. furcatus*, which exudes a white latex that quickly turns greenish, and *L. zonarius* in subgenus *Piperites*. However, the morphological similarities between this very rare American *L. furcatus* and the species of *Russula* subsection *Ochricompectae* are overwhelming.

Materials and methods

The relatedness of the different taxa in *Russula* subsection *Ochricompectae* among each other and between these and *L. furcatus* as well as the systematic position of the *Ochricompectae* within the family *Russulaceae* are here investigated. Our approach is both morphological and molecular, using phylogenetic analyses of DNA sequences from several nuclear ribosomal genes and from part of a protein-coding gene, the second largest subunit of RNA polymerase II (RPB2). An overview of representativity of sampling and nomenclatural authorities for all taxa are supplied in Tables 1 and 2.

Morphological analyses

The microscopical features for the discussed taxa of *Ochricompectae* were examined and compared with existing type specimens. All microscopic observations and measurements - except for basidiospores - were made in ammoniacal Congo red preparations from dried material, after a short aqueous KOH pretreatment to improve tissue dissociation and matrix dissolution. We refer the reader to Buyck (1991) for methodology and explanation of cystidial terminology. Contents of hymenial cystidia, dermatocystidia and lactifers were

tested for their reaction to sulfoaldehydes. All parts of the fruit bodies were examined for the presence of ortho- or metachromatic contents or incrustations in cresyl blue as explained in Buyck (1989). Observations and measurements on basidiospores and their ornamentation were made in Melzer's reagent.

The authors follow Larsson and Larsson (2003) for circumscription of systematic groups in *Russulales* and the russuloid clade, Sarnari (1998) for the systematics of European *Russula* and Heilmann-Clausen *et al.* (1998) for European *Lactarius*.

Photographic illustrations of the macroscopical aspect of the discussed taxa have been made available online (<http://www.mtsn.tn.it/-russulales-news/welcome.asp>).

Phylogenetic analyses

Taxon sampling and molecular techniques

For this study we sampled 67 taxa (Table 3) within the 'eurussuloid' clade sensu Larsson and Larsson (2003): the ingroup includes 28 *Lactarius* (representing all of the six recognized subgenera) and 30 *Russula* (representing five of the six recognized subgenera); the 9 species used as outgroup represent 'amylostereaceae' clade (1 species), 'auriscalpiaceae' clade (1 species), 'albatrellus' clade (1 species); 'bondarzewiaceae' (2 species), 'gloeocystidiellum 1' clade (1 species), 'peniophorales' clade (2 species), and 'stereales' clade (1 species).

DNA was extracted from dried specimens, using either Dneasy Plant Minikit (Qiagen, Crawley, U.K.) or PrepMan Ultra (Applied Biosystems, Foster City, CA), followed by purification with JETquick general DNA cleanup columns (Genomed, Löhne, Germany). PCR amplification followed Eberhardt (2002). Amplified PCR products were purified with QIAquick PCR (Qiagen Valencia, CA, USA) or Viogene PCR clean-up (Viogene, Sunnyvale, CA, USA) prior to automated sequencing using CEQ or BigDye chemistries and respectively a CEQ 2000 automated sequencer (Beckman Coulter,

Table 1. Representativity of sampling for the main infrageneric subdivision of *Russula* Pers. (following the classification of Sarnari 1998).

<p>Subgenus <i>Compacta</i> (Fr.) Bon Section <i>Archaeinae</i> R. Heim ex Buyck & Sarnari <i>R. camarophylla</i> Romagn., <i>R. earlei</i> Peck Section <i>Compactae</i> Fr. <i>R. acrifolia</i> Romagn., <i>R. albonigra</i> (Krombh.) Fr., <i>R. compacta</i> Frost cf. <i>R. sp.</i> (Madagascar), <i>R. nigricans</i> Fr., <i>R. ochricompacta</i> Fr. & O.K. Miller, <i>R. zonaria</i> Buyck & Desjardin Section <i>Lactarioides</i> (Bataille) Konrad & Joss. <i>R. delica</i> Fr. cf.</p>
<p>Subgenus <i>Heterophyllidia</i> Romagn. Section <i>Griseoflaccidae</i> Sarnari ad int. Section <i>Heterophyllae</i> Fr. <i>R. aeruginea</i> Fr., <i>R. cyanoxantha</i> (Schaeff.) Fr., <i>R. grisea</i> Fr., <i>R. heterophylla</i> (Fr.) Fr., <i>R. ochrospora</i> (Nicolaj) Quadr., <i>R. parazurea</i> Jul. Schäff., <i>R. vesca</i> Fr., <i>R. virescens</i> (Schaeff.) Fr.</p>
<p>Subgenus <i>Amoenula</i> Sarnari</p>
<p>Subgenus <i>Ingratula</i> Romagn. Section <i>Felleinae</i> (Melzer & Zvára) Sarnari Section <i>Ingratae</i> (Qué.) Maire <i>R. farinipes</i> Romell, <i>R. foetens</i> Pers. cf., <i>R. illota</i> Romagn., <i>R. pallescens</i> P. Karst., <i>R. pectinatoides</i> Peck Section <i>Subvelatae</i> Singer</p>
<p>Subgenus <i>Russula</i> Romagn. emend. Section <i>Messapicae</i> Sarnari Section <i>Paraincrustatae</i> Sarnari <i>R. lepida</i> Fr. Section <i>Polychromae</i> (Maire) Sarnari Section <i>Russula</i> (Romagn.) Sarnari <i>R. emetica</i> (Schaeff.: Fr.) Pers., <i>R. firmula</i> Jul. Schäff., <i>R. maculata</i> Qué. & Roze, <i>R. persicina</i> Krombh. Section <i>Tenellae</i> Qué. <i>R. gracillima</i> Jul. Schäff.</p>
<p>Subgenus <i>Incrustatula</i> Romagn. emend. Section <i>Amethystinae</i> (Romagn.) Sarnari <i>R. risigallina</i> (Batsch) Sacc. Section <i>Lilaceinae</i> (Melzer & Zvára) Konrad & Joss.</p>

Fullerton, CA, USA) or ABI PRISM 310 Genetic or 3700 DNA analysers (PE Applied Biosystems, Foster City, CA, USA). We amplified and sequenced the three following loci: The internal transcribed spacers and the 5.8S (ITS1-5.8S-ITS2) using primers ITS1F - ITS4 (White *et al.*, 1990), NL1 - NL4 (Gardes and Bruns, 1993), the nuclear ribosomal large subunit (nucLSU) using primers LR0R - LR7 (or LR5) (Vilgalys and Hester, 1990; <http://www.biology.duke.edu/fungi/mycolab/primers.htm>), and part of the second largest subunit of the RNA polymerase II (*RPB2*, region 6-7) using primer bRPB2 6f - fRPB2 7cr (Liu *et al.*, 1999; Matheny, 2005). Sequences

were assembled and edited using the software package SequencherTM 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Alignments of the ITS1-5.8S-ITS2 [including a small part of the ribosomal small nuclear subunit (nucSSU)], nucLSU and *RPB2* (6-7) sequences for the 67 taxa listed in Table 3 were prepared using PAUP* (Swofford, 2002) and MacClade 4.05 (Maddison and Maddison, 2002).

Phylogenetic analyses

Topological incongruence among our data sets (nucSSU+ITS+5.8S+ITS2+nucLSU and *RPB2*) was examined using 500 replicates

Table 2. Representativity of sampling for the main infrageneric subdivision of *Lactarius* Pers. (following Heilmann-Clausen *et al.*, 1998, with additions of extra-European taxa (*) following Verbeken, 2001).

Subgenus *Lactarius*

Section *Lactarius*

L. piperatus (Scop.:Fr.) Pers.

Subgenus *Lactifluus* (Burl.) Hesler & A.H. Sm

Section *Lactiflui* (Burl.) Hesler & A.H. Sm.

L. volemus (Fr.:Fr.) Fr.

***Section *Gymnocarpi* R.Heim ex Verbeken**

L. longisporus Verbeken

***Section *Rubroviolascetini* (Singer) Verbeken**

L. rubroviolascens R. Heim

Subgenus *Lactariopsis* (Henn.) R. Heim

Section *Albati* (Bataille) Singer

L. deceptivus Peck, *L. vellereus* (Fr.: Fr.) Fr.

***Section *Chamaeleontini* Verbeken**

L. emergens Verbeken, *L. madagascariensis* Verbeken & Buyck.

***Section *Lactariopsis* (Henn.) R. Heim**

L. pelliculatus (Beeli) Buyck, *L. velutissimus* Verbeken

Subgenus *Piperites* (Fr. ex J. Kickx f.) Kauffman

Section *Atroviridi* Hesler & A.H. Sm.

Section *Glutinosi* (Quél.) Bataille

L. flexuosus (Pers.: Fr.) Gray, *L. trivialis* (Fr.:Fr.)Fr.

Section *Uvidi* (Konrad) Bon (invalid)

Section *Zonarii* Quél.

L. citriolens Pouzar, *L. furcatus* Coker, *L.zonarius* (Bull.) Fr.

Section *Deliciosi* (Fr.: Fr.) Redeuilh, Verbeken & Walley

L. quieticolor Romagn.

Section *Torminosi* (Fr.: Fr.) Cooke

L. pubescens Fr.

Section *Colorati* (Bataille) Hesler & A.H. Sm.

Subgenus *Russularia* (Burl.) Kauffman

Section *Russularia* Fr. ex Burl.

L. subsericatus(Kuhner & Romagn. ex Bon

Section *Tabidi* Fr. (invalid)

Section *Olentes* (Bataille) Basso

L. camphoratus (Bull.) Fr.

Subgenus *Plinthogali* (Burl.) Hesler & A.H. Sm.

Section *Plinthogali* (Burl.) Singer

L. angiocarpus Verbeken & U. Eberh., *L. lignyotus* Fr., *L. romagnesii* Bon, *L. acris* (Bolton: Fr.) Gray

Section *Fumosi* Hesler & A.H. Sm.

Unclassified

***Section *Edules* Verbeken**

L. densifolius Verbeken & Karhula, *L. edulis* Verbeken & Buyck, *L. inversus* Gooss.-Font. & R. Heim, *L. nodosicystidiosus* Verbeken & Buyck, *L. phlebophyllus* R. Heim

Table 3. Taxon sampling and Genbank accession numbers for the regions sequenced (‘---’ indicates presence of missing data for part of the sequence in the analyses).

Taxon^a	Collection source^b	Location	Herbarium	GenBank acc. ITS1-5.8S-ITS2+nucLSU	GenBank acc. <i>RBP2</i> (region 6-7)
Eurussuloid clade					
/amylostereaceae					
<i>Amylostereum laevigatum</i>	olrim409/CBS623.84			AY781246+AF287843	AY218469
/auriscalpiaceae					
<i>Auriscalpium vulgare</i>	AFTOL1897/ DAOM128994			DQ911613+DQ911614	AY218472
/albatrellus					
<i>Albatrellus skamianus</i>	DAOM220694/ Bgthesis			--- +AF393044	AY218466
/bondarzewiaceae					
<i>Bondarzewia montana</i>	AFTOL452			DQ200923+DQ234539	AY218474
<i>Echinodontium tinctorium</i>	AFTOL455			AY854088+AF393056	AY218482
/gloeocystidiellum 1					
<i>Gloeocystidiellum porosum</i>	EB990923			AY048881	DQ408126
/peniophorales					
<i>Peniophora nuda</i>	AFTOL660			DQ411533+AF287880	DQ408129
<i>Scytinostroma alutum</i>	CBS 762.81			--- +AF393075	DQ408130
/russulales					
<i>Lactarius acris</i>	EU014	GERMANY	UPS	DQ421988	DQ421922
<i>Lactarius angiocarpus</i>	DA00-448	ZAMBIA	GENT	--- +DQ421981	DQ421921
<i>Lactarius camphoratus</i>	UE04.09.2004-5	SWEDEN	UPS	DQ422009	DQ421933
<i>Lactarius citriolens</i>	UE20.09.2004-03	SWEDEN	UPS	DQ422003	DQ421931
<i>Lactarius deceptivus</i>	AV04-181	USA	GENT	DQ422020	DQ421935
<i>Lactarius densifolius</i>	BB 12.1994	BURUNDI	PC	DQ421980	DQ421920
<i>Lactarius edulis</i>	AV99-041	ZIMBABWE	GENT	DQ421977	DQ421916
<i>Lactarius emergens</i>	AV 99-005	ZIMBABWE	GENT	AY606979 + ---	DQ421919

Table 3 (continued). Taxon sampling and Genbank accession numbers for the regions sequenced (‘---’ indicates presence of missing data for part of the sequence in the analyses).

Taxon^a	Collection source^b	Location	Herbarium	GenBank acc. ITS1-5.8S-ITS2+nucLSU	GenBank acc. <i>RBP2</i> (region 6-7)
<i>Lactarius flexuosus</i>	UE06.09.2002-1	SWEDEN	UPS	DQ421992	DQ421925
<i>Lactarius furcatus</i>	RH7804	COSTA RICA	NY	DQ421994	DQ421927
<i>Lactarius inversus</i>	AB63	GUINEA	GENT	DQ421978	DQ421917
<i>Lactarius lignyotus</i>	UE06.09.2003-5	SWEDEN	UPS	DQ421993	DQ421926
<i>Lactarius longisporus</i>	AV99-197	ZIMBABWE	GENT	DQ421971 (AV)	DQ421910 (BB)
	BB 00.1519	MADAGASCAR	PC		
<i>Lactarius madagascariensis</i>	BB 99-409	MADAGASCAR	PC	DQ421975 + ---	DQ421914
<i>Lactarius nodosicytidiosus</i>	BB 97-072	MADAGASCAR	PC	DQ421976	DQ421915
<i>Lactarius pelliculatus</i>	BB00-1335	MADAGASCAR	PC	DQ421974	DQ421913
<i>Lactarius phlebophyllus</i>	BB00-1388	MADAGASCAR	PC	DQ421979	DQ421918
<i>Lactarius piperatus</i>	UE09.08.2004-6	SWEDEN	UPS	DQ422035	DQ421937
<i>Lactarius pubescens</i>	UE15.09.2002-2	SWEDEN	UPS	DQ421996	DQ421929
<i>Lactarius quieticolor</i>	UE10.09.2004-1	SWEDEN	UPS	DQ422002	DQ421930
<i>Lactarius romagnesii</i>	UE29.09.2002-6	FRANCE	UPS	DQ421989	DQ421923
<i>Lactarius rubroviolascens</i>	BB 97.266	MADAGASCAR	PC	--- +DQ421972	DQ421911
<i>Lactarius subsericatus</i>	UE11.10.2004-8	SWEDEN	UPS	DQ422011	DQ421934
<i>Lactarius trivialis</i>	UE27.08.2002-17a	SWEDEN	UPS	DQ421991	DQ421924
<i>Lactarius vellereus</i>	UE20.09.2004-22	SWEDEN	UPS	DQ422034	DQ421936
<i>Lactarius velutissimus</i>	AV 99-185	ZIMBABWE	GENT	DQ421973	DQ421912
<i>Lactarius volemus</i>	UE09.08.2004-5	SWEDEN	UPS	DQ422008	DQ421932
<i>Lactarius zonarius</i>	UE27.09.2002-4	FRANCE	UPS	EU278678	EU278679
<i>Russula cyanoxantha</i>	UE29.09.2002-2	FRANCE	UPS	DQ422033	DQ421970
<i>Russula aeruginea</i>	AT2003017	SWEDEN	UPS	DQ421999	DQ421946
<i>Russula albonigra</i>	AT2002064	SWEDEN	UPS	DQ422029	DQ421966

Table 3 (continued). Taxon sampling and Genbank accession numbers for the regions sequenced (‘---’ indicates presence of missing data for part of the sequence in the analyses).

Taxon^a	Collection source^b	Location	Herbarium	GenBank acc. ITS1-5.8S-ITS2+nucLSU	GenBank acc. <i>RBP2</i> (region 6-7)
<i>Russula camarophylla</i>	PAM01081108	FRANCE	PC	DQ421982	DQ421938
<i>Russula compacta</i>	Duke s.n.	USA		AF287888	AY218514.1
<i>Russula cf. compacta</i>	AV04130	THAILAND	PC	DQ422001	DQ421948
<i>Russula cf. foetens</i>	UE18.07.2003-7	SWEDEN	UPS	DQ422023	DQ421962
<i>Russula cf. delica</i>	UE24.08.2004-20	SWEDEN	UPS	DQ422005	DQ421950
<i>Russula earlei</i>	WCRW00-412	USA	PC	DQ422025	DQ421963
<i>Russula emetica</i>	UE05.10.2003-11	SWEDEN	UPS	DQ421997	DQ421943
<i>Russula farinipes</i>	UE28.09.2002-4	FRANCE	UPS	DQ421983	DQ421939
<i>Russula firmula</i>	AT2004142	SWEDEN	UPS	DQ422017	DQ421958
<i>Russula gracillima</i>	UE23.08.2004-14	SWEDEN	UPS	DQ422004	DQ421949
<i>Russula grisea</i>	UE2005.08.16-01	SWEDEN	UPS	DQ422030	DQ421968
<i>Russula sp.</i>	BB99.250	MADAGASCAR	PC	DQ422028	DQ421965
<i>Russula heterophylla</i>	UE20.08.2004-2	SWEDEN	UPS	DQ422006	DQ421951
<i>Russula illota</i>	UE26.07.2002-3	SWEDEN	UPS	DQ422024	DQ421967
<i>Russula lepida</i>	HJB9990	BELGIUM	UPS	DQ422013	DQ421954
<i>Russula maculata</i>	HJB10019	BELGIUM	UPS	DQ422015	DQ421956
<i>Russula nigricans</i>	UE20.09.2004-07	SWEDEN	UPS	DQ422010	DQ421952
<i>Russula ochricompacta</i>	BB02.107	USA	PC	DQ421984	DQ421940
<i>Russula ochricompacta^c</i>	BB02.118	USA	PC	DQ421986+DQ422036	
<i>Russula ochrospora</i>	GD20.07.2004	ITALY	UPS	DQ422012	DQ421953
<i>Russula pallescens</i>	PL146/2002	NORWAY	TUR	DQ421987	DQ421941
<i>Russula parazurea</i>	BW06.09.2002-16/MF01.10.2003	SWEDEN	UPS	DQ422007 (MF)	DQ421945 (BW)
<i>Russula pectinatoides</i>	AT2001049	SWEDEN	UPS	DQ422026	DQ421964
<i>Russula persicina</i>	UE21.09.2003-01	SWEDEN	UPS	DQ422019	DQ421960

Table 3 (continued). Taxon sampling and Genbank accession numbers for the regions sequenced (‘---’ indicates presence of missing data for part of the sequence in the analyses).

Taxon^a	Collection source^b	Location	Herbarium	GenBank acc. ITS1-5.8S-ITS2+nucLSU	GenBank acc. <i>RBP2</i> (region 6-7)
<i>Russula risigallina</i>	UE03.07.2003-08	SWEDEN	UPS	DQ422022	DQ421961
<i>Russula vesca</i>	AT2002091	SWEDEN	UPS	DQ422018	DQ421959
<i>Russula virescens</i>	HJB9989	BELGIUM	UPS	DQ422014	DQ421955
<i>Russula zonaria</i>	DED7442	THAILAND	isotype PC	DQ421990	DQ421942
/stereales					
<i>Stereum hirsutum</i>	AFTOL492			AY854063+AF393078	AY218520

^a Classification follows Larsson and Larsson 2003.

^b Collection sources: AB=Amadou Bâ, AT=Andrew Taylor, AV=Annemieke Verbeken, BB=Bart Buyck, BW=Birgitta Wasstorp, DA=David Arora, Dennis. E. Desjardin, EU=Elisabeth Uhlmann, GD=Guiseppe Donelli, HJB=Henri J. Beker, MF=Marco Floriani, PAM=Pierre Arthur Moreau, PL=Perry Larsen, RH=Roy Halling, UE=Ursula Eberhardt, WCR=William C. Roody,

^c Sequences obtained to verify the *R. ochricompacta* species concept (100% ITS and nucLSU similarity between *R. ochricompacta* BB02.107 and BB02.118)

of maximum likelihood bootstrapping (ML-BS) with the GTRMIX model and gamma distribution conducted in RAxML-VI-HPC (RAxML-bs; Stamatakis *et al.*, 2005). The two data sets were partitioned as follows for combinability tests: five partitions for the ribosomal data (nucSSU, ITS1, 5.8S, ITS2, nuLSU), and two partitions for *RPB2* (1st and 2nd, 3rd position). To screen for putative conflict we used the program compat.py (available at www.lutzonilab.net), which compares ML-BS values of the loci. A conflict was assumed to be significant when two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values BS $\geq 70\%$ (Mason-Gamer and Kellogg, 1996).

A maximum likelihood search for the most likely tree on the data set combining the two data sets for 67 congruent taxa was completed with 500 replicates using RAxML with the same settings as applied in the bootstrap analyses. In addition, bayesian analyses using Bayesian Metropolis coupled Markov chain Monte Carlo algorithm (B-MCMCMC) as implemented in MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001) were completed on the three-locus data sets. Rooting of the phylogenies used *Stereum hirsutum*, according to Binder and Hibbett (2002), Binder *et al.* (2005) and Lutzoni *et al.* (2004), the latter being the only study that had recovered some significant support for internal relationships within the 'russuloid' clade. Bayesian analyses were implemented with four independent chains, with every 500th tree sampled for 20,000,000 generations, using a GTR model of nucleotide substitution, with an estimated proportion of invariable sites and a gamma distribution approximated by four categories. To verify that all runs converged to the same log-likelihood stationary level, we conducted three independent B-MCMCMC runs. The influence of different partitioning of the data on phylogenetic inference and support was examined with both ML and bayesian methods. Five different data set partitionings were used: 2 partitions (nucSSU+ITS1+5.8S+ITS2+nuLSU, *RPB2* 1st+2nd+3rd), 4 partitions (nucSSU+nuLSU+5.8S, ITS1+ITS2, *RPB2* 1st+2nd, *RPB2* 3rd), 5 partitions (nucSSU+nuLSU+5.8S, ITS1+ITS2, *RPB2* 1st, *RPB2*

2nd, *RPB2* 3rd), 6 partitions (nucSSU, nuLSU, 5.8S, ITS1+ITS2, *RPB2* 1st, 2nd, *RPB2* 3rd), 7 partitions (nucSSU, nuLSU, 5.8S, ITS1+ITS2, *RPB2* 1st, *RPB2* 2nd, *RPB2* 3rd).

Branch support for the phylogeny that combined three-locus data set was estimated with bootstrap values obtained from 500 replicates of ML bootstrapping conducted with RAxML and posterior probabilities (PP) derived from a majority-rule consensus tree built from the last 10'000 trees of the three independent bayesian runs (30'000 trees total). Bayesian posterior probabilities (PP) $\geq 95\%$ and ML bootstrap values (ML-bs) $\geq 70\%$ were considered to be significant.

We applied the SH-test statistic (Shimodaira-Hasegawa, 1999) as implemented in PAUP* to determine if unconstrained and constrained phylogenies were equally good explanations of the data (H_0) or not (H_1). This test was conducted with a GTR evolutionary model with all parameters estimated during search and running 1000 bootstrap replicates with full optimization (one-tailed test)

Results

Morphological evidence

The various species attributed to *Russula* subsect. *Ochricompactae* are here discussed in alphabetical order and followed by a commentary on *Lactarius furcatus*.

Russula aurantiophylla Buyck & Ducousso, Cryptogamie Mycologie 25: 127. 2004.

(Fig. 1)

Pileus very small, not beyond 26 mm in diam when dry, depressed in the center, pubescent-felty with the hairs being arranged in a concentric fashion towards the margin, continuous, dry, dull, not separable, chalk-white when young, later developing cream, ochre to brown tints; margin smooth, waving or irregular, distinctly involuted, later with distinct concentric depressions. *Gills* much narrower than the cap trama thickness, ca 2 mm, attenuating towards the cap margin, relatively close, frequently bifurcating, slightly decurrent with the hymenium forming an abruptly delimited orange band at the stipe apex; gill edge even and concolorous when young. *Stipe* shorter than the cap diam., central

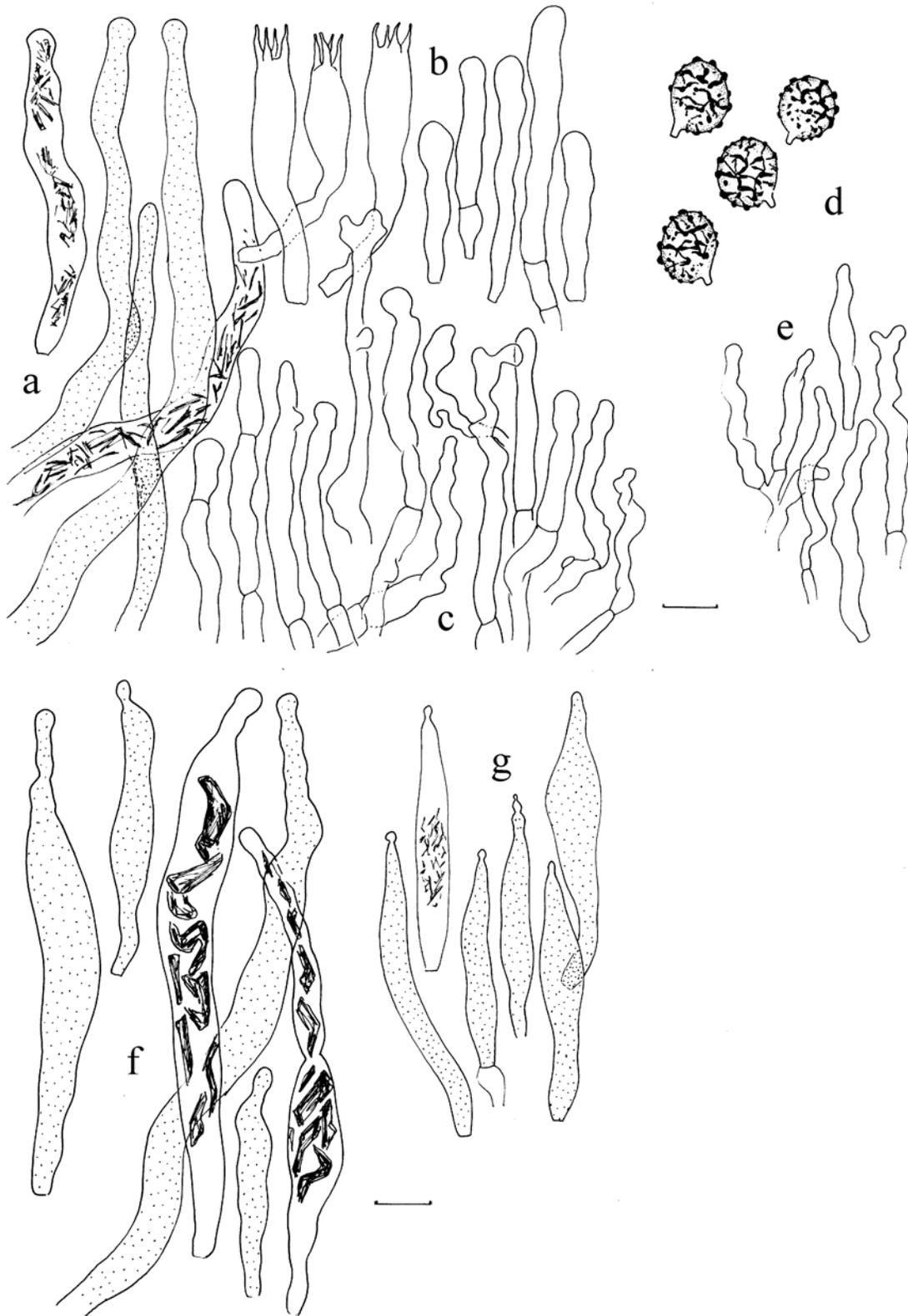


Fig. 1. *Russula aurantiophylla* (holotype). **a.** Dermatozystidia. **b.** Basidia and basidiola. **c.** Terminal cells of hyphae in the cap surface. **d.** Spores in Melzer's reagent. **e.** Marginal cells of the gills. **f.** Pleuromacrozystidia of type 1. **g.** Pleuromacrozystidia of type 2. Bar= 5 μm for spores, 10 μm for all other elements.

to slightly eccentric, cylindrical to tapering downwards, chalk white, wrinkled-furrowed near the base and not obtusely rounded but irregularly spreading in an almost root-like fashion, agglomerating and incorporating parts of soil, scrobiculate from large gelatinous, grayish 'droplets' or masses of variable size. *Context* 10-12 mm thick in pileus, white but developing lemon yellow colors towards the base, not exuding milk on injury. *Smell* and *taste* not noted. *Spore print* probably orange. *Exsiccatum* pale yellowish brown to dirty off white, with clear narrow concentric depressions near the cap margin, gills a dark pinkish brown, very thick, with undifferentiated edge.

Spores shortly ellipsoid to subglobose, very small, (5.8)6.2-6.5-6.8(7.1) × (5.1)5.4-5.62-5.9(6) μm, Q = (1.05)1.15(1.25), ornamentation relatively high compared to spore size, composed of strongly amyloid crests and convex warts locally interconnected by subtle tracts, the whole forming a subreticulate to almost reticulate, sometimes dense pattern; suprahilar spot relatively small, inamyloid. *Basidia* 44-50 × 7-8 μm, clavate, four-spored, with rather slender, long sterigmata, 5-6 × 1 μm; basidiola very sinuous and irregular in outline, subcylindrical to clavate; also with many very slender, irregular, sometimes abruptly branched or swollen dispersed elements. *Cystidia* very abundant on sides and edges of the gills, hardly emergent or projecting up to 20-30 μm beyond the basidia, with abundant, refringent, granular-crystalline contents, of two types: the first type rather slender and short, mostly 40-58 × 5-8 μm, originating in the hymenium-subhymenium, fusiform and minutely mucronate, filled with finely crystalline contents; the second type much more robust and larger, although of very variable size, originating in subhymenium-trama, (40)80-148 × (6)8-12 μm, possibly longer and continuing for considerable distance underneath the subhymenium, fusiformous, clavate-pedicellate to lageniformous, often largely capitate or appendiculate, with a neck of variable length, filled with coarsely refringent-crystalline contents, weakly SV+. *Marginal cells* not differentiated, but the gill edge with smaller, slightly more slender elements. *Lamellar trama* filamentous, hyphae

intermixed with many, variably long, cylindrical, sinuous, weakly SV+, cystidioid elements and the protruding bases of the type 2-pleurocystidia. *Subhymenium* extremely well developed, up to 150 μm deep, densely composed of narrow elements. *Pileipellis* one-layered, slightly gelatinized, entirely orthochromatic in cresyl blue, a relatively thick cutis of 3-6 μm wide hyphae with very irregular, nodose-tortuous extremities near the surface, tightly interwoven into a dense tissue, sometimes with pale brownish pigments, thin-walled or with slightly thickened wall near the very tip. *Pileocystidia* numerous, arising from underneath the surface, cylindrical to fusiformous, 5-9 μm diam., with distinct and abundant, granular or more generally coarsely crystalline contents. *Oleiferous hyphae* present, but rare, oily-refringent. *Stipitipellis* similar to the cap surface near the top, caulocystidia more slender and more capitate, in the lower half with very long rhizoids composed of thick-walled slender hyphae. *Clamps* absent.

Specimens examined: NEW CALEDONIA, near Koniambo, under *Nothofagus*, M. Ducouso K18-2 (**holotype**, PC).

Commentary: As this species was only published as a short latin diagnosis, we here provided a detailed illustrated description. In the field, *R. aurantiophylla* resembles a tiny specimen of *R. ochricompacta*. It possesses the same overall colour and lactarioid features (scrobiculae on the stipe, a concentrically zoned cap and inrolled cap margin), but it has thicker, more widely spaced gills.

The microscopic differences, however, are striking and clearly indicate a distinct species (Fig. 1). Although the tissues of the type of *R. aurantiophylla* do not inflate very well, the subhymenium is impressively developed and unusually deep. Both the hymenium and subhymenium contain abundant ripe basidiospores that are trapped in between the cells. This feature has never been observed in any species of *Russulaceae* and suggests some kind of repetitive growth or secondary extension of the hymenium. The latter feature has been described for many resupinate fungi but not for any agaricoid species. *Russula. aurantiophylla* possesses two types of hymenial cystidia: one is small and restricted to the surface (i.e. hymenium level), whereas the

second type is much more voluminous, originates deeply in the subhymenium or lamellar trama, and probably continues to elongate during the subsequent thickening of the gills. These are not really pseudocystidia, but rather endings of the abundant cystidioid elements present in the gill trama (in the sense of Buyck, 1991: cylindrical long cystidia of the context that are not lactifers since they do not ramify into a network, but are unbranched and of determinate length). Similar cystidioid elements are common in other, mostly acrid *Russula* spp. as well. The lamellar trama contains hardly any sphaerocytes as is typical for temperate *Lactarius* spp. but not for *Russula* and is likely indicative of ancient species.

Russula grossa Berk., Hook. J. Bot. 3: 39. 1851.

Specimen examined: INDIA: Siccim, coll. Berkeley, 1851, in Herbarium Hookerianum 1867 (holotypus, K sub nr. 109298).

Commentary: *R. grossa* was described on a single specimen from the Himalayan foothills of Sikkim, India. The original description (*Pileo cyathiformi viscoso maculato-squamoso, margine rugoso involuto; stipite crasso obeso subaequali; lamellis decurrentibus antice latioribus integris. Hab. Darjeeling.*) is accompanied by the following comments: “*Well characterized by its viscid, spotted pileus and coarse habit. The gills are yellowish when dry, but I cannot ascertain the colour of the spores.*”

The type is a slice of a sporophore that is very heavily mold-infested and damaged by insects. It is nevertheless easy to exclude it from *Ochricompectae* by all of its features. This taxon, which is also unrelated to *Russula melliolens* as suggested by Singer (1986), is not further considered here. It very likely belongs to section *Heterophyllae* as suggested by the viscid pileus, the inamyloid suprahilar spot on the spores and, especially, by the few typical ‘*Heterophyllae*’-extremities that we were able to observe in the pileipellis.

The earlier discussion and subsequent transfer of *R. grossa* to *Ochricompectae* (Bills and Pegler, 1988) was based on a misinterpretation of this taxon and its application to a

much more recent specimen collected by Atri in 1982 in the Himalayan mountains, which is indeed a good representative of *Ochricompectae* (see below).

Russula grossa sensu Bills & Pegler 1988 ac sensu Saini & Atri 1982, non Berkeley 1851. (Fig. 2)

Fruit bodies up to 10 cm high. *Pileus* up to 9.5 cm diam., infunduliform with involuted, irregular to almost wavy margin; surface dry with pruinose fibrillose scales, yellowish white (1A2). *Gills* decurrent, crowded, equal but dichotomously forked, broad, yellowish brown (5D8), unchanging when bruised; edge entire, concolorous. *Stipe* up to 3.7 × 2 cm, central, stout, tough, broad above and tapering below, white, pruinose, solid, unchanging when bruised. *Flesh* white, unchanging, not exuding milk on injury. *Taste* bitter. *Odor* disagreeable. KOH on cap surface orange yellow. Phenol on cap surface coffee brown. *Spore print* pale orange (5A3). *Exsiccatum* pale isabelline, gills dark reddish brown without crystalline paler deposits on gill edge.

Spores very small, ellipsoid to almost larmiformous, (5.9)6-6.39-6.8(7.1) × (4.3)4.5-4.79-5.1(5.5) μm, Q = (1.24)1.34(1.44); ornamentation overall low, composed of small convex warts interconnected or incompletely so by fine lines or forming short crests, some warts nevertheless very distinct and strongly amyloid; suprahilar spot inamyloid but distinct. *Basidia* short, (30)35-46 × 6.5-8 μm, stout, sinuous to shortly clavate, widest at the tip, 4-spored; sterigmata stout for their small size. *Cystidia* large, 77-160 × 9-22 μm, imbedded or hardly projecting above the basidia-level, fusiform-lageniform, minutely capitate to rostrate, often strongly inflated near or below the trama-subhymenium transition, thin-walled, with rather poor crystalline contents. *Pseudocystidia* not observed. *Marginal cells* not or hardly differentiated, some elements more inflated near the tip and broadly capitate. *Subhymenium* difficult to observe due to rapidly collapsing cells, taking hardly any color in Congo Red. *Lamellar trama* almost without sphaerocytes, a dense tissue of slender hyphae.

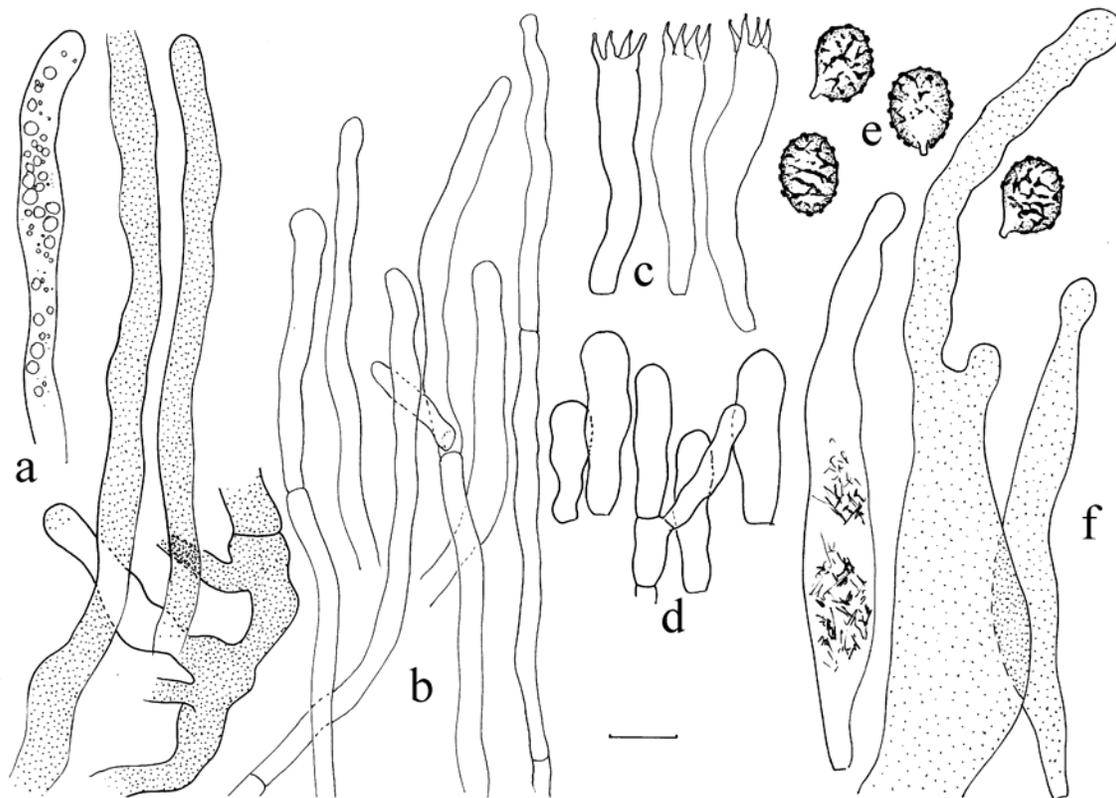


Fig. 2. *Russula grossa* sensu Bills & Pegler 1988 ac sensu Saini & Atri 1982, non Berkeley 1851. (isotype K). **a.** Dermatomycetia. **b.** Terminal cells of hyphae in the cap surface. **c.** Basidia. **d.** Basidiola. **e.** Spores in Melzer's reagent. **f.** Pleuromacrocystidia. Bar= 5 μ m for spores, 10 μ m for all other elements.

Pileipellis poorly developed, entirely orthochromatic in Cresyl blue, single layered, a thin cutis of entangled, narrow and very thin-walled, easily collapsing hyphae measuring 3-5 μ m diam., sparsely septate, simply rounded at the tip, not strongly ramifying. *Pileocystidia* absent. *Pseudocystidia* not observed, but the majority of the hyphae just underneath the cap surface have an oleiferous aspect, although their contents are probably of a different nature being strongly refringent and locally appearing as if perforated possible as the result from deposition of some substance. Typical oleiferous hyphae are present in deeper layers. *Clamps* absent.

Specimen examined: INDIA: Himachal Pradesh, Simla, Summer Hill, scattered on humicolous soil in *Pinus roxburghii* forest, 1983 m alt., 23 August 1979, N.S.Atri 10 (PUN 272 isotype, K **holotype**).

Commentary: The microscopic features illustrated by Saini and Atri (ut *R. grossa*) correspond very well to our observations but lack precision, in particular with respect to the spore ornamentation which is not isolate but of

the same type as in *R. ochricompacta*, yet is mostly also composed of some dispersed higher warts and, on the whole, better developed than in the latter species. Further arguments for recognizing this collection as an independent species include the ecology, its geographical isolation and the different smell. The other differences are quantitative and thus taxonomically very difficult to exploit, although the exsiccatum suggest that there should also be differences in overall color of the fruit bodies.

Russula ochricompacta Bills & O.K. Mill., *Mycologia* 76: 976. 1984.

Pileus 69-172 mm diam., depressed in the center, felty to granular or distinctly roughened but continuous, mostly dry and dull, less often shiny but not viscous, not pruinose, not separable, whitish to chalk-white, later developing cream, ochre to pale brown to orange brown tints; margin smooth, thin when expanded, often undulate or irregular, strongly involuted, sometimes with a distinct concentric

zonation in the form of paler, almost whitish, slightly depressed circles. *Gills* much narrower than the cap trama thickness, 3-5 mm high, attenuating towards the cap margin, crowded (18L+1/cm at 1 cm from margin), frequently bifurcating, adnate to strongly decurrent with a tooth of up to more than 1 cm along stipe, quickly orange yellow from the centre towards the margin, gill edge with an irregularly deposited white fringe. *Stipe* 32-75 × 16-41 mm, central to strongly eccentric, shorter than the cap diam., cylindrical or tapering downwards, chalk white, not squamose but entirely pruinose to pulverulent, strongly wrinkled-furrowed near the base and not obtusely rounded but irregularly spreading in an almost root-like fashion, agglomerating and incorporating soil particles, sometimes distinctly scrobilucate from gelatinous, greyish 'droplets' of variable size, firm and hard but very young already completely hollow. *Context* not exuding milk on injury, 10-12 mm thick in pileus, white but with greyish zones when water-soaked and differently organized in stipe and cap, the greyish zones being visible as irregular, relatively small, circular-spherical dots or islands in the stipe but in the form of a clear concentric zonation in the cap that extends over the entire thickness of the flesh (from surface to dorsal gills), without bruising reactions but developing sometimes lemon yellow colors towards the base. *Smell* strong and persistent, of citronella, remaining present for a long time in dried specimens. *Taste* mild or slightly astringent to nauseous. *Spore print* bright orange (5A6-7). *Exsiccatum* with grayish white pileus and stipe, the pileus surface very uneven as if 'dried up' in a mosaic-like structure. Gills dirty greenish brown with the edge irregularly covered in off-white crystalline-like deposits

Spores very small, elliptical, (4.8)5.1-5.39-5.7(5.8) × 3.9-4.18-4.4(4.6) μm, Q = (1.19) 1.29(1.38); ornamentation subreticulate but very low, although variable in height, sometimes producing clear amyloid irregular, laterally extending warts and short to long ridges forming an incomplete network, at other times hardly visible; suprahilar spot not amyloid but distinct. *Basidia* slender, 4-spored, *Cystidia* (45)70-180(250) × 11-20(35) μm,

dispersed (600-700/mm²), hardly emergent, but deeply embedded in the trama and often rostrate with a long narrow neck ascending in the hymenium leaving the wider body of the cystidium in the subhymenium or underlying trama, with coarsely crystalline to refringent contents. *Marginal cells* abundant, small, subcylindrical and more or less nodulose-moniliformous, often shortly forked. *Pseudocystidia* absent. *Subhymenium* dense, very difficult to observe. *Lamellar trama* with many sphaerocytes and some long, relatively thick, oleiferous-like, refringent hyphae. *Pileipellis* poorly developed, not gelatinized, orthochromatic in cresyl blue, composed of cylindrical, very thin-walled, loosely intertwined and sparsely septate hyphae, 3-6 μm wide; the terminal cell often somewhat constricted subapically or more or less undulating, neither zebroid wall-incrustations nor pigmented contents; a network of abundant and often larger refringent-granular, contorted-nodulose and oleiferous-like hyphae present throughout the pellis and with the tips of ca 3-5 μm diam. protruding towards the cap surface, remaining yellowish refringent in cresyl blue; well-characterized pileocystidia absent. *Stipitipellis* with distinct agglomerated bundles of thin-walled, narrow hyphae (trichoids), some with a refringent, yellowish content or wall deposit (difficult to judge), without caulocystidia. *Clamps* absent.

Specimens examined: USA. Texas, Newton Co., Bleakwood, along highway 87, D.Lewis property, in mixed oak-gum floodplain forest, 4 July 2002, Buyck 02.107 (PC); *ibid.*, 5 July 2003, Lewis 6738 (PC), *ibid.*, 18 July 2007, Buyck 07.010, *ibid.*, 24 July 2007, Buyck 07.060; Tyler Co., canyon rim trail, monospecific beech forest, on sandy soil, 5 July 2002, Buyck 02.118 (PC); Mississippi, without locality, 12 July 1997, D.Lewis 5824 (PC); North Carolina, near Asheville, mixed hardwoods, brought in from NAMA foray, 17 July 2004, Buyck 04-279 (PC). Virginia, Poverty Hollow, Montgomery Co., on soil in mixed woods of *Quercus*, *Pinus rigida*, *Acer* and *Liriodendron*, Bills 146 (**holotype**, VPI).

Commentary: *Russula ochricompacta* is the type species of subsection *Ochricompactae*. It is an unmistakable taxon in the field because of its whitish, tomentose (when dry) to almost velvety (if wet) cap and stipe, the orange spore print, the bright orange, regularly forked gills

and the - for the *Russulaceae* - very unusual but distinct citronella smell which persists in dried specimens. The intensity of the spore print colour, described originally as “ochraceous, close to coding IVE in Romagnesi’s chart” is in fact far more intense and well beyond the spore print colour of any other known dark-spored *Russula*-species; it is not exactly darker, but a much brighter, vivid orange !

Buyck (1995) published a commentary on the original description of the features of the type collection, which lacked accuracy for microscopic features. More specifically, Buyck (l.c.) reexamined and illustrated the hymenial features of the type collection, demonstrating that these were almost identical to some resupinate species in the genus *Gloeocystidiellum* (*Corticaceae* s.l.). Molecular studies by Larsson and Larsson (2003) have since shown that certain taxa of *Gloeocystidiellum* with verrucose spores, as well as some species of *Boidinia* are indeed very closely related to agaricoid *Russulaceae*.

The more recent collections cited here are particularly interesting because they exhibit unique morphological features that were perhaps not very evident or overlooked in the type collection (coloured illustrations have been posted at <http://www.mtsn.tn.it/russulales-news/multifurca.asp>). Indeed, *R. ochricompacta* possesses several typical *Lactarius*-features not discussed in the original description. Some of the collections from Texas have a distinctly scrobiculate stipe, for example. This character has never been observed in any other group of *Russula* and was hitherto interpreted as a typical feature of *Lactarius*. The scrobiculae themselves are somewhat different from typical scrobiculae in *Lactarius* as they seem to consist of permanent, large, waxy droplets deposited on the lower stipe surface. In addition, one can easily observe with a hand lens the strong pulverulent to almost velvety nature of the stipe surface in between the scrobiculae of *R. ochricompacta*. Under the microscope, this pulverulence corresponds to the presence of large trichoids - or bundles - of hyphal extremities, a feature described and illustrated by Buyck (1989b: 135-136, Figs 77-78) for some tropical African taxa in *Russula* subsect. *Fistulosinae*, another group that is hard to

distinguish from *Lactarius* in the field. Once again, this is a feature that is typical of many taxa in *Lactarius* but unrecorded among temperate *russulas*.

The concentric zonation of the cap, already illustrated in Metzler and Metzler (1992), in combination with the strongly inrolled cap margin are perfectly comparable to typical *Lactarius* spp., but do not find a match in any of the known species of *Russula*. Also the strongly decurrent, regularly forked gills, sometimes running down the stipe for more than 1 cm, is another example of a previously unrecorded feature for *Russula*.

In conclusion, *R. ochricompacta* can morphologically be summarized as a bright orange spored *Russula* because of the absence of lactifers and pseudocystidia, but with a typical *Lactarius* habit, regularly forked gills and the hymenial features of a *Gloeocystidiellum*.

Russula zonaria Buyck & Desjardin, *Cryptogamie Mycologie* 24(2): 112. 2003.

Specimens examined: THAILAND, Chiang Mai, Doi Suthep, Sangra Sabhasri lane to Huai Kok Ma Village, scattered in soil under *Dipterocarpus costatus* in montane primary forest, elev. 1200 m, 3 July 2002, D.E.Desjardin 7442 (SFSU, BBH, PC); Chiang Mai prov., Mae Teng distr., Tung Yaow village, elev. 1350 m, hill ridge with *Castanopsis*-dominated broadleaved forest disturbed by fire, 21 July 2004, Verbeke A. & Walley R. 2004-032 (GENT).

Commentary: A modern description was supplied by Buyck and Desjardin (2003). The discussion accompanying this description underlined the problematic choice of a correct genus for this taxon, which was not a straightforward decision. *R. zonaria* possesses indeed features that argue both against and in favour of a placement in *Russula*: it has pseudocystidia ending in the hymenium, exactly as in *Lactarius*, without however, possessing the extensive ramified lactiferous system so typical for the latter genus. On the other hand, it exudes no latex and it shares striking overall similarities with *R. ochricompacta*. Although either genus could fit this taxon, the authors decided on *Russula* because of the evident close relationship to *R. ochricompacta*, being unaware at the time of the equally close resemblance to *Lactarius furcatus* Coker (see below).

Lactarius furcatus Coker, J. Elisha Mitchell Sci. Soc. 34: 18. 1918.

Specimens examined: COSTA RICA: Puntarenas, Coto Brus, Las Mellizas, La Amistad Lodge, near Parque interacional La Amistad, 3 July 1998, R. Halling 7804, 8361 (NY). USA: Texas, Newton Co., near Lewis' residence one mile north of Bleakwood, off State Highway 87, 6 July 2000, D.P. Lewis 6330 (PC).

Commentary: A detailed modern description was provided by Montoya *et al.* (2003). For nearly one century, this taxon had been solely known from the description of the (lost) North American type collection. *Lactarius furcatus* was very recently rediscovered in Costa Rican *Quercus* forests by Montoya *et al.* (2003) who suggested that it is very close to *L. zonarius*. The latter species possesses a very similar general aspect, but is unrelated as evident from our molecular analysis.

Having been visually confronted with these recent specimens of *L. furcatus* very shortly after the description of *Russula zonaria*, it was immediately clear that the systematic position of *Ochricompectae* had to be reconsidered and that this was impossible on the basis of morphological arguments alone. Once dried, it is impossible to distinguish between specimens of *R. zonaria* and *L. furcatus* without a microscope.

Under the microscope, *L. furcatus* is a typical representative of *Lactarius*, not only because of the exudation of a latex due to the possession of an extensive ramified lactiferous system that is strongly reacting to sulfoaldehydes and ending in abundant pseudocystidia in the hymenium, but also because of the typical configuration of the context in many individual spherocyte rosettes. None of the *russulas* here discussed possesses these features. Other differences with the *russulas* of subsection *Ochricompectae* concern the colour of the gills, which are white when immature, and the strong, viscose-glutinous aspect of the humid cap for the Costa Rican collections.

Molecular evidence

Our data consist of 194 sequences, of which 55 sequences for ITS1-5.8S-ITS2 (missing for 3 taxa: *L. rubroviolascens*, *L. angiocarpus* and *Russula compacta*), 55

sequences for nucLSU (missing for 3 taxa: *Lactarius emergens*, *L. madagascariensis* and *L. zonarius*), and 57 *RPB2* 'russulales' sequences were newly generated for this study. The remaining 27 sequences were retrieved from GenBank. Alignment of the three loci totaled 1709 characters (nucSSU-ITS1-5.8S-ITS2: 327 char., nucLSU: 851 char., *RPB2*: 531 char.) once ambiguous regions were excluded (nucSSU-ITS1-5.8S-ITS2: 542 char., nucLSU: 169 char.). Alignments are available at the TREE-base website (www.treebase.org).

Based on our congruence criterion (see above), apparent conflicts appeared in three cases: nucSSU-ITS-5.8S-ITS2+nucLSU: *Russula* sp. + *R. aff. compacta* (ML-BS: 73%), *RPB2*: *Russula* aff. *compacta* + *R. compacta* (ML-BS: 100%); nucSSU-ITS-5.8S-ITS2 + nucLSU: *Russula risigallina* + *R. firmula* (ML-BS: 77%), *RPB2*: *Russula risigallina* + *R. cf. maculata* (ML-BS: 74%); nucSSU-ITS-5.8S-ITS2+nucLSU: *Lactarius romagnesii* + *L. angiocarpus* + *L. acris* (ML-BS: 74%), *RPB2*: + *Lactarius acris* + *L. romagnesii* + *L. lignyotus* (ML-BS: 86%). As these conflicts had moderate support (ML-BS < 75% based on one of the two data sets screened for conflict) and concerned only terminal relationships between morphologically closely related taxa, we ignored them and used all 67 taxa for combined analyses.

The three-locus analyses for 67 taxa inferred ML and Bayesian methods. Fig. 3 depicts the ML tree ($\ln = -16292.840891$) and highlights significant branch support recovered by ML bootstrapping as well as posterior probabilities (PP) derived from the Bayesian majority-rule consensus tree (ML-BS: $\geq 70\%$, PP: $\geq 95\%$)

The reported analyses used a 2-partition data set (nucSSU + ITS1 + 5.8S + ITS2 + nucLSU and *RPB2*^{1st}, 2nd 3rd). The different partition settings for the data did not influence significantly the results of the ML analyses, i.e. they recovered the same topology and equivalent support. However, for Bayesian analyses, fewer partitions for the data (2 versus 4-7 partitions) resulted in higher posterior probabilities for some of the internal branches of the resulting phylogeny (results not shown). These nodes lead to clades including taxa that

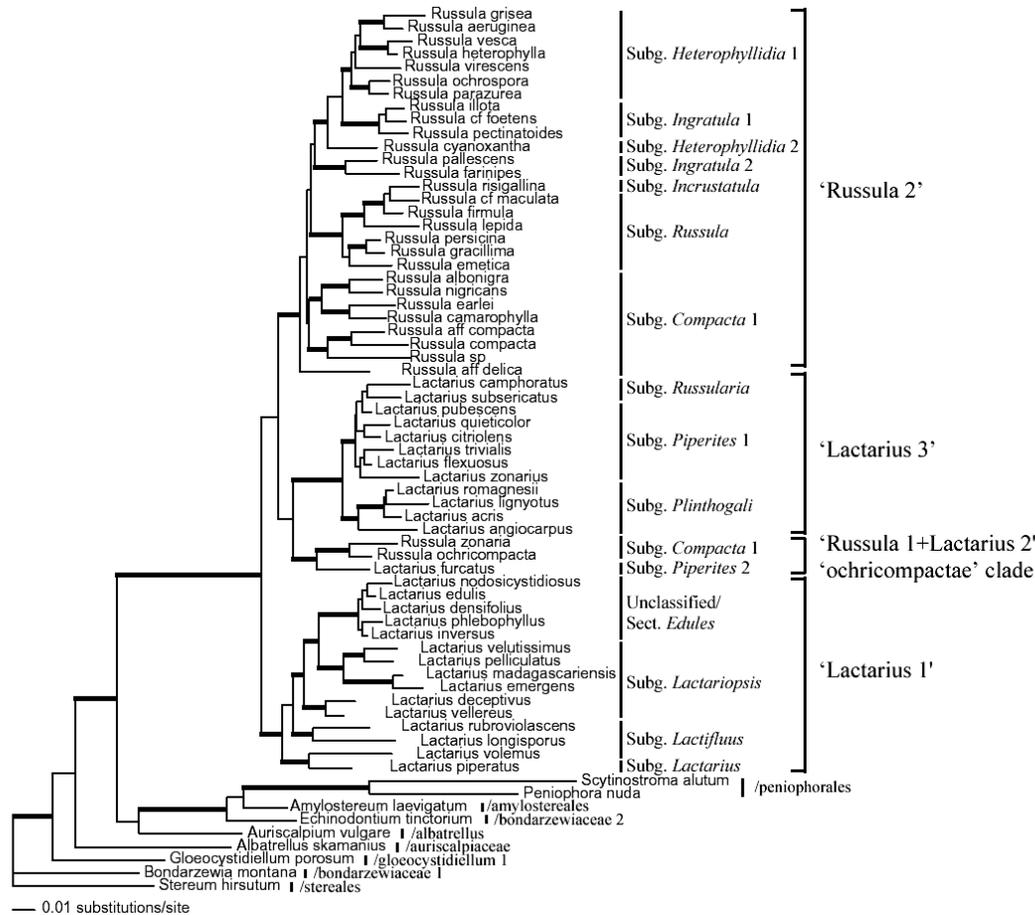


Fig. 3. Phylogenetic relationships inferred by ML analysis combining ITS1-5,8S-ITS2, nucLSU and *RPB2* sequence data for 67 taxa. Thick black branches received ML bootstrap values $\geq 70\%$ and Bayesian posterior probabilities $\geq 95\%$ (see text for bootstrap values and posterior probabilities associated with branches). Thick gray internodes were significantly supported only by ML-BS bootstrap values. Classification follows Heilmann-Clausen et al. (1998) for *Lactarius* and Sarnari (1998) for *Russula*.

miss ITS or/and nucLSU (monophyly of clade '*Lactarius 1*' and also of '*Piperites 1-Russularia*' suggesting that Bayesian inference is more sensitive than ML inference to missing data.

Combining the three loci (nucSSU-ITS-5.8S-ITS2, nucLSU and *RPB2*), four major clades were recovered within the '*russulales*' sensu Larsson and Larsson (2003) (Fig. 3).

'*Lactarius 1*' (ML-BS: 78%, PP: 98%) comprises representatives of three subgenera (*Lactariopsis*, *Lactifluus* and *Lactarius*) and one unclassified section (sect. *Edules* Verbeke). The latter section comprises exclusively tropical African taxa and was never placed in any subgenus in the past. Most of the taxa in the '*Lactarius 3*' clade belong to groups that have an exclusively or predominantly

tropical distribution. Another interesting observation concerns the complete absence of zonate and viscose to glutinose caps in this clade which, on the other hand, contains all 'veiled' caps or known 'annulate' species in the genus. There is strong support (ML-BS: 94%, PP: 100%) for a monophyletic and sister relationship of subgenus *Lactariopsis* (monophyletic, ML-BS: 100%, PP: 100%) and section *Edules* (monophyletic, ML-BS: 100%, PP: 100%). This clade can furthermore be regarded as the *Lactarius* core clade as it contains the type species of the genus, *L. piperatus*. The definition of the various represented subgenera is, however, seriously questioned because of the strongly supported monophyletic group (ML-BS: 94%, PP: 100%) formed by the two northern hemisphere type-

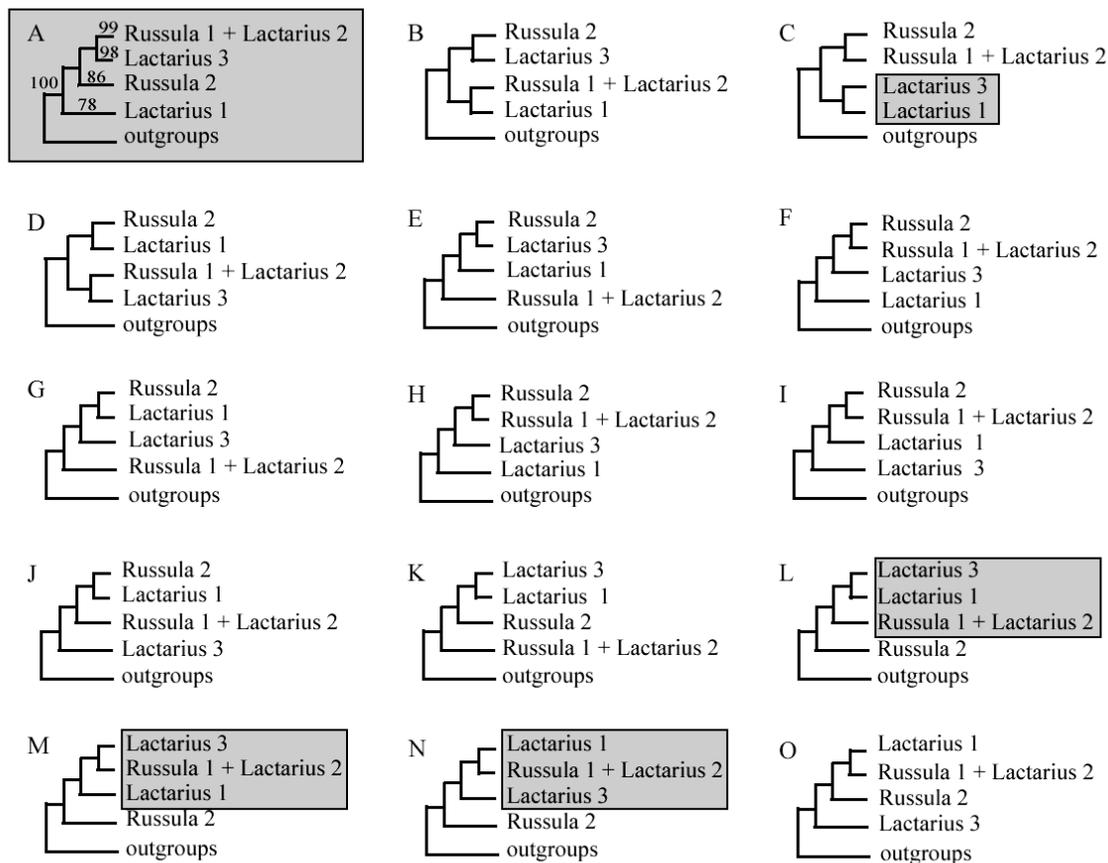


Fig. 4. Possible resolution of basal relationships between the four major clades identified within *Russulaceae* (15 rooted bifurcating trees A-O). A: recovered topology with associated ML bootstrap values. B-O: other possible arrangements of the four major supported clades. Grey boxes highlight groups that are important for the discussion below.

species of subgenera *Lactifluus* (*L. volemus*) and *Lactarius* (*L. piperatus*), the two being separated from the tropical members of *Lactifluus*. Subgenus *Lactifluus* therefore remains partly unresolved with a monophyletic *L. rubroviolascens* - *L. longisporus* (ML-BS: 71%, PP: 99%). Subgenus *Lactariopsis* appears paraphyletic, because the African section *Edules* is sister to a monophyletic subgroup of four African species of this subgenus (sections *Chamaeleontini* and *Lactariopsis*; ML-BS: 100%, PP: 100%) whereas the northern hemisphere sect. *Albati* of the same subgenus, here represented by *L. deceptivus* and *L. vellereus* (ML-BS: 100%, PP: 100%), occupy a more basal position (ML-BS: 89%, PP: 100%) and may not be closely related to the African sections that are classified in this subgenus.

‘*Russula 1 + Lactarius 2*’, a mixed group that combines with maximal support (ML-BS:

100%, PP: 100%) a monophyletic *R. zonaria* and *R. ochricompacta* (subgenus *Compacta* sect. *Compactae*, subsection *Ochricompactae*) with *Lactarius furcatus* (ML-BS: 100%, PP: 100%), a species classified in *Lactarius* subgenus *Piperites* sect. *Torminosi* subsect. *Zonarii* by Montoya *et al.* (2003). Subgenus *Piperites* becomes consequently polyphyletic.

‘*Lactarius 3*’ (ML-BS: 98%, PP: 100%) includes the sampled species of three subgenera of the genus *Lactarius*: *Piperites* (with the exception of *L. furcatus*), *Russularia* and a monophyletic *Plinthogali* (ML-BS: 71%, PP: 99%), the latter being the only subgenus within that clade with an important tropical component. Subgenera *Piperites* and *Russularia* are monophyletic with low support (ML-BS: 71%, PP: <50%). None of the subgenera in this clade is represented in the ‘*Lactarius 3*’ clade and many species belong to

exclusively temperate to cold-adapted infrageneric sections, such as *Deliciosi*, *Torminosi*, *Uvidi* or *Glutinosi* for example.

'*Russula* 2' is the core clade of *Russula* (ML-BS: 86%, PP: 100%) and includes all sampled *Russula* species with the exception of *R. zonaria* and *R. ochricompacta*. All subgenera of the genus are here represented except for subgenus *Amoenula*. The latter subgenus is only recognized at this level by Sarnari (1998) – a view not supported by recent multigene phylogenies (Buyck *et al.*, 2005). In this clade subgenus *Compacta* remains partly unresolved with one supported clade combining section *Archaeinae* (*R. camaro-phylla* and *R. earlei*) with species belonging to section *Compactae* (*R. albonigra* and *R. nigricans*) (ML-BS: 81%, PP: 98%). Subgenus *Russula* appears monophyletic/paraphyletic with subgenus *Incrustatula* (ML-BS: 100%, PP: 100%). Subgenus *Heterophyllidia* is suggested to be paraphyletic with part of subgenus *Ingratula* nested within section *Heterophyllae* (ML-BS: 77%, PP: 100%) and with the two remaining species of subgenus *Ingratula* (*R. pallescens* and *R. farinipes*) forming a highly supported, more basal subclade (ML-BS: 99%, PP: 100%).

The phylogenetic placement of the mixed subclade '*Russula* 1 + *Lactarius* 2' suggests a polyphyletic *Russula* and a polyphyletic/paraphyletic *Lactarius* questioning the delimitation between these two genera. Applying a SH-test statistics, our ML unconstrained phylogeny (Figs 3 and 4A) was not a significantly better explanation of the data than a tree constraining the monophyly of *Lactarius* s. l. – transferring '*Russula* 1' in *Lactarius* ('*Lactarius* 1' with '*Lactarius* 3' and '*Russula* 1+*Lactarius* 2'; P = 0.218).

Using *Stereum hirsutum* as the outgroup we recovered a monophyletic '*peniophorales*'-'*amylostereales*'-'*bondarzewiaceae* 2' clade (ML-BS: 100%, PP: 100%). Other basal relationships within the 'russuloid' clade correspond to those recovered by Binder *et al.* (2005) but are not significantly supported.

Discussion

Russula versus *Lactarius*

The modern formal distinction between *Russula* and *Lactarius* relies entirely on

characters associated with the lactiferous system (Buyck, 1999), which is not ramified in *Russula* and does not extend into the hymenium in the shape of pseudocystidia as does the ramified lactiferous system of *Lactarius*. Typically, *Lactarius* exudes milk when injured while species of *Russula* do not. Because fresh *Lactarius* readily exude latex - at least in the temperate to arctic regions of the northern hemisphere - the distinction between both genera is normally easy, even in the field. *Lactarius* also tends to have uniformly and dull-coloured caps and stipes as well as regularly inserted shorter lamellulae among the gills. *Russula*, on the other hand, has typically brightly coloured caps contrasting with the much paler gills and stipe, and only few species have regularly inserted lamellulae among the gills. Several other features such as concentrically zonate caps, scrobiculate stipes (and sometimes even caps) and hairy cap margins are also restricted to certain infrageneric groups in *Lactarius* and virtually unknown in *Russula*.

With the exploration of the tropical African *Russulaceae* (Buyck, 1993, 1994, 1997; Heim 1938; Verbeken 1996), however, a highly original and endemic flora was described that challenged and expanded the northern hemisphere concept of both genera, i.e. very few *Lactarius* species have hairy cap margins and scrobicules on the stipe while many *Russula* spp. have dull and uniform cap and stipe colours. Moreover, as the absence of latex-exudation is not an uncommon phenomenon in tropical *Lactarius* - even when lactifers are abundantly present in the context - many species cannot readily be assigned to either *Russula* or *Lactarius* in the field (Heim 1938; Buyck 1989b, 1995, 1999). Both genera also include a considerable number of annulate taxa, a feature unknown from the northern hemisphere. On top of this, both Buyck (1989b) and Verbeken (1996) documented several examples of highly similar taxa in both genera. However, these look-alikes remained so far quite comfortably classified in their respective generic and infrageneric groups based on the features of the lactiferous system. Though stunning, the likeness with several groups of species between both genera might therefore be due to convergence. The

usefulness of this sole remaining criterion to distinguish between *Russula* and *Lactarius* - presence/absence of a well developed lactiferous system extending into the hymenium as pseudocystidia - was only questioned in a very limited number of cases such as Buyck and Desjardin (2003) for *R. zonaria* or Verbeke (1996) for *L. ruvubuensis* for example.

The most surprising fact of the 'ochricompactae'-clade resides in the microscopic diversity compared to the strong macroscopic homogeneity of this group, the opposite of the situation in the other genera of the *Russulaceae*. Indeed, the microscopical differences in hymenial and tramal features among the few individual species of this clade are equivalent to those separating the variously known genera in the entire russuloid clade (coloured illustrations posted at <http://www.mtsn.tn.it/russulales-news/multifurca.asp>):

- *Russula ochricompacta* as well as *R. grossa* sensu Bills & Pegler ac Saini & Atri are very similar and both are unique in the fact that hymenial features are identical to those of some of the resupinate species classified in *Gloeocystidiellum* (Buyck, 1995). The content of the cystidia does not react with sulfoaldehydes and is different from that of *L. furcatus*. Both species have a unique smell among *Russulales*.
- *Russula aurantiophylla* is morphologically identical, only much smaller, but differs from the former in having different cystidia as well as long cylindrical elements in the lamellar trama that react with sulfoaldehydes. It furthermore possesses a unique hymenium development reminiscent of resupinate fungi.
- *Russula zonaria* has pseudocystidia as in *Lactarius* but lacks an extensively branching, lactiferous system as in *L. furcatus*, also the content of these elements is chemically different from the latter.
- *Lactarius furcatus*, finally, is in every respect a typical *Lactarius*, and possesses a branched, lactiferous system with abundant pseudocystidia,

as well as the typical 'rosette'-structure of the context.

With regard to the other features of *Ochricompactae*, none of the morphological characters of this mixed *Russula-Lactarius* clade are restricted to this small group of species. It is the combination of the various characters that makes this group one of the most easily recognizable ones in the family.

Regularly forked gills, for example, have been described for other taxa, both in *Russula* and *Lactarius*. More than a dozen African *russulas* have regularly forked gills as a constant and reliable feature. Unpublished sequence data by the first author place all of these species firmly within the *Russula* clade. Also several *Lactarius* species have regularly forked gills such as *L. phlebophyllus* in 'Lactarius 1' clade.

The sole *russulas* that possess a (mostly obscure) concentric zonation of the cap (but only when wet and only on the cap surface, not in the underlying trama) are in subsection *Fistulosinae* (Buyck, pers. obs.), a very different group of species with mostly acrid taste, white spore prints, polydymous gills, a reddening-blackening context and a very distinctive pellis structure that is in every aspect reminiscent of *Lactarius* sect. *Plinthogali*.

Apart from *R. grossa*, which is here discarded from *Ochricompactae*, *R. ochricompacta* was not related to any other species in *Russula* or *Lactarius* by previous studies until Buyck described very recently two new species in *Ochricompactae* from the southern hemisphere (Buyck and Desjardin, 2003; Buyck, 2004). *Ochricompactae* clearly stand out from all other *russulas*, although Buyck (in Buyck and Desjardin, 2003) had suggested possible close affinities with *Russula* subsections *Pallidosporinae* (subgenus *Compacta*) and *Ilicinae* (subgenus *Heterophyllidia*) based on some shared characters: "Sparse hymenial cystidia, that originate deep in the lamellar trama, orthochromatic and structurally hardly differentiated pileo- and stipitipellis tissues, subreticulate, crested spores without an amyloid spot and a tendency towards dark spore prints, are all typical features of *Pallidosporinae* and *Ilicinae*".

The phylogeny presented in Fig. 3 now suggests that the above mentioned morphological similarities between the species in *Ochricompactae* and some other infrageneric groups in *Russula* are not the result of a direct relationship, and highly supports the clustering of *Russula* subsection *Ochricompactae* with *Lactarius furcatus* clearly separated from the *Russula* core clade.

Choosing a systematic position for the *Ochricompactae* clade

In the light of the morphological and molecular data presented here, the formal distinction between *Russula* and *Lactarius* appears to be artificial. But since the relationships between the here retrieved four major clades in the *Russulaceae* lack significant support, it is possible to imagine different systematic scenarios (Fig. 4).

(1) A possible solution would consist in considering all the ingroup taxa of this analysis, and thus by extension not only all species presently classified in *Russula* and *Lactarius* but also all related gasteroid, secotioid and pleurotoid genera, to be part of a single supergenus. In practice, it would consist in transferring all taxa presently classified in *Russulaceae* in *Russula*, as this has nomenclatural priority. Given the ease with which it is possible to distinguish between *Lactarius* and *Russula* in the northern hemisphere, this solution would probably cause a lot of understandable criticism from the international mycological community.

(2) Another solution consists in the transfer of all species of *Russula* subsection *Ochricompactae* to *Lactarius*. This solution would be phylogenetically acceptable on the condition that all clades containing *Lactarius* species ('*Lactarius* 1', '*Russula* 1 + *Lactarius* 2' and '*Lactarius* 3') appear monophyletic and sister group to the '*Russula* 2' clade. In other words, this solution is only acceptable if the outgroup taxa connect to the ingroup somewhere on the branch that separates the *Russula* core clade (*Russula* 2) from all clades that contain *Lactarius* species (Fig. 4, L, M, N). Such a topology has been recovered with significant support (PP: 95-97%) based on a single locus (nucLSU) by Eberhardt and Verbeken (2004). However, in the same paper,

their combined nucLSU + ITS analysis suggested a paraphyletic *Russula* with no significant support.

Our 3-locus phylogeny now suggests - although without significant support - a paraphyletic genus *Lactarius*. The results of the SH test suggest that the recovered ML topology does not fit the data significantly better than a topology constraining the monophyly of all the clades that comprise *Lactarius* species (which would thereby implement a transfer of *Russula* subsection *Ochricompactae* in *Lactarius*). For 12 out of the 15 possible systematic scenarios (Fig. 4: excluding L, M, N), this transfer, however, would still leave the genus *Lactarius s. l.* either paraphyletic (Fig. 4: E-K, O) or polyphyletic (Fig. 4: B-D).

The possession of several typical 'lactarioid' features could at first sight be interpreted as arguing in favour of the inclusion of *Ochricompactae* in *Lactarius*. There are, however, several 'caveats': (1) even if it is indeed so that *Ochricompactae* possess a scrobiculate stipe, the scrobiculae are of a different nature than those in *Lactarius* and do not result in a pitted aspect as they do in the latter genus, but the drops remain intact and voluminous; (2) - some species being completely devoid of lactifers and pseudocystidia, the incredible variation in hymenial features among the *Ochricompactae* results in a very uncomfortable position of most species in *Lactarius* - in the same way as *L. furcatus* would be unfit in *Russula*; (3) the zonate caps in *Ochricompactae* are certainly more reminiscent of *Lactarius* than they are for *Russula*, but also here there is a difference with the other *Lactarius*-species as the zonation is clearly the result of an organisation of the underlying trama (in section the zonation extends from the hymenium level up to the cap surface) unlike other *Lactarius*.

(3) The third option finally consists in the recognition of additional genera in *Russulaceae*: each of the four supported monophyletic clades in our analysis would then represent a separate genus, except for scenario C (Fig. 4) where the recognition of the mixed clade as a separate genus would leave '*Lactarius* 1' and '*Lactarius* 3' monophyletic. In all cases, however, this option implicates a

fragmentation of *Lactarius* in its present concept.

In this context, we would like to draw the attention to the position of the type species of both *Russula* and *Lactarius*. Indeed, whereas the position of *R. emetica* – the nomenclatural type of *Russula* – is well within the clade that includes nearly all species of *Russula*, the type of *Lactarius*, *L. piperatus*, occupies a very isolated systematic position as it is classified in a separate subgenus. In our analysis, the type of *Lactarius* sits in the clade composed of predominantly tropical taxa. Without changing the type species of *Lactarius*, a name change for nearly all northern temperate taxa of *Lactarius* would be necessary in case clades ‘*Lactarius* 1’ and ‘*Lactarius* 3’ become separate genera.

The description of a separate genus for the ‘*ochricompactae*’ clade would be the most elegant and easily defensible solution from a morphological, nomenclatural and phylogenetic point of view. Indeed, morphologically speaking, the ‘*ochricompactae*’ clade has sufficient features that argue for its recognition as a separate genus: the consistently forking gills of all included taxa, the dark spore print, the zonation of the context, the microscopic diversity of hymenial features which seems indicative of a relict group, etc.

Conclusion

In conclusion from the above, we therefore propose the following new taxa in *Russulaceae*:

Genus *Multifurca* Buyck & V. Hofst. **genus nov.**

Mycobank: 511332

Pileus concentric modo zonatus, siccus subtomentosusque, umido interdum viscidus, albus, albidus vel aurantiaco-ochraceus, saepe obscurior in vetere ac locale ferrugineus vel ochraceo-brunneus. *Lamellae* adnatae vel subdecurrentes, regulariter furcatae, angustae, interdum moderate crassae, aurantiacae, roseo-aurantiacae vel ochraceae. *Stipes* centralis vel eccentricus, pileo concolor, cavus, firmus, generaliter scrobiculis gelatinosis instructus, basi soli particulis agglomeratis. *Caro albida*, interdum umido grisea, immutabilis, firma, concentric modo zonata. *Latex* nullus vel moderate abundans et albus, dein virescens. *Odor* nulla vel fortiter resinacea. Sapor

amarescens vel acris. *Sporae* in cumulo aurantiacae, minutissimae, plus minusve sub-reticulatae; macula suprahilaris inamyloidea. *Dermatocystidia*, macrocystidia, pseudocystidia vel hyphae lactiferae praesentia vel absentia. *Fibulae* nullae.

Pileus concentrically zoned, dry and (sub)tomentose, velvety to sometimes strongly viscid when wet, white to buff or pale ochre, often developing darker, pale rusty brown or ochre brown tints. *Gills* adnate to subdecurrent or descending with a tooth along stipe, regularly forked, narrow, often relatively thick, orange, pinkish-orange to honey yellow. *Stipe* central to eccentric, concolorous with cap, hollow yet firm, scrobiculate, the base often ill-delimited and agglomerating the soil underneath. *Context* white to grayish when water soaked, unchanging, firm, in section presenting the same concentric zonation as evidenced on the cap surface over the whole thickness of the cap. *Milk* when present white, staining context dirty green. *Smell* mild to strong and distinct (resinaceous, citronella, etc.). *Taste* varying from slightly bitter to acrid. *Spore print* orange. *Spores* very small, with a faint, obscurely to distinctly subreticulate ornamentation, suprahilar spot not amyloid. With or without (dermato)cystidia, pseudocystidia and lactifers. Without clamps.

Type species.

Multifurca ochricompacta (Bills & O.K. Miller) Buyck & V. Hofst., **comb. nov.**

Mycobank: 511334

Basionym: *Russula ochricompacta* Bills & O.K. Mill., *Mycologia* 76: 976. 1984.

Attributed species:

- *Multifurca furcata* Buyck & V. Hofst., **comb. nov.**

Mycobank: 511335

Basionym: *Lactarius furcatus* Coker, *J. Elisha Mitchell Sci. Soc.* 34: 18. 1918.

- *Multifurca zonaria* (Buyck & Desjardin) Buyck & V. Hofst., **comb. nov.**

Mycobank: 511337

Basionym: *Russula zonaria* Buyck & Desjardin, *Cryptogamie Mycologie* 24: 112. 2003.

- *Multifurca aurantiophylla* (Buyck & Ducouso) Buyck & V. Hofst., **comb. nov.**

Mycobank: 511336 (Fig. 1)

Basionym: *Russula aurantiophylla* Buyck & Ducouso, *Cryptogamie Mycologie* 25: 127. 2004.

- *Multifurca roxburghiae* Buyck & V. Hofst.,
sp. nov. (Fig. 2)

MycoBank: 511333

Synonym: *R. grossa* sensu Bills & Pegler 1988 ac
sensu Saini & Atri 1982, non Berkeley 1851.

A *R. ochricompacta* praecipue differt odore resinaceo,
consociatione *Pino roxburghii*, distributione montis
Emodi, sporarum ornamentatione distinctiore.

Holotypus INDIA: Himachal Pradesh, Simla,
Summer Hill, 1983 m alt., N.S.Atri, August 23, 1979 (K
109299 **holotype**).

Key to the species

All species of *Multifurca* are very rare
and known only from the type or from a very
limited number of collections.

1. Greenish-greyish milk exuded on injury. Taste acrid
..... *Multifurca furcata* (= *L.furcatus*)
1. No traces of milk at all on injury. Taste mostly bitter
or nauseous. (2)
2. Pseudocystidia present in hymenium, continuing in
trama as vascular hyphae. Taste bitter or nauseous.
Medium-sized, pale yellowish brown taxon only known
from Thailand. Under dipterocarps.....
..... *Multifurca zonaria* (= *R.zonaria*)
2. No pseudocystidia present in hymenium, but cystidia
sometimes originating from deep in lamellar trama.....
..... (3)
3. Spores predominantly subglobose, with distinct
mostly linear subreticulate ornamentation. Hymenium
also with numerous, slender, capitulate, emergent
cystidia. Small white taxon (<5 cm diam.) under
Nothofagus. Taste unknown. Only known from New
Caledonia.....
..... *Multifurca aurantiophylla* (= *R.aurantiophylla*)
3. Spores not subglobose, with very faint ornamentation.
Without many slender capitulate hymenial cystidia.
Taste slightly bitter, nauseous. Medium-sized taxa
growing with *Pinaceae* and possibly also *Fagaceae*.
..... (4)
4. Spores very faintly ornamented. Penetrating smell of
citronella. Eastern USA and Gulf of Mexico.....
..... *Multifurca ochricompacta* (= *R.ochricompacta*)
4. Spores with distinct ornamentation (especially bigger
warts) although remaining overall quite low. Smell
resinaceous. Under *Pinus roxburghii* in Himalaya. India.
..... *Multifurca roxburghiae* **sp. nov.**

Further exploration of the tropics and the
southern hemisphere will undoubtedly reveal
many more undescribed and interesting
Russulaceae, yet, the here newly described
genus *Multifurca* will never be a group with

many species. All included taxa seem to be
extremely rare and geographically restricted.
Such a pattern - 1 or 2 rare taxa per continent -
is in our experience highly indicative of a relict
group. The corticioid nature of some of the
hymenial features of several species and the
extremely small size of the spores (as in
Russula sect. *Archaeinae*) suggest indeed that
Multifurca is a very ancient group.

Glaciations may be responsible for the
absence of this group in Europe. *Multifurca*
is also conspicuously absent from Africa and
Madagascar and those African taxa with
regularly furcating gills (e.g. *L. phlebophyllus*)
are not closely related as shown by our
sequence data.

From the above, one might conclude that
Multifurca is composed of northern hemisphere
species of medium to higher altitudes within an
area that corresponds quite well to the
distribution pattern of *Pinaceae*. *Pinus* and
Quercus were cited as possible host trees for
the type locality of *M. ochricompacta* and
pines were also present in the beech forest
where it was later found by the senior author.
Pinus roxburghii Sargent, host to *M.*
roxburghiae sp.nov., occurs between 2100-
2200 m elevation in the Himalaya mountains of
Bhutan, N-India, Kashmir, Nepal, Pakistan,
Sikkim and South Tibet (Wu and Raven,
1999). This pine is particular in shedding its
needles after no longer than one year,
resembling thereby deciduous trees (Richardson
and Rundel, 1998). It has been introduced
in other continents, such as Africa and
America.

Fagaceae are certainly another host
family, in particular for *Multifurca furcata*,
which accompanies *Quercus* in the Gulf of
Mexico down to Costa Rica (Montoya *et al.*,
2003), whereas *Nothofagus* is host to *M.*
aurantiophylla in New Caledonia. On the other
hand, *Dipterocarpaceae* are host to *M. zonaria*.
In this paper, we refrain from suggesting
systematic changes in *Lactarius*. These will be
treated in a separate paper. A proposal to
change the nomenclatural type of *Lactarius*
(Buyck *et al.*, in prep.) will allow for the future
emendation of genus *Lactifluus* (Pers.) Roussel
1806 for nearly all taxa presently classified in
subgenera *Lactifluus*, *Lactarius* and *Lactari-*
opsis.

Acknowledgements

The authors thank Roy Halling (NYBG), Dennis Desjardin (SFSU) for giving access to their collections, David Lewis (Texas) for field assistance and access to his personal herbarium and Bernard Duhem (PC) for preparing thin sections of the gills for species of *Ochricompectae*. The work was financially supported by Artdatabanken (Sweden) to Ursula Eberhardt and by FWO Vlaanderen to Annemieke Verbeken.

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