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Hypogeous gasteroid *Lactarius sulphosmus* sp. nov. and agaricoid *Russula vinosobrunneola* sp. nov. (Russulaceae) from China

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Abstract

Two new species of Russulaceae from China are herein described and illustrated based on their morphologies and phylogenies. A hypogeous gasteroid species, *Lactarius sulphosmus* sp. nov. and an agaricoid species, *Russula vinosobrunneola* sp. nov. are introduced. The latter is morphologically distinguished from *R. sichuanensis*, although the ITS-based phylogeny was unable to distinguish them. Therefore, a multi-gene phylogenetic analysis of the *nLSU*, *ITS*, *mtSSU*, and *tef-1α* gene sequences of *Russula* subsection *Laricinae* was carried out, which supports the assertion that they are different species.

Key words – Basidiomycota – phylogeny – Agaricomycetes – Russulales – taxonomy

Introduction

Sequestrate and angiocarpic basidiomata have frequently been observed in many groups of Agaricomycetes (Calonge & Martín 2000, Watling & Martín 2003, Danks et al. 2010, Henkel et al. 2010, Orihara et al. 2012, Lebel & Vellinga 2013, Trierveiler-Pereira et al. 2014, Hongsanan et al. 2015, Li et al. 2018a). Various potential selective pressures have been proposed to explain the hymenium transformation from free or attached gills to enclosed labyrinthoid chambers and the disappearance of the stipe (Miller et al. 2001). It may be regarded as a protective mechanism in the macrofungi to prevent the effects of extreme environmental conditions, such as low temperature and aridity (Thiers 1984a, Bruns et al. 1989, Bougher & Lebel 2001, Wilson et al. 2011). Gasteroid to secotioid basidiomata and hypogeous habitats can provide protection against moisture loss and ice gelivation (Binder & Bresinsky 2002, Trappe & Claridge 2005). In tropical areas, gasteromycetization of fructifications make angiocarpous species less dependent on seasonal changes. The abundance of angiocarpic basidiomata is often overlooked (Verbeken et al. 2014).

Members of Russulaceae are characterized by a combination of chalky texture, caused by sphaerocytes in the context, and amyloid spore ornamentation in Melzer's reagent. In some Russulaceae, the context exudes latex when injured (Lebel & Trappe 2000, Miller et al. 2001, Lebel & Castellano 2002, Verbeken & Nuytinck 2013, Verbeken et al. 2014). Russulaceae species form ectomycorrhizal associations with plants (Henkel et al. 2000, Tedersoo & Nara 2010, Kong et al. 2015, Buyck et al. 2018). This family has often been regarded as a large and complex group that

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includes agaricoid, corticioid, gasteroid, pleurotoid, and secotioid members (Buyck & Horak 1999, Calonge & Martín 2000, Larsson & Larsson 2003, Miller et al. 2006, Larsson 2007). Previous analyses indicated that the transformation from agaricoid ancestors to secotioid and gasteroid species has happened many times in evolutionary history of the Russulaceae (Miller et al. 2001, 2006). Thus, the concept that the secotioid Russulaceae genera are synonyms of the agaricoid ones has been gradually accepted during recent years. *Zelleromyces* and *Arcangeliella* were included in *Lactarius*, while *Cystangium*, *Elasmomyces*, *Gymnomyces*, *Macowanites*, and *Martellia*, are secotioid members of *Russula* (Eberhardt & Verbeken 2004, Lebel & Tonkin 2007, Buyck et al. 2010, Verbeken et al. 2014, Sang et al. 2016).

Secotioid Russulaceae species have been thoroughly studied in Australia, New Zealand (Bougher 1997, Bougher & Lebel 2001, Lebel 2001, 2002, 2003a, b, Lebel & Castellano 2002), and North America (Zeller & Dodge 1919, 1936, Singer & Smith 1960, Smith 1963, Thiers 1984b, Miller & Lebel 1999, Desjardin 2003, Smith et al. 2006). However, there are fewer records from Southeast Asia (Verbeken et al. 2014) and China (Zeller & Dodge 1935, Zhang & Yu 1990, Tao & Chang 1993, Liu 1998, Li et al. 2012, Sang et al. 2016). In this study, a hypogeous secotioid species *Lactarius sulphosmus* from the subtropical area of southeast China, was found to be new to science based on morphological characteristics and phylogenetic analysis. Another novel species *Russula vinosobrunneola* from northeast China is agaricoid in morphology. However, it has a close phylogenetic relationship with the semi-gasteroid species *R. sichuanensis* G.J. Li & H.A. Wen. These two species are herein described and illustrated in detail. Their morphological and phylogenetic analyses with closely related taxa are also provided.

Materials & Methods

Specimens and morphology

Specimens were photographed and collected in the field and their macroscopic characteristics were recorded at the same time under daylight. Color name and codes followed the standard of Ridgway (1912). Specimens were dehydrated using a Vatti fruit dryer (Zhongshan, China) at 60 °C overnight. Specimens of the two new species were deposited in the Herbarium Mycologicum, Academiae Sinicae (HMAS). Herbarium name abbreviations followed those of Thiers (2018). Small pieces of dry specimens were rehydrated in 5% KOH solution. Rehydrated lamellae and pileus pellis were sliced by hand using a Flying Eagle razor blade (Shanghai, China). Microscopic characters were observed under a Nikon Eclipse 80i optical microscope (Tokyo, Japan). Observations, measurements, and line drawings of basidiospores, basidia, and cystidia elements of the pileipellis and stipitipellis were performed with the help of Congo Red. Melzer's reagent and Sulphovanillin (SV) were employed to test the reactions of basidiospore ornamentations and cystidium contents. The lengths of the apiculus and spore ornamentations were excluded from measurements. At least 50 basidiospores and 20 other elements were measured for each characteristic. Scanning electron microscope (SEM) photos of basidiospores were captured through a Hitachi SU8010 Field Emission SEM (Tokyo, Japan). The abbreviation [*a*/*b*/*c*] represented that *a* basidiospores were measured from *b* fruit bodies of *c* specimens. Basidiospore widths and lengths were displayed as (*w*–) *x*–*y* (–*z*), where *x*–*y* was the range including 95% or more of the measured values, while *w* and *z* were the extremes of all measured values. Q stands for the ratio of basidiospore length/width. A bold **Q** stands for the average value of Q plus or minus the standard deviation. Further details of the microscopic observations in this study are available in Li et al. (2012, 2015), Li (2014).

DNA extraction, polymerase chain reaction (PCR) and sequencing

Small pieces of dried fruiting bodies were pulverized with a Xinyi 48 tissue grinder (Ningbo, China) by shaking for 80 s at 30 Hz 2 times in a 2-mL tube with one 3 mm steel ball. Genomic DNA was extracted using a using a modified magnetic bead method with a GeneOn Plant DNA Extraction Kit (Changchun, China) following the manufacturer's instructions. Gene regions were

amplified using following pairs of primers: ITS1/ITS4 (White et al. 1990) for the 5.8S and internal transcribed spacers ITS1 and ITS2 regions (*ITS*), LROR/LR5 (Moncalvo et al. 2000, 2002) for the large subunit of the nuclear ribosomal DNA (*nLSU*), MS1/MS2 (White et al. 1990) for the small subunit of the mitochondrial ribosomal DNA region (*mtSSU*), and EF1-983F/EF1-1567R (Morehouse et al. 2003) for the translation elongation factor alpha (*tef1-α*). PCRs were performed in a 50 μL reaction mixture that contained the following: 21 μL ddH_2O, 25 μL 2 × Plus Master Mix (Biomed Biotech Beijing), 1.5 μL each primer (10 mmol/L), and 1 μL DNA template, in an ABI Verti 96 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). PCR procedures followed an initial denaturation at 95 °C for 4 min, followed by 35 cycles of 94 °C for 50 s, 51 °C for 55 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min for *ITS*, *nLSU*, and *mtSSU*; an initial denaturation at 95 °C for 4 min, followed by 15 cycles of 94 °C for 50 s, 65–50 °C for 55 s (reduced by 1 °C per cycle), 20 cycles of 94 °C for 50 s, 50 °C for 55 s, 72 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min for *tef-1α*. The PCR products were purified and sequenced by Biomed Biotech Company using an ABI 3130 DNA sequencer and the ABI BigDye 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

Phylogenic analyses

Raw forward and reverse sequences were assembled using DNAStar v7.1 (Lasergene, WI, USA). GenBank and UNITE accession numbers of ITS sequences are shown in Tables 1, 2. Alignments were performed in Mafft 7.376 (Katoh & Standley 2013). Necessary edits and adjustments were made using Bioedit (Hall 1999). Ambiguously aligned regions and gaps in matrixes were removed from the analyses with Gblocks 0.91b (Talavera & Castresana 2007). Sequences for multi-gene analysis were combined in SequenceMatrix 1.8 (Vaidya et al. 2011). Bayesian analysis was conducted in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). The best-fit model of evolution was estimated using MrModelTest 2.3 (Nylander 2004) before analysis. Posterior probabilities (PP) were calculated with the Markov chain Monte Carlo (MCMC) algorithm (Larget & Simon 1999). Runs of 1,000,000 generations with the tree sampled every 100th generation were initiated for three heated and one cold Markov chains. The analyses were automatically terminated when the average standard deviation of the split frequencies reached a value below 0.01. The first 25% of trees were discarded as burn-in. Maximum Likelihood (ML) analysis was performed with RAxMLHPC2 v. 8.2.4 (Stamatakis 2014) as implemented on the CIPRES portal (Miller et al. 2010), using a GTRGAMMA model with 1000 rapid bootstrap (BS) replicates. The alignment matrices for Bayesian and ML analyses were deposited in TreeBASE (ID 22573). The phylogenetic tree was displayed in TreeGraph 2.4.0 (Stöver & Müller 2010).

Table 1 Specimens and their GenBank/UNITE accession numbers analyzed in ITS phylogenetic analyses. Specimens with newly acquired sequences for this study are shown in bold.

Taxon	Location	Voucher No.	Sequence accession No.	Reference
Arcangeliella borziana	Switzerland	WSL (F. Ayer) Nr. 96-05-3344	AF286204	Peter et al. (2001)
Arcangeliella borziana	Switzerland	NA	AF373599	Peter et al. (2001)
Arcangeliella camphorata	Japan	OSC 119445	EU644702	NA
Arcangeliella camphorata	Japan	OSC 74230	EU644701	NA
Arcangeliella crassa	USA	OSC 41826	KT968563	NA
Arcangeliella sp.	Thailand	AC7R3	FJ454900	Roy et al. (2009)
Arcangeliella sp.	Australia: Tasmania	BH2148F	JF960610	Horton (2011)
Gloeocystidiellum aculeatum	China: Taiwan	Wu890714-52	AF506433	Larsson & Larsson (2003)

Table 1 Continued.

Taxon	Location	Voucher No.	Sequence accession No.	Reference
Lactarius aff. *wenquanensis*	Thailand	GENT LTH143	EF141537	Le et al. (2007)
Lactarius angiocarpus	Zambia: Copperbelt	GENT Arora 00-448	AY606942	Eberhardt & Verbeken (2004)
Lactarius akahatsu	Japan: Tottori	TMI 22601	EF141556	Le et al. (2007)
Lactarius alboscrobiculatus	Thailand	GENT LTH175	EF141538	Le et al. (2007)
Lactarius alboscrobiculatus var. *roseopurpureus*	Thailand	GENT LTH148	EF141540	Le et al. (2007)
Lactarius alnicola	USA	MONT EB0064-14	KX394276	Barge & Cripps (2016)
Lactarius alpinus	Europe	FNL GNP-125	KX094937	Nuytinck & Voitk (2016)
Lactarius austrozonarius	Thailand: Chiang Mai	GENT FH12-007	KF432965	Wisitrassameewong et al. (2014)
Lactarius austrotorminosus	Thailand	GENT LTH111	EF141531	Le et al. (2007)
Lactarius camphoratus	Sweden	UPS UE04.09.2004-5	DQ422009	NA
Lactarius chichuensis	China: Yunnan	HKAS Wang1236	KF241541	Verbeken et al. (2014)
Lactarius chrysorrheus	Italy	UPS UE04.10.2002-8	KF133261	Verbeken et al. (2014)
Lactarius citriolens	Sweden	UPS UE20.09.2004-03	DQ422003	NA
Lactarius crassus	USA	Trappe 17996	KT968581	NA
Lactarius cyathuliformis	Sweden	UPS UE04.09.2004-2	KF133266	Verbeken et al. (2014)
Lactarius deliciosus	Slovakia	GENT JN2001-046	KF133272	Verbeken et al. (2014)
Lactarius deterrimus	Slovakia	GENT JN 2001-053	DQ922515	Nuytinck & Verbeken (2007)
Lactarius echinus	Sri Lanka	AV07-168	KF133273	Verbeken et al. (2014)
Lactarius falcatus	Thailand	GENT KVP08-038	KF133274	Verbeken et al. (2014)
Lactarius formosus	Thailand	GENT LTH382	EF141549	Le et al. (2007)
Lactarius hatsudake	Vietnam: Lam Dong	GENT JN2011-065	KF432967	Wisitrassameewong et al. (2014)
Lactarius helvus	Sweden	UPS UE08.09.2004-1	KF133263	Verbeken et al. (2014)
Lactarius illyricus	Austria	VM 14644	JF908315	Osmundson et al. (2013)
Lactarius lignicola	China: Yunnan	KUN-HKAS 59474	MF508946	Wang (2017)
Lactarius pallido-ochraceus	China: Yunnan	KUN-HKAS 59446	MF508943	Wang (2017)
Lactarius olympianus	North America	ED 08-018	KR364089	De Crop et al. (2016)

Table 1 Continued.

Taxon	Location	Voucher No.	Sequence accession No.	Reference
Lactarius pallidizonatus	China: Yunnan	KUN-HKAS 62019	MF508931	Wang (2017)
Lactarius pomiolens	Sri Lanka	AV07-159	KF133282	Verbeken et al. (2014)
Lactarius porninsis	Slovakia	GENT JN 2001-082	DQ922547	Nuytinck & Verbeken (2007)
Lactarius psammicola	USA: Michigan	BPL869	KY848507	NA
Lactarius purpureus	Thailand: Chiang Mai	GENT FH12-008	KF432966	Wisitrassameewong et al. (2014)
Lactarius purpureus	Thailand	GENT LTH120	EF141542	Le et al. (2007)
Lactarius purpureus	Papua New Guinea	E4552	EF141543	Le et al. (2007)
Lactarius quietus	Sweden	UPS UE16.09.2004	KF133264	Verbeken et al. (2014)
Lactarius repraesentaneus	Europe	NA	AY331011	Nuytinck et al. (2003)
Lactarius rubriviridis	USA	DED7312	EF685088	Nuytinck et al. (2007)
Lactarius rufus	Europe	ED2008-06	KT165278	Wisitrassameewong et al. (2016)
Lactarius sanguifluus	Belgium	GENT JN 2000-008	AY332547	Nuytinck & Verbeken (2003)
Lactarius salmonicolor	Europe	NA	AF140265	Eberhardt et al. (2000)
Lactarius saturnisporus	Sri Lanka	AV07-170	KF133283	Verbeken et al. (2014)
Lactarius scrobiculatus	Slovakia	GENT JN01-058	KF432968	Wisitrassameewong et al. (2014)
Lactarius speciosus	USA: North Carolina	AV 04/206	EF141547	Le et al. (2007)
Lactarius sphagneti	UK	PL2805	KF133268	Verbeken et al. (2014)
Lactarius spinosporus	China: Sichuan	BJTC FAN445	KY270490	Sang et al. (2017)
Lactarius stephensii	Belgium	RW2930	AY331012	Nuytinck et al. (2003)
Lactarius subbrevipes	China: Yunnan	KUN-HKAS 94947	MF508934	Wang (2017)
Lactarius subdulcis	Belgium	JV2006-024	KF133279	Verbeken et al. (2014)
Lactarius subsericatus	Sweden	UPS UE11.10.2004-8	DQ422011	NA
Lactarius sulphosmus	**China: Fujian**	**HMAS 279586**	**MG819726**	**This study**
Lactarius sulphosmus	**China: Fujian**	**HMAS 276808**	**MG719937**	**This study**
Lactarius torminosus	Canada	OUC 97057	DQ367908	Durall et al. (2006)
Lactarius uvidus	Europe	NA	AY606957	Eberhardt & Verbeken (2004)
Lactarius vinaceorufescens	Canada	GENT JN2007-018	KF241542	Verbeken et al. (2014)
Lactarius yumthangensis	India	BSHC Das 11-147	KJ742418	Das et al. (2015)
Russula aurantioflammans	Finland: Enontekiö	TUR A796	UDB011116	NA

Table 1 Continued.

Taxon	Location	Voucher No.	Sequence accession No.	Reference
Russula cessans	Estonia: Hiiu maakond	TU 101805	UDB015971	NA
Russula cessans	North America	S. Miller 9678	AY061730	Miller & Buyck (2002)
Russula cf. *aurantioflammans*	China: Neimenggu	HMAS 252635	KX441038	Unpublished
Russula cf. *fulvograminea*	China: Neimenggu	HMAS 267791	KX441108	Unpublished
Russula cf. *graminea*	China: Yunnan	HMAS 271166	KX441221	Unpublished
Russula cf. *laricina*	China: Liaoning	HMAS 267755	KX441101	Unpublished
Russula emetica	Germany	TUB lw081	UDB000300	NA
Russula emetica	United Kingdom	DG44	UDB001628	NA
Russula font-queri	Estonia: Valga maakond	TU101814	UDB015978	NA
Russula font-queri	China: Jilin	HMAS 267744	KX441096	Unpublished
Russula fulvograminea	Finland: Paltamo	TU 101621	UDB016259	NA
Russula fulvograminea	Finland: Vehmersalmi	JR 2810F	UDB011112	NA
Russula fulvograminea	China: Jilin	HMAS 252626	KX441036	Unpublished
Russula laricina	Europe	E Watling 25556	AY061685	Miller & Buyck (2002)
Russula laricina	China	HMAS 252564	KF850405	Li (2014)
Russula laricina	Europe	575/08.681	JN944008	Schoch et al. (2012)
Russula laricina	China: Sichuan	HMAS 269630	KX441153	Unpublished
Russula nauseosa	Estonia: Saare maakond	TU 118126	UDB011239	NA
Russula nauseosa	Estonia: Saare maakond	TU 106370	UDB011164	NA
Russula nauseosa	Estonia: Tartu maakond	TU 101692	UDB011360	NA
Russula nauseosa	United Kingdom	DG 05-47	UDB001716	NA
Russula nauseosa	Estonia: Pärnu maakond	TU 101789	UDB011333	NA
Russula nauseosa	Europe	FH 12173	KT933985	Looney et al. (2016)
Russula nauseosa	Chian: Guizhou	MYH 2013100345	KX655853	NA
Russula sichuanensis	China: Sichuan	HMAS 268888	KX441125	Unpublished
Russula sichuanensis	China: Sichuan	HKAS 53885	JX391968	Li et al. (2013)
Russula sichuanensis	China: Sichuan	HKAS 53792	JX391969	Li et al. (2013)
Russula sichuanensis	**China: Gansu**	**HMAS 255316**	**MG786566**	**This study**
Russula sp.	China: Heilongjiang	HMJAU 32220	KX095013	NA
Russula sp.	China: Heilongjiang	HMJAU 32153	KX095024	NA
Russula sp.	China: Heilongjiang	HMJAU 32222	KX095022	NA
Russula sp.	China: Yunnan	HKAS 78378	KF002768	Guo et al. (2014)
Russula sp.	China: Heilongjiang	HMJAU 32217	KX094991	NA
Russula sp.	China: Heilongjiang	HMJAU 32216	KX094990	NA
Russula sp.	China: Yunnan	HKAS 78371	KF002761	Guo et al. (2014)
Russula sp.	China: Yunnan	HKAS 78377	KF002767	Guo et al. (2014)
Russula sp.	China: Yunnan	HKAS 78370	KF002760	Guo et al. (2014)

Table 1 Continued.

Taxon	Location	Voucher No.	Sequence accession No.	Reference
Russula vinosobrunneola	**China: Heilongjiang**	**HMAS 278896**	**MG719923**	**This study**
Russula vinosobrunneola	**China: Heilongjiang**	**HMAS 281131**	**MG719924**	**This study**
Russula vinosobrunneola	**China: Heilongjiang**	**HMAS 278885**	**MG719925**	**This study**
Russula vinosobrunneola	**China: Heilongjiang**	**HMAS 278960**	**MG719926**	**This study**
Russula vinosobrunneola	**China: Heilongjiang**	**HMAS 281138 (Type)**	**MG719927**	**This study**
Russula violaceoincarnata	Finland	UPS2005.09.06-11	UDB002549	NA
Zelleromyces cinnabarinus	USA	FLAS-F-60604	MH016796	NA
Zelleromyces gardneri	USA	NA	DQ453696	Frank et al. (2006)
Zelleromyces gardneri	USA	NA	JN022500	NA
Zelleromyces giennensis	Spain	NA	AF230900	Calonge & Martín (2000)
Zelleromyces hispanicus	Spain	MA-Fungi 37497	AF231911	Calonge & Martín (2000)
Zelleromyces hispanicus	Spain	MA-Fungi 37498	AF231912	Calonge & Martín (2000)
Zelleromyces sp.	Australia: Tasmania	BH3528R	JF960852	Horton (2011)

Table 2 Specimens and their GenBank accession numbers analyzed in multi-gene phylogenetic analyses. Specimens with newly acquired sequences for this study are shown in bold.

Taxon	Voucher No.	*LSU*	*ITS*	*tef-1α*	*mtSSU*
Russula cf. *aurantioflammans*	HMAS 252635	KX441285	KX441038	MF893370	KX441532
Russula cf. *fulvograminea*	HMAS 267791	KX441355	KX441108	MF893440	KX441602
Russula cf. *graminea*	HMAS 271166	KX441468	KX441221	MF893553	KX441715
Russula cf. *laricina*	HMAS 267755	KX441348	KX441101	MF893433	KX441595
Russula cf. *melliolens* sp.1	HMAS 269070	KX441378	KX441131	MF893463	KX441625
Russula cf. *melliolens* sp.2	HMAS 276813	LT602938	LT602961	KX442503	LT602984
Russula font-queri	HMAS 267744	KX441343	KX441096	MF893428	KX441590
Russula fulvograminea	HMAS 252626	KX441283	KX441036	MF893368	KX441530
Russula laricina	HMAS 269630	KX441400	KX441153	MF893485	KX441647
Russula sichuanensis	HMAS 268888	KX441372	KX441125	MF893457	KX441619
Russula sichuanensis	**HMAS 255316**	**MG786572**	**MG786566**	**MG812160**	**MG792323**
Russula vinosobrunneola	**HMAS 278896**	**MG786567**	**MG719923**	**MG812155**	**MG792318**
Russula vinosobrunneola	**HMAS 278960**	**MG786568**	**MG719926**	**MG812156**	**MG792319**
Russula vinosobrunneola	**HMAS 281138**	**MG786569**	**MG719927**	**MG812157**	**MG792320**
Russula vinosobrunneola	**HMAS 278885**	**MG786570**	**MG719925**	**MG812158**	**MG792321**
Russula vinosobrunneola	**HMAS 281131**	**MG786571**	**MG719924**	**MG812159**	**MG792322**

Results

Phylogenetic analyses

For *Lactarius*: The best-fit model of Bayesian analysis was GTR+I+G. The Maximum Likelihood and Bayesian trees had almost identical topology except for several minor terminal rearrangements, which were not statistically supported. The new species *L. sulphosmus* clustered with *L. illyricus* Piltaver in section *Zonarii* (Quél.) Bon in *Lactarius* subgenus *Piperites* (Fr. ex J. Kickx fil.) Kauffman (BS 76%, PP 0.98, Fig. 1). A blast against the GenBank database showed that the ITS sequence of the new species has 95% identity with those of *L. illyricus*, *L. purpureus* R. Heim, and *L. psammicola* A.H. Sm. The topologies generally agree with those described by Le et al. (2007) and Verbeken et al. (2014). The monophyly of both subgenus *Piperites*, sections *Colorati* (Bataille) Hesler & A.H. Sm., *Uvidi* (Konrad) Bon, *Piperites* (Fr. ex J. Kickx fil.) Burl., and *Zonarii* was not supported.

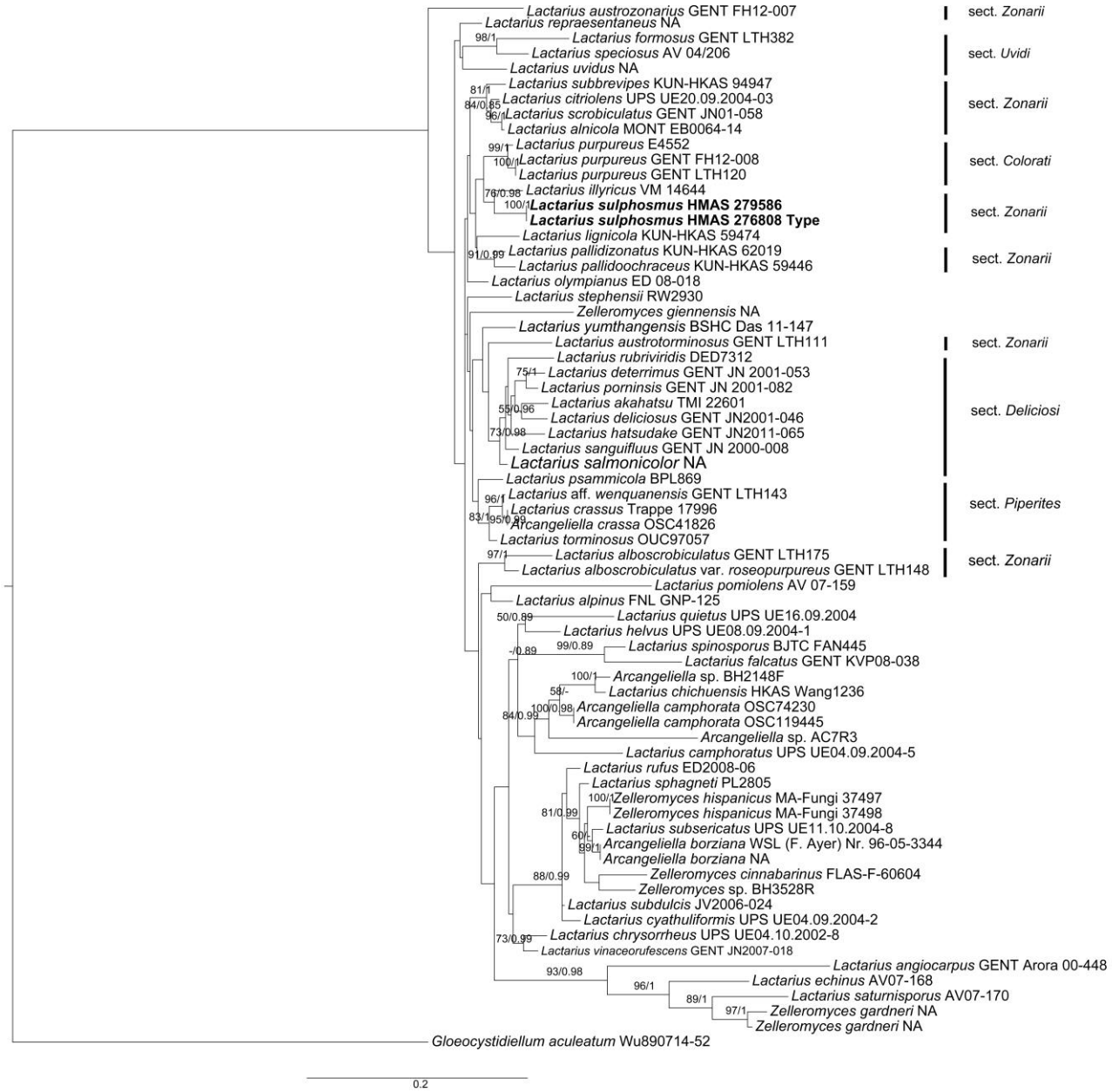

Figure 1 – Phylogeny of *Lactarius* subgenus *Piperites* and other closely related species generated from the Maximum Likelihood analysis of the *ITS* sequences. Bootstrap support (BS) > 50 % and Bayesian posterior probability (PP) values > 0.8 are given at the internodes (BS / PP).

For *Russula*: The best-fit models of Bayesian analyses were HKY (ITS1), HKY+G (ITS2 and ITS1-5.8S-ITS2), GTR+I+G (*nLSU* and 5.8S), F81+G (*mtSSU*), and SYM+G (*tef-1α*). The *ITS*-based phylogeny of *Russula* section *Tenellae* (Quél.) Sarnari subsection *Laricinae* (Romagn.) Bon was unable to distinguish three common species *R. nauseosa*, *R. laricina*, and *R. cessans* in Clade A (Fig. 2), which had poor statistical support (BS 52%, PP-). Clade B was highly supported by 96% BS and 1 PP values (Fig. 2). It included the agaricoid species *R. vinosobrunneola*, the semi-gasteroid species *R. sichuanensis* G.J. Li & H.A. Wen, some unrecognized *Russula* taxa from China, and *R. nauseosa* (TU 118126 from Estonia). Although the morphological differences of *R. sichuanensis* and *R. vinosobrunneola* were remarkable, the ITS-based phylogeny could not separate them. *Russula font-queri* Singer, *R. aurantioflammans* Ruots., Sarnari & Vauras, *R. fulvograminea* Ruots., Sarnari & Vauras, *R. violaceoincarnata* Knudsen & T. Borgen, and three more taxa (*R.* cf. *aurantioflammans*, *R.* cf. *fulvograminea*, and *R.* cf. *graminea)* formed a clade that was supported by a PP value of 0.77 (Clade C, Fig. 2).

Therefore, a multi-gene phylogenetic analysis of *nLSU*, *ITS*, *mtSSU*, and *tef-1α* was carried out for a better specific level resolution. The multi-gene phylogenetic topology (Fig. 3) showed that *R. vinosobrunneola* and *R. sichuanensis* were separated into two clades with strong support (*R. vinosobrunneola*: BS 98%, PP 0.99, *R. sichuanensis*: BS 100%, PP 1.00).

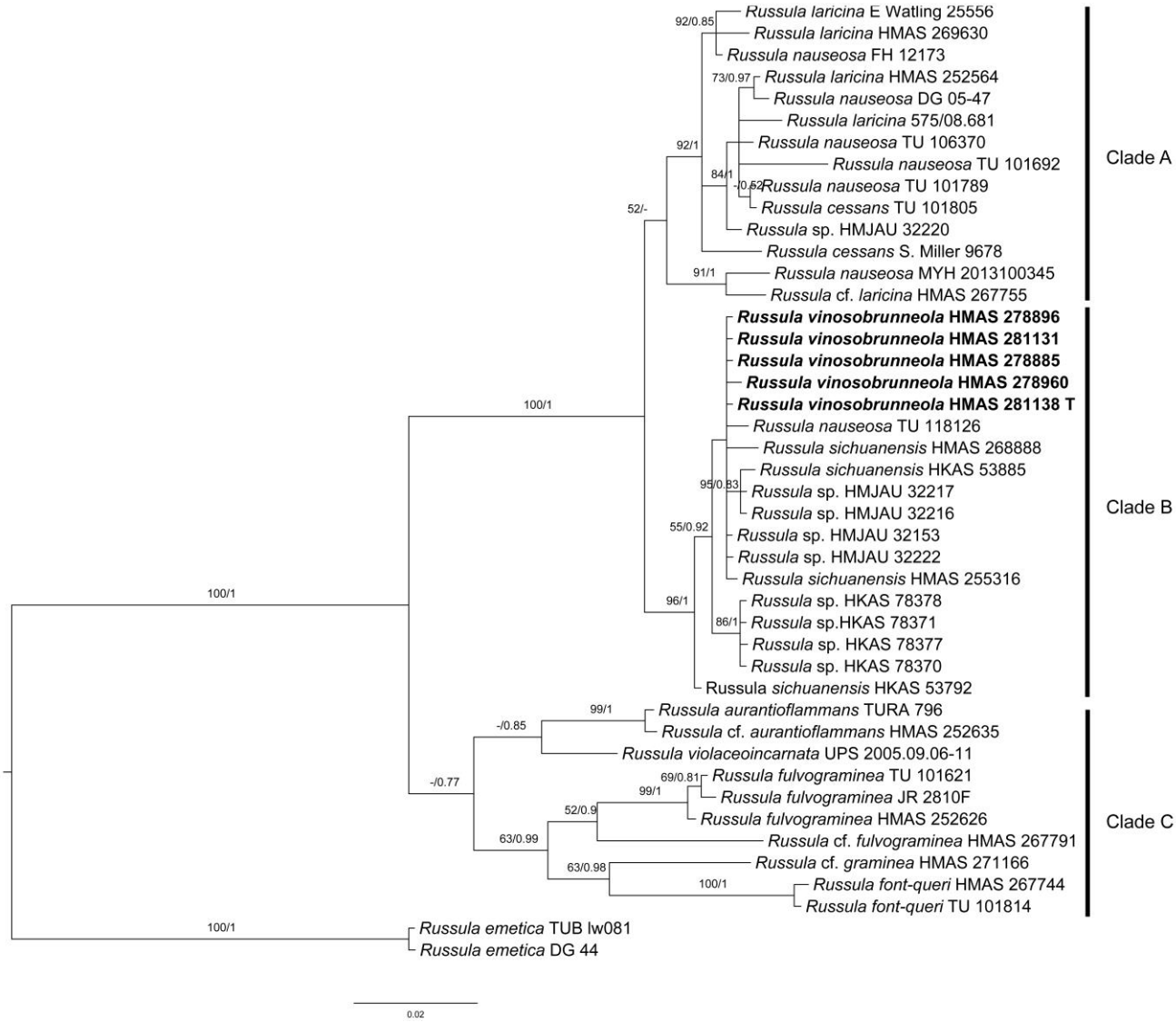

Figure 2 – Phylogeny of *Russula* section *Tenellae* subsection *Laricinae* generated from the Bayesian analysis of the ITS sequences. Bootstrap support (BS) values > 50 % and Bayesian posterior probability (PP) values > 0.8 are given at the internodes (BS / PP).

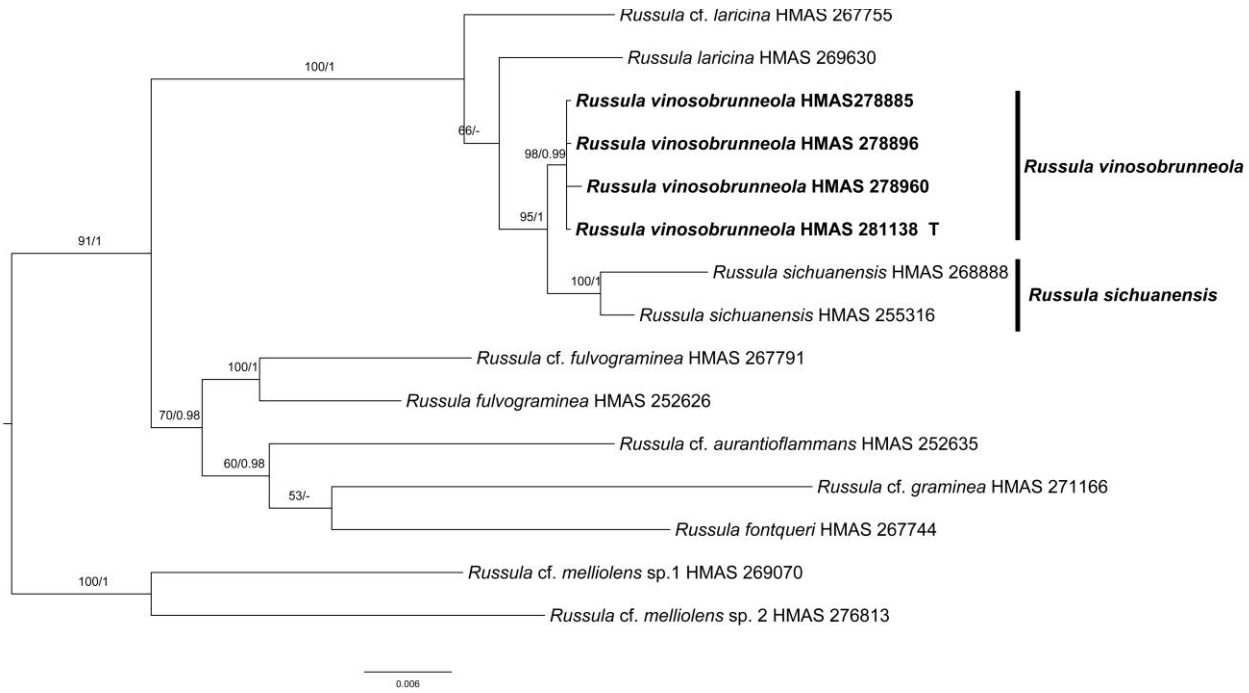

Figure 3 – Phylogeny of *R. vinosobrunneola* and *R. sichuanensis* and allied species generated from the Bayesian analysis of a combination of *ITS*, *nLSU*, *mtSSU* and *tef-1α* gene sequences. Bootstrap support (BS) values > 50 % and Bayesian posterior probability (PP) values > 0.8 are given at the internodes (BS / PP).

Taxonomy

Lactarius sulphosmus G.J. Li & R.L. Zhao, sp. nov. Figs 4a–b, 5

MycoBank number: MB824979; Facesoffungi number: FoF03950

Etymology – refers to the strongly sulphide-like smell of basidiomata.

Typus – China, Fujian Province, Wuyishan City, Wuyishan National Nature Reserve, Huangxizhou, N27°43' E117°46', elevation 823 m, 6 June 2015, collector Rui Lin Zhao, Guo Jie Li, Mao Qiang He, and Sheng Yu Su, *ZRL20150189.* (HMAS 276808).

Diagnosis –Basidiomata 35–46 × 25–31 mm, irregularly subglobose. Peridial surface pale yellow, dull. Gleba cream, loculate. Columella absent. Latex abundant. Smell strongly like sulphide, reminiscent of hair burning. Basidiospores globose to subglobose, with isolated and curved spines branched at apex. Basidia four-spored. Pseudocystidia rare. True cystidia rare.

Description – *Basidiomata* small to medium-sized, 35–46 × 25–31 mm, irregularly globose to subglobose, planoconcave at base. Peridium irregularly shaped, often with furrows up to 5 mm in depth, smooth, dull, not viscid when wet, pale yellowish tinged, light buff (XV17′) to pale ochraceous-buff (XV15′f), often intermixed with a darker ocher tinge of chamois (XXX19″b) to honey yellow (XXX19″). *Stipe* and *columella* absent. *Gleba* first compact, crowdedly loculate when mature, rarely labyrinthoid (2–3 per mm), cream colored, cartridge buff (XXX19″f) to ivory yellow (XXX21″f), partly turning ochraceous tawny (XV15′i) to honey yellow (XXX19″). *Latex* white, rather abundant, thick and sticky, mild tasting, forming a thin and white layer on gleba when dry. Smell strongly like sulphide, reminiscent of hair burning. *Taste* mild.

Basidiospores [500/2/2] (11.3–) 12.3–15.4 (–15.9) × (10.5–) 11.2–13.3 (–14) µm, Q = 1.00–1.25 (–1.28) (Q = 1.14 ± 0.07), globose to subglobose, rarely broadly ellipsoid, echinulate; ornamentation composed of amyloid warts that are isolated, curved, rounded, or shortly double or triple forked at the apex, 1.8–2.2 µm in height; suprahilar area amyloid but indistinct. *Basidia* 25–29 × 6–8 µm, four-spored, rarely two-spored, sterigmata 1–2 µm long, subcylindrical to cylindrical, rarely subclavate, hyaline, unchanging in KOH. *True cystidia* rare, 32–43 × 9–11 µm, clavate to subclavate, sometimes fusoid, subfusoid to subcylindrical, apex rounded to obtuse, thin–walled,

hollow, unchanging in SV. *Pseudocystidia* rare, cylindrical, 4–6 µm in diameter (diam.) Peridiopellis a trichoderm, composed of thin-walled, mostly ascending to suberect, unbranched, cylindrical hyaline hyphae 3–6 µm wide; pileocystidia absent; terminal cells cylindrical, apex round to obtuse, rarely tapered. *Clamp connections* absent in all tissues.

Habit and habitat – Hypogeous, single in broad-leaved forest (dominated by e.g., *Castanopsis eyrie*, *C. faberi*, *C. fargesii*, *Eurya japonica*, *Lithocarpus hancei*, *Machilus thunbergii*, *Manglietia fordiana*, and *Schima superba*) at 800–900 m altitude.

Other specimens examined – China, Fujian Province, Wuyishan City, Wuyishan National Nature Reserve, Huangxizhou, N27°43' E117°46', elevation 823 m, 6 June 2015, collector Rui Lin Zhao, Guo Jie Li, Mao Qiang He and Sheng Yu Su, *ZRL20154537*. (HMAS 279586).

Distribution – China (Fujian Province). Season. June.

Notes – The combination of abundant white latex, strong sulphidic, burning hair smell, spore ornamentation composed of high spine with double or triple forked tips, is distinct for the new species *L. sulphosmus*. The ITS-based phylogenetic analysis (Fig. 1) showed that *L. sulphosmus* and *L. illyricus* from Slovenia form a clade (BS 76%, PP 0.98), which indicates that they are closely related in phylogeny. However, they are obviously different species in morphology, since *L. illyricus* is an agaricoid species (Le et al. 2007, Piltaver 1992). This new species has pale yellow basidiomata, not hairy pileus and white, unchanging latex, which are characters of section *Zonarii* (Bon 1980).

In morphology *L. sulphosmus* is most similar to the Chinese species *Zelleromyces ramispinus* (B.C. Zhang & Y.N. Yu) Trappe, T. Lebel & Castellano (basionym: *Martellia ramispina* B.C. Zhang & Y.N. Yu) because both have gasteroid basidiomata, spore surface with spines over 2 µm high and double or triple forked tips, and a habitat of subtropical forest. However, *Z. ramispinus* has white peridium surface, and larger basidiospores (15–20 µm in diam, Zhang & Yu 1990).

Russula vinosobrunneola G.J. Li & R.L. Zhao, sp. nov. Figs 4c–d, 6

MycoBank number: MB824980; Facesoffungi number: FoF04080

Etymology – refers to the vinaceous brown tinged, small to medium-sized pileus.

Typus – China, Heilongjiang Province, Qitaihe City, Boli County, Xidaquan National Forest Park, N 45°45′ E 130°34′, elv. 753 m., 19 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20160434*. (HMAS 281138).

Diagnosis – the morphological characteristics of *R. vinosobrunneola* are similar to those of *R. laricina*, but *R. vinosobrunneola* has amyloid ornamentation, composed of warts mostly linked by fine lines as ridges, reticulate, rarely isolated, forming an incomplete to complete reticulum.

Description – *Basidiomata* small to medium-sized. *Pileus* 11–54 mm in diam., first subglobose to hemispheric, plano-convex when mature, sometimes depressed at center, slightly striate about 3–5 mm from the edge inwards when old, rarely cracked, slightly viscid when wet, sometimes glabrous, peeling 1/5–1/3 from the edge, often vinaceous brown tinged, hazel (XIV11′k), liver brown (XIV7′m) to vinaceous russet (XXVIII7″) at the edge, a paler tinge of avellaneous (XL17‴b), fawn color (XL13‴) to olive buff (XL21‴d) towards the center. *Lamellae* adnate, 2–4 mm in height, 11–16 per cm at the edge, not forked, often interveined, first pale yellowish tinges of martius yellow (III23f), turning Apricot Yellow (XL21‴d) to Light Cadmium (IV19) when mature, Orange-Citrine (IV19k) to Medal Bronze (III19m) when old and dry, lamellulae absent. *Stipes* central to subcentral, 4.2–6.5 × 0.9–1.6 cm, subcylindrical to cylindrical, surface dry, rugulose longitudinally, dull, without annulus, slightly attenuate upwards, White (LIII), a brown tinge of Tawny (XV13′i) when bruised and dry, stuffed at first, becoming hollow when old. *Context* up to 1–3 mm at the center of the pileus, White (LIII), unchanging, slowly turning greyish brown, tinges of Sudan Brown (III15k), Ochraceous-Tawny (XV15′i) to Buckthorn Brown (XV17′i) when bruised, fragile; odor not distinct; taste mild. *Spore print* Yellow (Romagnesi IV b–c).

Basidiospores [500/2/2] 7.7–9.6 (–10.1) × 6.4–8 (–8.6) µm, Q = 1.06–1.33 (–1.38) (Q = 1.19 ± 0.07), subglobose to broadly ellipsoid, rarely globose to ellipsoid, verrucose; ornamentation

amyloid, composed of verrucose to conical warts 0.7–1 µm in height, mostly linked by fine lines as ridges, reticulate, rarely isolated, forming an incomplete to complete reticulum; suprahilar area amyloid and distinct. *Basidia* 34–42 × 8–11 µm, clavate, ventricose towards upper half, four-spored, projecting 15–25 µm beyond hymenium, hyaline in KOH; sterigmata 4–6 µm, pointed, straight to slightly tortuous. *Pleurocystidia* scattered, 47–72 × 6–7 µm, originating from subhymenium, projecting 20–35 µm beyond hymenium, thin-walled, subfusiform to subclavate, sometimes clavate or fusiform, apex obtuse to round, at times subacute, contents crystalline, blackish grey in SV. *Cheilocystidia* not observed; lamellar edge sterile. *Subhymenium* 20–50 µm thick, composed of slender cylindrical cells 4–10 µm wide under basidia and inflated elliptical to globose cells 15–25 µm in diam. next to trama. *Pileipellis* 150–270 µm thick, composed of an epipellis (60–90 µm thick) and a subpellis (90–230 µm thick); epipellis a cutis or trichoderm, composed of mostly ascending to repent hyphae, intermixed with a few suberect elements, 2–4 µm in diam.; pileocystidia abundant, 37–55 × 3–5 µm, cylindrical to subcylindrical, with round to obtuse tips, multi-septate, contents dense, black in SV; subpellis composed of long, rarely septate, equally wide hyphae 2–4 µm in diam. interweaved with sphaerocytes 15–30 µm in diam. *Stipitipellis* a cutis composed of thin-walled, septate, cylindrical, hyaline to slightly yellowish hyphae 3–5 µm wide; terminal cells of stipitipellis hyphae with obtuse apex; caulocystidia not observed. *Trama* composed of sphaerocystes 15–60 µm in diam. and septate filamentous hyphae. *Clamp connections* and *lacticiferous hyphae* absent in all tissues.

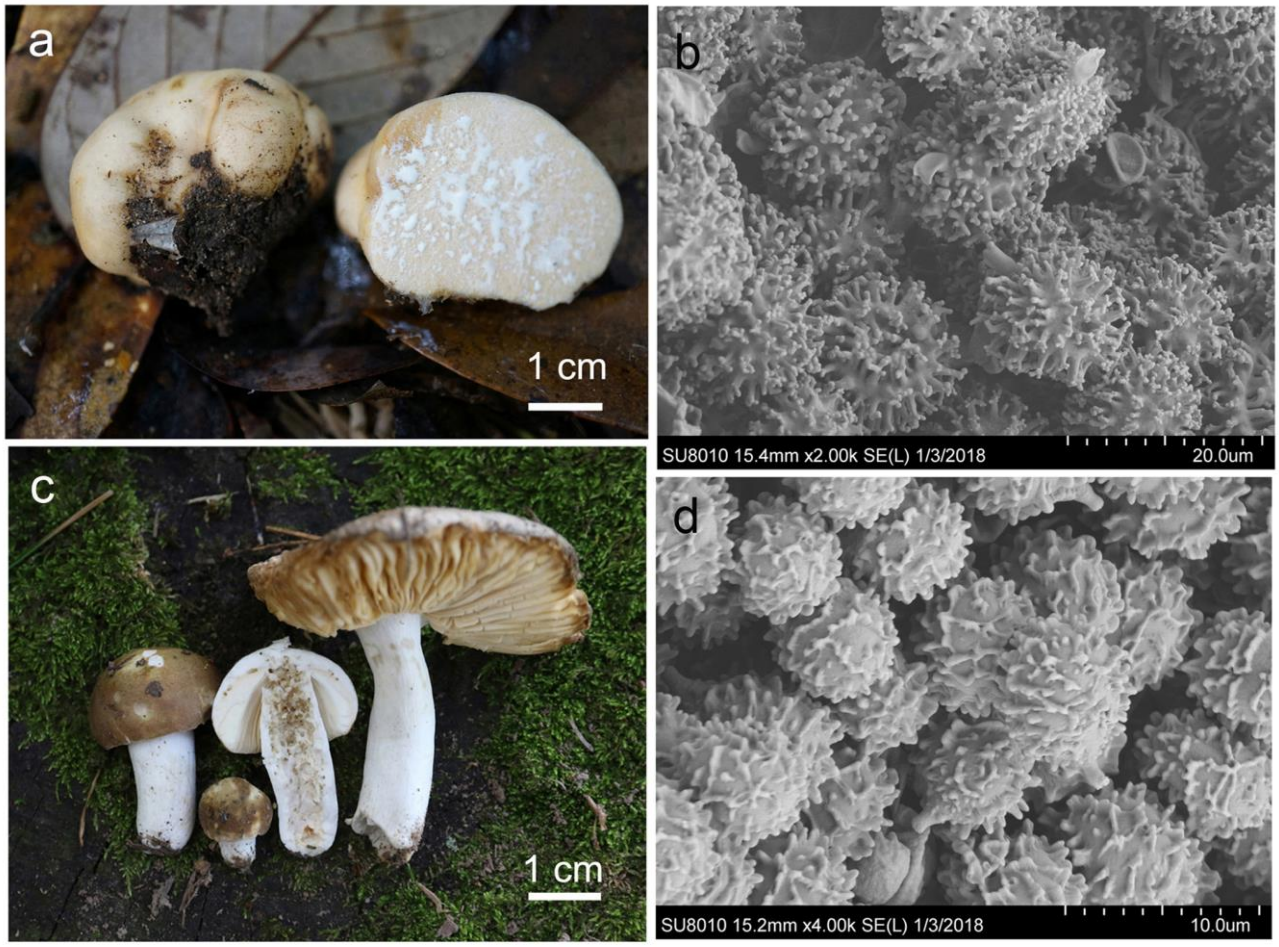

Figure 4 – Basidiomata and scanning electron micrograph (SEM) of basidiospores. a–b *Lactarius sulphosmus*: *ZRL20150189* (HMAS 276808 holotype). c–d *Russula vinosobrunneola*: *ZRL20160434* (HMAS 281138 holotype).

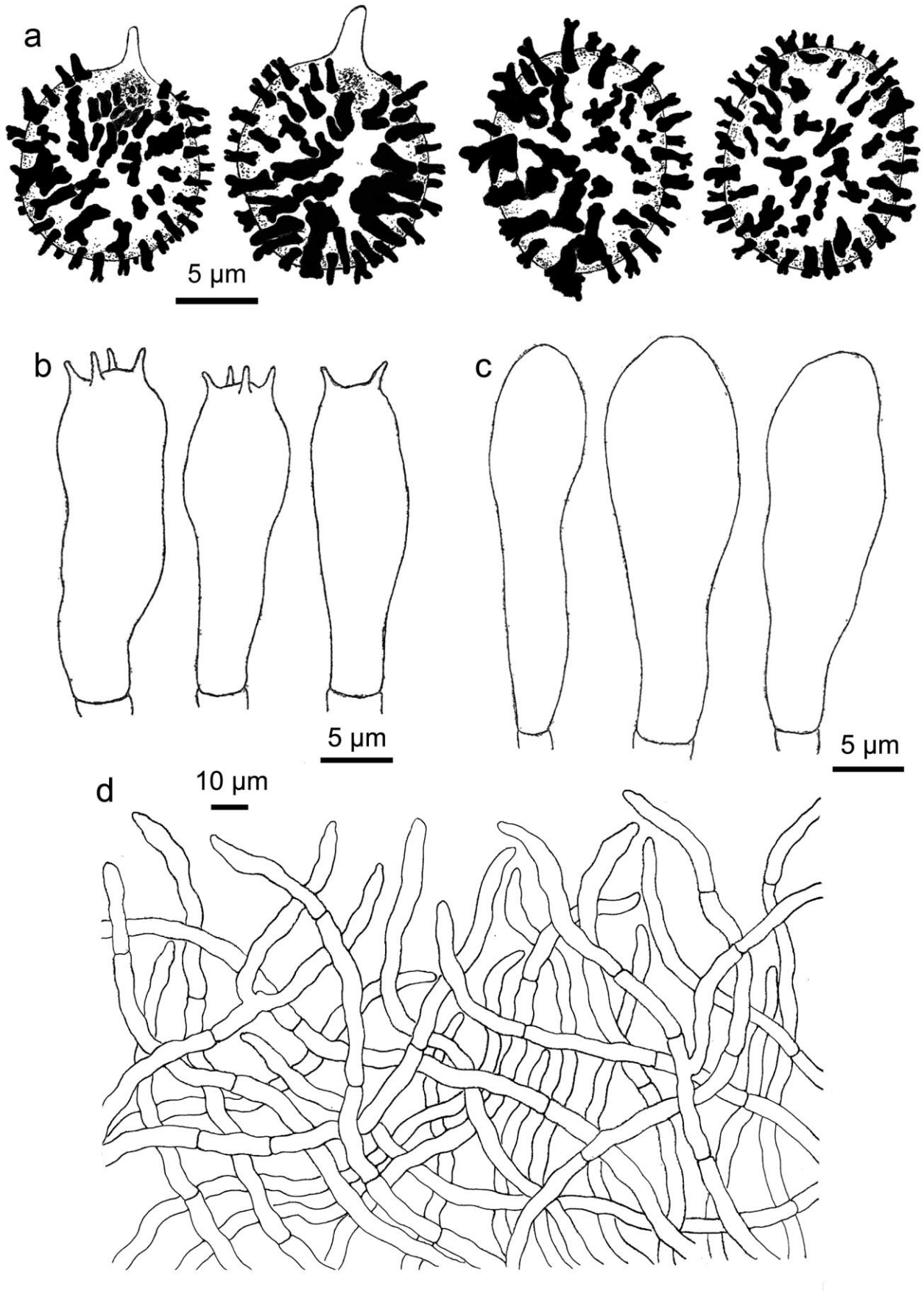

Figure 5 – *Lactarius sulphosmus*: *ZRL20150189* (HMAS 276808 holotype) a Basidiospores. b Basidia. c True cystidia. d Pileipellis (epipellis).

Habit and habitat – Scattered in coniferous and broad-leaved intermixed forests (dominated by e.g., *Abies nephrolepis*, *Pinus bungeana*, *P. koraiensis*, *P. sylvestris* var. *mongolica*, *Tilia tuan*, and *Xylosma racemosum*) at 600–800 m altitude.

Other specimens examined – China, Heilongjiang Province, Kiamusze City, Tangyuan County, Daliangzihe National Forest Park, N 47°04′ E 129°71′, elv. 619 m., 18 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20160383*. (HMAS 281131); Qitaihe City, Boli County, Xidaquan National Forest Park, N 45°45′ E 130°34′, elv. 827 m., 19 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20160428*. (HMAS 278960); elv. 721 m., *ZRL20160394*. (HMAS 278885); Yichun City, Dailing District, Liangshui National Nature Reserve, N 47°18′ E 128°87′, elv. 657 m., 17 July 2016, Rui-Lin Zhao, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20160273*. (HMAS 278896).

Distribution – China (Heilongjiang Province). Season. July.

Notes – A combination of yellow spore print, greyish brown turning context, and coniferous habitat indicates that *R. vinosobrunneola* is a member of *Russula* section *Tenellae* subsection *Laricinae* (Sarnari 2005). The semi-gasteroid species *R. sichuanensis* is the closest species to *R. vinosobrunneola* phylogenetically, but they can be readily distinguished because *R. sichuanensis* has secotioid basidiomata, dirty-white to pale-pinkish pileus, globose to subglobose, larger basidiospores 8.8–15.5 × 7.6–13.1 µm with warts often higher than 1 µm, shorter and wider basidia 24–35 × 11–15 µm, and an ixotrichoderm pileipellis (Li et al. 2013). Compared with other members of subsect. *Laricinae*, *R. vinosobrunneola* most resembles *R. laricina* Velen., due to the similar pileus colors and dimensions of basidiospores, basidia, and pleurocystidia. The only difference is that the basidiospore ornamentation of *R. laricina* is composed of mostly isolated warts (Sarnari 2005), while those of *R. vinosobrunneola* is mostly reticulum. The basidiospore ornamentation reticulum from this new species is also helpful to separate it from *R. nauseosa* (Pers.) Fr. (Sarnari 2005). Although *R. cessans* A. Pearson has similar basidiospore ornamentations to those of *R. vinosobrunneola*, it can be distinguished for having pink to vinaceous pileus, subacrid and unchanging context, and wider pleurocystidia up to 12 µm. The other uncommon species of *R.* subsect. *Laricinae*, such as *R. olivina* Ruots. & Vauras, differ from *R. vinosobrunneola* in their two-spored basidia and larger basidiospores of 10–14 × 8–11.5 µm and *R. sapinea* Sarnari differs from *R. vinosobrunneola* by its strongly yellowish context and ocher spore print (Sarnari 2005). *Russula vinosobrunnea* (Bres.) Romagn., a species of *R.* section *Polychromidia* Romagn., subsection *Olivaceinae* Singer, is taken for *R. vinosobrunneola* because they both have vinaceous brown pileus, yellow spore prints, and basidiospores 7–10 × 6–8 µm. The similarity of specific epithets can also lead to the confusion of these two species. However, *R. vinosobrunnea* can be distinguished by its large stout basidiomata with pileus up to 120 mm in diam., long pleurocystidia up to 130 µm in length, pileipellis with primodial hyphae without incrustation, and a habitat of *Fagus* forest (Sarnari 2005).

Discussion

The coexistence of agaricoid, secotioid, and gasteroid lineages within one group has been reported many times in Agricomycetes (Wilson et al. 2011). The phylogenetic relationships between the agaricoid and gasteroid lineages have been analyzed in many studies (Hopple & Vilgalys 1994, Mueller & Pine 1994, Kretzer & Bruns 1997, Miller et al. 2001, Wilson et al. 2011) and agaricoid basidiomata has been shown to be the plesiomorphy. The agaricoid new species of this study *R. vinosobrunneola* has a close phylogenetic relationship with secotioid *R. sichuanensis* (Fig. 2). *Russula sichuanensis* is distributed in coniferous forests of the Qinghai–Tibet Plateau (Li et al. 2013, Saba & Khalid 2015). The differences between the habitats of these two species corroborate the viewpoint that the stage of gasteromycetization is a coping mechanism for the hostile environment (Hibbett et al. 1994, Buyck 1995, Trappe & Claridge 2005, Smith et al. 2006, Verbeken et al. 2014). The results of phylogenetic analyses also showed that these two species could not be distinguished by ITS phylogeny, indicating the speciation may have occurred recently in their evolutionary history.

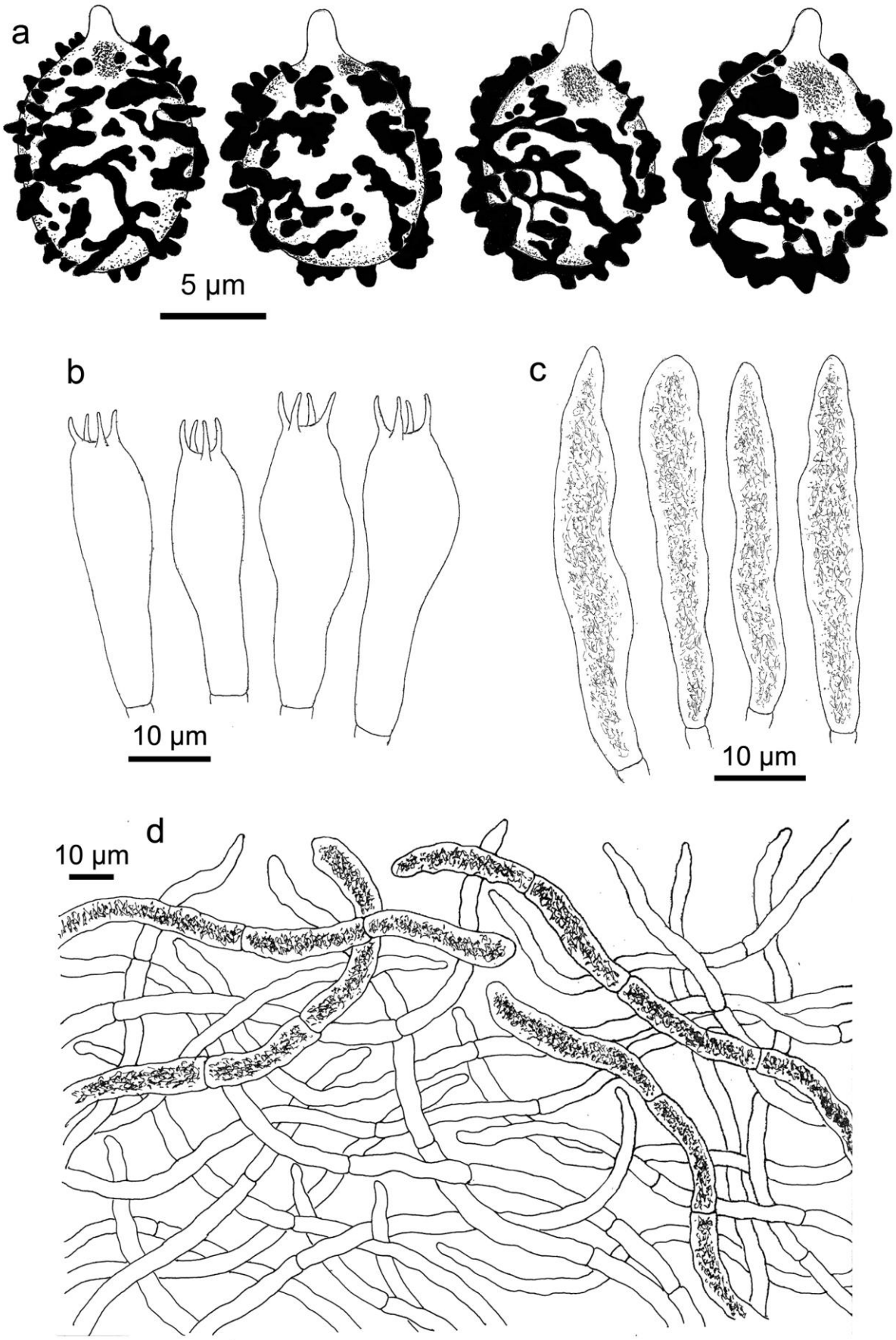

Figure 6 – *Russula vinosobrunneola*: *ZRL20160434* (HMAS 281138 holotype) a Basidiospores. b Basidia. c True cystidia. d Pileipellis (epipellis).

Verbeken et al. (2014) revealed a striking diversity of sequestrate Russulales in Southeast Asia. A total of ten species have been recognized in this area. In China, most species of sequestrate Russulales, including *L. sulphosmus* of this study, are described from subtropical regions. Only *Zelleromyces sinensis* B. Liu, K. Tao & Ming C. Chang is from a temperate region in China (Tao & Chang 1993). Compared with the recent morphological descriptions of the agaricoid species in detail (Buyck & Adamčík 2013, Buyck et al. 2013, 2015, Adamčík et al. 2015a, 2018), those of above angiocarpous taxa are often brief. Considering that angiocarpous mushrooms are often overlooked (Verbeken et al. 2014), a majority of recent new *Russula* species are agaricoid, whatever their collecting localities are in Africa (Sanon et al. 2014), Asia (Das et al. 2013, 2017a, 2017b, 2018, Ariyawansa et al. 2015, Hyde et al. 2016, Li et al. 2016, 2018b, Jiang et al. 2017, Song et al. 2018), America (Adamčík et al. 2015b, Hyde et al. 2017), and Europe (Trendel et al. 2017). Thus further investigations of gasteroid species are more needed than agaricoid ones. As divergence time can be used as a universal criterion for taxa ranking (Zhao et al. 2016, 2017). We suggest that the divergence times of the agaricoid, secotioid, and gasteroid Russulaceae lineages can be estimated in future evolution analyses. Because it is not only helpful in evolutionary relationship clarification these lineages, but also useful in the systematic analyses of corresponding species.

Acknowledgements

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