Two new species of *Russula* from Northeast China

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Li GJ, Zhang CL, Zhao RL, Lin FC 2018 – Two new species of *Russula* from Northeast China. Mycosphe 9(3), 431–443, Doi 10.5943/mycosphe/9/3/1

Abstract

Two new species of the genus *Russula* from northeastern China are described and illustrated based on phylogenetic analysis of internal transcribed spacer (ITS) ribosomal DNA sequences and morphological examination. These two new species are *R. heilongjiangensis* (from subsect. *Maculatinae*) and *R. khinganensis* (from subsect. *Puellarinae*). The morphological and molecular analyses support their taxonomic classification. A comparison of these new species and other similar species is also addressed in this paper.

Key words – Agaricomycetes – morphology – phylogeny – Russulaceae – Russulales – taxonomy

Introduction

The genus *Russula* Pers. is one of the most conspicuous gill mushroom groups among Basidiomycetes. It is characterized by a heteromerous context and amyloid spore ornamentation (Romagnesi 1967, 1985, Sarnari 1998, 2005, Li 2014). This genus contains hymenoid and sequestrate species, and the latter claim has been widely proved by molecular phylogenetic analyses (Lebel & Tonkin 2007, Buyck et al. 2010). Basidiocarps of *Russula* are common in various boreal forests (Romagnesi 1967, 1985, Sarnari 1998, Li 2014) and form an ectomycorrhizal system in association with diverse host plants (Trappe 1962, Roberts et al. 2004). The basidiocarps of a number of *Russula* species are collected for food and medicine (Buyck 2008, Dai & Yang 2008, Dai et al. 2010, Li et al. 2010), and a few red-capped species of *Russula* have an acrid taste when eaten raw or undercooked (Li et al. 2010, Bau et al. 2014, Li 2014); however, some species are poisonous (Li et al. 2010, Li 2014). Phylogenetic analyses of *Russula* and Russulaceae were carried out to investigate the relationship of infrageneric groups and monophyly of the genus (Eberhardt 2002, Miller & Buyck 2002, Park et al. 2013, Adamčík et al. 2016a, 2016b). Although *Russula* was shown to be paraphyletic in some early phylogenetic analyses (Miller et al. 2001, 2006, Shimono et al. 2004), additional analyses suggested that *Russula* is a monophyletic genus (Eberhardt 2002, Larsson & Larsson 2003, Buyck 2008, Buyck et al. 2010, Zhao et al. 2017). To establish new *Russula* taxa, internal transcribed spacer (ITS) phylogeny is commonly used as evidence. In recent years, Asia has become a hot spot for the exploration of unknown *Russula* species (Li 2014, Li et al. 2015).

Northeast China, historically known as Manchuria, consists of the three provinces of Heilongjiang, Jilin, and Liaoning and the eastern part of the Inner Mongolia Autonomous Region.

The temperate continental climate of this region often means a long and cold winter and a short and rainy summer. In terms of flora, Northeast China includes the Eurasian forest subregion and Sino-Japanese subregion of the Holarctic region (Wu 1979). The three main mountain formations are the Changbai, Greater, and Lesser Khingan mountains, which contain subfrigid coniferous forests, temperate coniferous and broad-leaved forests, and broad-leaved deciduous forests. The main tree species of Northeast China are *Betula platyphylla*, *B. schmidtii*, *Larix gmelinii*, *Pinus koraiensis*, *P. sylvestris* var. *Mongolica*, *Populus davidiana*, and *Quercus mongolica* (Xu 1998). Many mountainous areas contain the best conserved forests in China (Gu 1986, Ding & Kang 1994), which facilitate the fruiting of *Russula*. Although dozens of *Russula* species were identified in different parts of the local fungal flora (Dai & Bau 2007, Li & Bau 2003, Bau et al. 2007, Bau 2012, Wang & Bau 2015), a thorough study of this genus in Northeast China is still not available. The flora of *Russula* in this region is an intermixture of European, North American, and Asian species (Li 2014). Three new species and three new Chinese records of *Russula* have been reported in this region (Li et al. 2012, 2013, Liu et al. 2017, Jiang et al. 2017).

During a mushroom survey in Northeast China in 2016, two novel *Russula* species were identified, based on morphological and molecular phylogenetic analyses. They are described and illustrated herein with morphological details. Other species similar in morphology and phylogeny to these new species are also discussed.

Materials & Methods

Morphological analyses

Specimens were photographed with a Cannon EOS 7D Mark II digital camera (Utsunomiya, Japan). Macromorphological characteristics were recorded in detail, in the field and in daylight, based on fresh basidiocarps. Names and codes of the colors in the description follow those of Ridgway (1912). For permanent conservation, the specimens were dehydrated in a fruit dryer at 60°C until their water content fell below 10%. Rehydration of the dried specimens was carried out in 5% KOH. A solution of Congo Red was used for microscopic measurements and line drawing. Sulfovanillin (SV) and Melzer's reagent were employed for chemical reaction tests of basidiospore ornamentation and cystidium content. The rehydrated lamellae and pileus epidermis were sliced by hand with a razor blade before microscopic examination under a Nikon Eclipse 80i microscope (Tokyo, Japan). Basidiospores, basidia, and cystidia elements of the pileipellis and stipitipellis were examined and measured. Lengths of the apiculus and spore ornamentation were excluded from measurements. At least 20 elements were measured for each characteristic. An FEI Quanta 200 electron microscope (Hillsboro, OR, USA) was used for scanning electron microscopy. Herbarium name abbreviations followed those of Thiers (2018). The abbreviation [*a/b/c*] indicates that *a* basidiospores were measured in *b* fruit bodies of *c* specimens. Basidiospore dimensions are displayed as (*w*–) *x*–*y* (–*z*), *x*–*y*, which is the range including 95% or more of the measured values, while *w* and *z* are extremes of all the measured values. *Q* stands for the ratio basidiospore length/width. Bold *Q* represents the average value of *Q* plus or minus the standard deviation. Additional microscopic examination details in this study are explained in articles by Li et al. (2012, 2015), Li (2014). Comparison of selected morphological characteristics of the new species and closely related species were provided in Tables 1, 2. Specimens of the two new taxa were deposited in Herbarium Mycologicum, Academiae Sinicae (HMAS).

Phylogenetic analyses

DNA extraction procedures were performed with the GeneOn Plant DNA Extraction Kit (GeneOn BioTech, Changchun, China) by the magnetic bead method. The ITS sequences were amplified with primer pairs ITS1/ITS4, LROR/LR5, and NS1/NS4 (White et al. 1990, Hibbett 1996, Moncalvo et al. 2000, 2002). Polymerase chain reaction (PCR) was carried out on a Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific, MA, USA) according to the reaction conditions of Li (2014). PCR products were purified and sequenced with the same primers as mentioned above

Table 1 A comparison of selected morphological characteristics of *R. heilongjiangensis* and closely related species.

Taxon	Pileus color	Pileus diameter (mm)	Basidiospore dimensions (μm)	Basidiospore ornamentation	Basidia dimensions (μm)	Pileocystidia dimensions (μm)	Habitats
<i>Russula dryadicola</i>	purple, often fading to yellow	40–60	8.5–11.5 × 7.5–10	mostly isolated, 0.9–1.3 μm in height, rarely linked, not reticulate	50–62 × 10.5–15.5	75–130 × 9.5–16.5	alpine zone, associated with <i>Dryas</i> and <i>Juniperus</i>
<i>Russula heilongjiangensis</i>	bright red	28–52	6.8–10 × 5.6–8.3	mostly isolated, rarely linked as short crests 0.7–1 μm in height, not reticulate	34–46 × 9–13	68–101 × 9–12	coniferous and broad-leaved intermixed forests, with <i>Betula</i> , <i>Pinus</i> , and <i>Xylosma</i>
<i>Russula globispora</i>	variously colored, sometimes violet to cooper brown	40–100	9.7–12.4 × 8.1–9.7	Ornamentation 0.8–1.1 μm high, mostly isolated, rarely fused in pairs	41–53 × 12–17	60–95 × 12–16.5	deciduous forest
<i>Russula maculata</i>	red to orange	45–130	8.3–10.2 × 6.5–8.6	ornamentation 0.6–0.7 μm in height, linked as lines, partly reticulate, rarely isolated	39–60 × 12–15	59–90 × 11.5–14	deciduous forest, often under oaks of calcareous soil
<i>Russula nymphaeum</i>	cream, rose to orange	up to 100	8.3–10.8 × 6.5–7.9	ornamentation 0.3–0.6 μm high, often connected with fine lines, rarely reticulate	39–62 × 9–14	60–140 × 8–15	deciduous forest, often under <i>Quercus</i> and <i>Arbutus</i>

Table 2 A comparison of selected morphological characteristics of *R. khinganensis* and closely related species.

Taxon	Pileus color	Pileus diameter (mm)	Basidiospore print (Romagnesi)	Basidiospore dimensions (μm)	Basidia dimensions (μm)	Pileocystidia dimensions (μm)	Context odor
<i>Russula khinganensis</i>	bright red	28–52 mm	III b–c	6.8–10 × 5.6–8.3	34–46 × 9–13	68–101 × 9–12	not distinct
<i>Russula minutalis</i>	reddish violet to brown	–	IIc	6.5–8 × 5–6.5	–	–	–
<i>Russula puellaris</i>	reddish violet to brown	32–60 mm	IIc	7–9 × 5.7–7.2	34–50 × 11.5–16	50–74 × 10–15	not distinct
<i>Russula terenopus</i>	violet to brown	20–50 mm	IId	6.5–8.2 × 5.4–6.4	32–45 × 8–12	50–70 × 6–14	<i>Pelargonium</i> smell, strong and lingering
<i>Russula versatilis</i>	reddish violet, fading to yellow	30–60 mm	III b–c	7–9 × 5.5–7	30–40 × 8–11.5	40–75 × 8–13.5	<i>Pelargonium</i> smell, fainter and less lingering

by Majorbio Co., Ltd. (Shanghai, China) on an ABI 3730XL DNA analyzer with the ABI BigDye 3.0 Terminator Cycle Sequencing Kit (Applied Biosystems, CA, USA). Newly obtained raw sequences were spliced and deposited in GenBank (accession numbers are shown in Figs 1, 2). A total of 73 ITS sequences of closely related *Russula* taxa, including two specimens of *R. emetica* (Schaeff.) Pers. as an outgroup, were retrieved from the GenBank database. The alignment matrices were deposited in TreeBASE (ID 22710). Bayesian analyses (BA) were conducted in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). The evolutionary best-fit models of Bayesian analysis (BA) were conducted in MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). The evolutionary best-fit model were evaluated by means of MrModelTest 2.3 (Nylander 2004) before analysis. Posterior probabilities (PPs) were calculated by the Markov chain Monte Carlo algorithm (Larget & Simon 1999). A run of 1,000,000 generations with the trees sampled every 100th generation was carried out for three heated and one cold Markov chain. Automated termination was executed when the average standard deviation of split frequencies fell below 0.01 during the analysis. The first 25% of trees were discarded as burn-in data (Hall 1999). Maximum parsimony (MP) analysis was carried out in the PAUP* software v.4.01 (Swofford 2004). Gaps in the alignment were treated as missing data, and all sites were regarded as unordered and unweighted. The tree bisection–reconstruction algorithm was used in a heuristic search, and bootstrap support (BS) was analyzed with 1000 replicates (Felsenstein 1985). The “Maxtrees” parameter was set to 5000, branches of zero length were collapsed, and all the most parsimonious trees were saved. Clade stability was assessed in a bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa. The Kishino–Hasegawa test (Kishino & Hasegawa 1989) was performed to determine whether trees were significantly different. Trees were depicted in TreeGraph 2.4.0 (Stöver & Müller 2010).

Results

The dataset for phylogenetic analyses of *Russula* subsect. *Maculatinae* contained 36 ITS sequences (average length 561 bp), representing 21 taxa; these sequences were studied by the MP method and BA. In the MP analysis, 380 characters were constant, 16 variable characters were parsimony-uninformative, and 205 characters were parsimony-informative. One most parsimonious tree was found in the heuristic search. The tree has a consistency index of 0.640, a retention index of 0.887, a rescaled consistency index of 0.568, a homoplasy index of 0.360, and a tree length of 450. Only a few minor differences in terminal topology were observed among trees by MP analysis and BA. Phylogenetic topology obtained by BA is shown in Figure 1. Clades of *R. globispora* and *R. maculata* complexes in this analysis corresponded to those of Adamčík et al. (2016a). *Russula heilongjiangensis* clustered with *R. dryadicola*, *R. maculata*, and two unrecognized taxa with strong support (PP 1.00, BS 99%).

The dataset for phylogeny analyses of *R.* subsect. *Puellarinae* Singer contained 43 ITS sequences (average 601 bp), representing 17 taxa, were analyzed by MP and BA. In the MP analysis, 382 characters were constant, 44 variable characters were parsimony-uninformative, and 135 characters were parsimony-informative. One of the four most parsimony trees which was found in the heuristic search was presented in Figure 2. The tree has a CI of 0.639, an RI of 0.777, an RC of 0.496, an HI of 0.361, and a TL of 360. Some rearrangements in terminal topology could be observed between parsimony trees and the Bayesian tree. Members of *R.* subsect. *Puellarinae*, including the new species *R. khinganensis*, formed a well-supported monophyletic clade (PP 1.00, BS 100%). Although the phylogenetic topology of *R.* subsect. *Puellarinae* were still not stable, the long phylogenetic distance between *R. khinganensis* and the other members of this section was enough to support it as an independent species.

Taxonomy

Russula heilongjiangensis G.J. Li & R.L. Zhao, sp. nov.

Fungal Names number: FN570531; Facesoffungi number: FoF03951

Fig. 3

Etymology – refers to Heilongjiang Province, the locality of the type specimen.

Typus – China, Heilongjiang Province, Mudanjiang City, Muleng County, Liufengshan National Forest Park, N 44°11' E 130°21', elev. 501 m., 21 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20160516* (HMAS 255142), ITS: MG719932, nLSU: MH021611, nSSU: MH021633.

Diagnosis – the morphological characteristics of *R. heilongjiangensis* resemble those of *R. maculata*, but *R. heilongjiangensis* has smaller basidiocarps and shorter and narrower basidia.

Description – *Basidiocarps* small to medium-sized. Pileus 28–52 mm in diam., first hemispheric, then plano-convex, applanate when mature, often slightly depressed at the center, striate ~3 mm from edge inward, sometimes cracked, slightly viscid when wet, peeling 1/4–1/3 from the edge, bright red tinges of Carmine (IIi), Begonia Rose (IIb), and Rose Doree (I3b) intermixed with small dark red patches of Dark Vinaceous (XXVIII"), Deep Corinthian Red (XXVII3"i), and Hydrangea Red (XXVIII"i), fading orange, pale yellowish brown to yellow tinges of Zinc Orange (XV13'), Sayal Brown (XXIX15") to Pale Orange Yellow (III17) at center. *Lamellae* adnate, 3–5 mm in height, 13–18 pieces per centimeter at edge, sometimes forked near stipe, often intervened, pale yellowish tinges of Martius Yellow (III23f), often slightly dotted with orange to pale brownish tinges of Yellow Ocher (XV17') to Sudan Brown (III15k) when bruised, 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 upward, White (LIII), brownish yellow tinge of Tawny (XV13'i) when bruised and dry, stuffed at first, becoming hollow when old. Context up to 2–3 mm at center of pileus, White (LIII) first, turning Ochraceous-Orange (XV15'), Ochraceous-Tawny (XV15'i) to Buckthorn Brown (XV17'i) when bruised, fragile, no distinct odor; taste moderately acrid. *Spore print* Yellow (Romagnesi IV d–e).

Basidiospores [100/2/2] (6.8–) 7.4–9.4 (–10) × (5.6–) 5.9–8.3 µm, $Q = (1.06–) 1.12–1.33$ ($Q = 1.21 \pm 0.07$), hyaline, subglobose to broadly ellipsoid, rarely ellipsoid; ornamentation composed of amyloid warts that are mostly isolated, rarely linked as short crests and ridges, not forming network, warts 0.7–1 µm high; suprahilar area amyloid and distinct. *Basidia* 34–46 × 9–13 µm, four-spored, sterigmata 3–6 µm long, hyaline, rarely yellowish in KOH, subclavate to clavate, rarely cylindrical. *Pleurocystidia* scattered, 68–101 × 9–12 µm, distinctly projecting 30–50 µm beyond basidia, clavate to subclavate, sometimes fusoid, subfusoid to subcylindrical, apex rounded to obtuse, sometimes mucronate, thin-walled, contents fibrous to crystal, blackish gray in SV. *Cheilocystidia* not observed; lamellar edge sterile. *Subhymenium*: cellular layer 15–40 µm thick composed of inflated cells 8–20 µm in diam., hyaline, sometimes pale yellowish in KOH. *Pileipellis* is composed of epipellis and subpellis; epipellis trichoderm 100–250 µm thick, composed of thin-walled, unbranched cylindrical hyaline hyphae 3–6 µm wide; pileocystidia rare, 6–8 µm wide with crystal contents greyish in SV, unseptate, clavate to cylindrical, apex round; subpellis: cutis 100–120 µm thick, composed of gelatinized, interweaved hyaline hyphae 2–5 µm wide. *Stipitipellis*: cutis, upper layer composed of parallel filamentous hyphae 3–6 µm in diam., inner layer hyaline filamentous hyphae interweaved with sphaerocytes 15–25 µm in diam., some filamentous hyphae pale yellow in KOH; caulocystidia absent. *Trama* composed of sphaerocytes 15–40 µm in diam. and scattered by filamentous and cystidioid hyphae. *Clamp connections and laticiferous hyphae* absent in all tissues.

Habit and habitat – Single in coniferous and broad-leaved intermixed forests (dominated by e.g. *Betula dahurica*, *B. platyphylla*, *Pinus bungeana*, *P. koraiensis*, and *Xylosma racemosum*) at 400–600 m altitude.

Other specimens examined – China, Heilongjiang Province, Mudanjiang City, Muling County, Liufengshan National Forest Park, N 44°11' E 130°21', elev. 512 m., 21 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, *ZRL20164231* (HMAS 279587), ITS: MG719933, nLSU: MH021614, nSSU: MH021636.

Distribution – China (Heilongjiang). Season July and August.

Notes – The combination of a red pileus, yellow spore print, acrid-tasting context, and the absence of diverticula in pileipellis hyphae and pileocystidia contents turning grey in SV indicated

that the new species *R. heilongjiangensis* is a member of *Russula* subsect. *Maculatinae* (Romagn.) Konrad & Joss. described in Romagnesi (1967, 1985) and subsubsect. *Urentes* Maire described by Sarnari (1998, 2005), for which the name *Maculatinae* was more commonly used in some studies (Adamčík & Jančovičová 2013, Adamčík et al. 2016a).

The most similar species in morphology is *R. maculata* from the *R. maculata* complex, which also has a red pileus without a purple tinge, yellow-brownish context discoloration, lamellae with yellow-brownish spots, basidiospores not exceeding $10.5 \times 9 \mu\text{m}$, basidiospore ornamentation composed of amyloid warts often merged or connected by line connections. Nevertheless, these two species can be distinguished because *R. maculata* has a larger pileus [45–110 mm in diam. (Sarnari 1998)], longer and wider basidia ($39\text{--}60 \times 12\text{--}15 \mu\text{m}$ (Adamčík & Jančovičová 2013)), and habitats of *Quercus*, *Fagus*, *Carpinus*, and *Tilia* forest (Adamčík et al. 2016a). The other recognized species from the *R. maculata* complex is *R. nympharum* F. Hampe & Marxm., which is different from *R. heilongjiangensis* by its lower basidiospore ornamentation $0.3\text{--}0.6 \mu\text{m}$ and numerous pileocystidia (Adamčík et al. 2016a).

R. heilongjiangensis is close to the *R. globispora* complex in phylogeny. Presently, the *R. globispora* complex contains two species, which are closely related to *R. heilongjiangensis*. They can be distinguished from the new species by the following characteristics: *R. dryadicola* has a yellow and purple pileus without bright red tinges, high basidiospore ornamentation ($0.9\text{--}1.3 \mu\text{m}$) composed of mostly isolated warts, and an alpine habitat associated with *Dryas* and *Juniperus* (Sarnari 1998); *R. globispora* has larger basidiospores ($9.7\text{--}12.4 \times 8.1\text{--}9.7 \mu\text{m}$) and wider basidia ($12\text{--}17 \mu\text{m}$ (Sarnari 1998, Adamčík & Jančovičová 2013)). Thus, the morphological and phylogenetic analyses both support *R. heilongjiangensis* as a new species.

Russula khinganensis G.J. Li & R.L. Zhao, sp. nov.

Fig. 4

Fungal Names number: FN570532; Facesoffungi number: FoF03952

Etymology – refers to Lesser Khingan Mountains, the locality of the type specimen.

Typus – China, Heilongjiang Province, Yichun City, Dailing District, Liangshui National Natural Reserve, N $47^{\circ}18'$ E $128^{\circ}87'$, elev. 378 m., 17 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, ZRL20160285 (HMAS 278895), ITS: MG719928, nLSU: MH021612, nSSU: MH021634.

Diagnosis – *Russula khinganensis* is similar to *R. versatilis* in morphology, but *R. khinganensis* has smaller basidiocarps, narrower pleurocystidia, basidiospore ornamentation composed of short to long ridges, and a habitat of broad-leaved and coniferous intermixed forest.

Description – Basidiocarps small to medium sized. Pileus 26–38 mm in diam., hemispherical when young, turning applanate after maturation, with age depressed above stipe, slightly viscid when wet, greyish vinaceous tinges, Livid Brown (XXXIX1'''), Deep Livid Brown (XXXIX1'''i) to Russet Vinaceous (XXXIX9''') at center; margin incurved first, straight to slightly curled upward when mature, sometimes undulate and cracked, striate 5–10 mm from edge inward, peeling 1/4–1/2 toward the center, pale vinaceous tinges of Purplish Vinaceous (XXXIX1'''b), Pale Purplish Vinaceous (XXXIX1'''f), and Pale Brownish Vinaceous (XXXIX3'''f) toward the margin; margin slightly striate. Lamellae adnate, 2–5 mm in height, 11–15 pieces per centimeter at edge, not forked, slightly interveined, ocherous-tinged, Pale Ochraceous-Salmon (XV13'f) to Pale Ochraceous-Beige (XV15'f) first, Light Ochraceous-Salmon (XV13'd) to Ochraceous-Beige (XV15'b) when mature, unchanging when bruised, lamellulae absent. Stipes central to subcentral, $4.5\text{--}7.7 \times 0.8\text{--}1.5$ cm, subcylindrical to cylindrical, surface dry, rugulose longitudinally, dull, without annulus, tapering toward base, White (LIII), unchanging when old and bruised, stuffed at first, becoming hollow when old. Context 2–3 mm thick from lamellae attachment to stipe at pileus center, White (LIII), slowly and partly turning ocherous color of Yellow Ocher (XV17') after bruising for long time, compact under pileus; taste mild, sometimes slightly acrid in lamellae, no distinct odor. Spore print ocherous (Romagnesi III b–c).

Basidiospores [100/2/2] ($6.0\text{--}6.3\text{--}7.3$ (-7.6) $\times$ $5.2\text{--}6.4$ (-6.7) μm , $Q = (1.07\text{--}) 1.10\text{--}1.25$ (-1.33) ($Q = 1.18 \pm 0.05$), hyaline to pale cream colored, subglobose to broadly ellipsoid, rarely

globose or ellipsoid, verrucose; ornamentation amyloid, composed of verrucous to conical warts 0.5–1 μm in height, rarely linked by fine lines as short to long ridges, partly reticulate, sometimes isolated; suprahilar area amyloid and distinct. *Basidia* 35–43 $\times$ 10–11 μm , hyaline, clavate, ventricose toward upper half, four-spored, projecting 10–15 μm beyond hymenium; sterigmata 4–7 μm , pointed, straight to slightly tortuous. *Pleurocystidia* 51–65 $\times$ 6–9 μm , originating from subhymenium, projecting 15–35 μm beyond hymenium, thin-walled, clavate to subclavate, sometimes subfusiform to fusiform, apex mucronate, at times constricted, often with moniliform or capitate appendage, contents crystal, blackish in SV. *Cheilocystidia* not observed; lamellar edge sterile. *Subhymenium* 20–40 μm thick, composed of slender cylindrical cells 4–10 μm wide under basidia and inflated elliptical to globose cells 25–50 μm in diam. next to trama. *Pileipellis* 120–250 μm thick, composed of epipellis (50–70 μm thick) and subpellis (70–180 μm thick); epipellis trichoderm, composed of mostly ascending to suberect hyphae, intermixed with several repent and erect elements, 2–4 μm in diam.; pileocystidia 37–55 $\times$ 3–5 μm , cylindrical to subcylindrical, with round to obtuse tips, septate, contents dense, black in SV; subpellis composed of long, rarely septate, equally wide hyphae 2–4 μm in diam. interweaved with sphaerocytes 20–35 μm in diam. *Stipitipellis*: cutis composed of thin-walled, septate, cylindrical, hyaline to slightly yellowish hyphae 3–5 μm wide; terminal cells of stipitipellis hyphae with obtuse to tapered apex; caulocystidia not observed. *Trama* composed of sphaerocytes 20–50 μm in diam. and septate filamentous hyphae. *Clamp connections and lacticiferous hyphae* absent in all tissues.

Habit and habitat – Single or scattered in coniferous and broad-leaved intermixed forests, with *Betula platyphylla*, *Larix gmelinii*, *Populus davidiana*, and *Picea koraiensis*, at an altitude of 200–500 m.

Other specimens examined – China, Heilongjiang Province, Yichun City, Dailing District, Liangshui National Natural Reserve, N 47°18' E 128°87', elev. 385 m., 17 July 2016, Ming-Zhe Zhang, Xu-Ming Bai, Rong-Chun Dai, Guo-Jie Li, ZRL20162112 (HMAS 279576), ITS: MG719929, nLSU: MH021613, nSSU: MH021635.

Distribution – China (Heilongjiang). Season July.

Notes – This new species belongs to *R.* subsect. *Puellarinae* owing to its small and slim basidiocarps, purple pileus without a red tinge, mild context taste, basidia less than 50 $\times$ 12 μm , and septate pileocystidia without incrustation (Sarnari 1998). This section has been referred to as “series *Puellaris* ad int.” (nom. inval.) within *R.* subsect. *Puellarinae* because of its yellowish context, ocher spore print, and isolated to subridged basidiospore ornamentation (Sarnari 2005).

In “series *Puellaris* ad int.,” the species most similar to this new species is *R. versatilis* Romagn., because both have spore print IIb–IIc, an indistinct context odor, and a slightly less yellowish context of the “series.” Nonetheless, *R. versatilis* is distinguishable from *R. khinganensis* by a larger pileus (up to 55 mm in diam.), which is often variable and fading, wider pleurocystidia (8–13.5 μm), basidiospore ornamentation composed of mostly isolated warts, and a habitat of a hornbeam forest. The other two species of “series *Puellaris* ad int.” (*R. minutalis* Britzelm. and *R. puellaris* Fr.) have paler, dark cream to ocherous spore print IIc–IId; *R. puellaris* Fr. also differs because of its brownish ocher fading pileus, all over a yellowish turning context, and larger basidiospores, 7–9 $\times$ 5.7–7.2 μm . *Russula terenopus* Romagn. can be distinguished from the new species by its strong context odor of *Pelargonium* (Romagnesi 1967, Sarnari 2005). Thus, we proposed our specimens as a new species: *R. khinganensis*.

Discussion

The concept of *R.* subsect. *Maculatinae* was initially proposed under the name “*Maculatae*” (Konard & Jossierand 1934). Nonetheless, its morphological concept was still vague at the time. Not only the two commonly known species, *R. maculata* and *R. badia* Quél., but also two alien species, *R. rubra* Bres. and *R. pseudointegra* Arnould & Goris, were regarded as its members (Konard & Jossierand 1934, Romagnesi 1967). This group was upgraded to the section level by Romagnesi using the current name (Romagnesi 1962). Its characteristic morphology was detailed in ref. (Romagnesi 1967), including the mainly reddish pileus, yellowish basidiospore print,

Figure 1 – Phylogeny of *Russula* subject. *Maculatinae* in sense of Romagnesi (1967) and species with yellow-brownish generated from the Bayesian analysis of the ITS sequences. Bayesian posterior probability (PP) values and Bootstrap support (BS) values > 50 % are given at the internodes (PP/BS).

Figure 2 – Phylogeny of *Russula* subject. *Puellarinae* generated by the MP analysis of ITS sequences. Bayesian posterior probability (PP) values and Bootstrap support (BS) values > 50 % are given at the internodes (PP/BS).

Figure 3 – *Russula heilongjiangensis*: ZRL20160516 (HMAS255142 holotype). a–b Basidiocarp. c Basidiospores. d Basidia. e Pleurocystidia. f Pileipellis (epipellis).

Figure 4 – *Russula khinganensis*: ZRL20160285 (HMAS278895 holotype) a, b Basidiocarp. c Basidiospores. d Basidia. e Pleurocystidia. f Pileipellis (epipellis).

acid-tasting context, pileipellis hyphae without diverticula, and greyish turning pileocystidia in SV. Members of this group were involved in *R.* subsect. *Urentes* Maire in ref. Sarnari (1998), which has a wider morphological concept. Precise and statistically supported descriptions for members of *R.* subsect. *Maculatinae* were presented in a recent work, detailed with widely used and newly introduced morphological characteristics (Adamčík & Jančovičová 2012, 2013). At least three species can be recognized within the generally accepted morphological concept of *R. maculata* in phylogenetic analyses of the ITS region, including *R. maculata*, *R. nymphaeum* F. Hampe & Marxm., and an unknown Pakistani species (Adamčík et al. 2016a). Although some specimens from Northeast and Southwest China were identified as members of *R.* subsect. *Maculatinae*, such as *R. maculata* and *R. decipiens*, none of these identifications was supported by phylogenetic analyses (Li 2014). The ITS phylogenetic topology in our study not only supports the independence of *R. heilongjiangensis* but also shows that there are still unknown species of *R.* subsect. *Maculatinae* in China.

Compared with the elaborate morphological and phylogenetic analyses of *R.* subsect. *Maculatinae*, less attention has been given to *R.* subsect. *Puellarinae*, even in Europe. The numerous members, e.g. 12 species in ref. Romagnesi (1967) and 17 species in Sarnari (2005), increase the difficulty with the taxonomy of *R.* subsect. *Puellarinae*. Five species of this subsection are distributed in China, among which the taxonomic identity of the European species *R. puellaris* is supported by both morphological and phylogenetic evidences (Li 2014). *Russula khinganensis* is the second species in Northeast China that was described after *R. changbaiensis* G.J. Li & H.A. Wen (Li et al. 2013b).

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (Project IDs GJL: 31500013, RLZ: 31470152 and 31360014), the National Project on Scientific Groundwork for Basic Science of the Ministry of Science and Technology (No. 2014FY210400), and the Foundation of Innovative Group of Edible Mushrooms Industry of Beijing (Project ID: BAIC05-2017). Xu-Ming Bai, Ming Zhe Zhang, and Rong-Chun Dai are acknowledged for helping with specimen collection. The authors are grateful to Liu Yang for the loan of herbarium specimens, to Chun-Li Li and Xiao-Lan Zhang (Institute of Microbiology, Chinese Academy of Sciences) for helping with scanning electron microscopy, and to Yan-Lei Ding and Xin-Yu Zhu (Baotou Normal College) for assistance with DNA extraction and sequencing.

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