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Russula jilinensis sp. nov. (*Russulaceae*) from northeast China

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Abstract — *Russula jilinensis* (subg. *Coccinula* sect. *Laetinae*), is described from Changbai Mountains, northeast China. The new species is distinguished by its bright red glabrous pileus with a cinnamon tinged disc, slightly yellowish context, dark yellow to ocher spore print, and pileipellis with septate pileocystidia. The morphological characteristics are illustrated in detail and compared with those of similar species. Identification of *R. jilinensis* was supported by the molecular phylogenetic analysis based on the ribosomal DNA internal transcribed spacer regions (ITS).

Key words —*Russulales*, taxonomy, morphology, *Basidiomycota*

Introduction

Northeast China, including Liaoning, Jilin, and Heilongjiang Provinces and the eastern part of Inner Mongolia Autonomous Region, covers an area of 1.236×10^6 km^2 (39°–53°30′ N 115°–135°E) within the temperate to boreal continental climate zones. Plant communities range from grassland (eastern Inner Mongolia) to broadleaf forest (southern Liaoning), while the three main mountain systems (Great Hinggan, Lesser Hinggan, Changbai) are mostly covered by coniferous or mixed coniferous–broadleaf forests. The main trees in northeast China are *Pinus pumila*, *P. koraiensis*, *Larix gmelinii*, *Betula platyphylla*, *Abies nephrolepis*, *Picea jezoensis*, and *Quercus mongolica* (Jiang et al. 2003, Xu et al. 2008). In summer and early autumn, these ectomycorrhizal commensal plants facilitate the fruiting of ectomycorrhizal basidiomycetes, among which the members of *Russula* Pers. are very common.

Russula is widely distributed from western Europe to North America in the northern hemisphere (Romagnesi 1967, Singer 1986, Sarnari 1998, Miller & Buyck 2002, Bau et al. 2008). Although some new species and varieties have been reported from southern and southwestern China (Singer 1935, Chiu 1945, Ying 1983, 1989, Bi & Li 1986, Wen & Ying 2001, Wang et al. 2009), *Russula*

species have not been studied systematically elsewhere in China, including the north. Because of its taxonomic difficulty and the insufficiency of intensive taxonomic studies in China (Wang et al. 2009), many *Russula* species reported from northeastern China are identified using European or American names and lack voucher support (e.g., Teng 1996, Xie et al. 1986, Li & Bau 2003, Bau 2004). As now in North America (Adamcik & Buyck 2010, 2011; Buyck & Hofstetter 2011), the lack of endemic systematic studies has probably led to incorrect estimates of the extent of *Russula* diversity in northeastern China,.

Although many wood-inhabiting fungi were recently reported from northeastern China (Dai & Penttilä 2006, Yuan et al. 2006, Wei & Dai 2007, Dai et al. 2008, Xiong et al. 2008, Dai 2010), only a few *Russula* species have been reported from this area (Song et al. 2007). In addition, many virgin and natural forests in this area have not yet been intensively investigated. During a survey on *Russulales* in the Changbai Mountains, one interesting *Russula* was found, which is described here as a new species.

Materials & methods

Specimens were collected from the Changbai Mountains, Antu County of Jilin Province, from 2008 to 2010. Macromorphological characteristics of the fresh fruiting bodies were recorded in field notes. Color names and codes follow Ridgway (1912) and spore print colors were categorized according to Romagnesi (1967). Collections were oven-dried at 50–60 °C until their water content was <15% prior to microscopic examination. Solutions of 10% $FeSO_4$ and sulfovanillin (SV) were used for testing the chemical reaction of dried specimens. Specimens for microscopic examinations were hand-sectioned and rehydrated in a 5% KOH solution prior to observation under a Nikon Eclipse 80i microscope. Basidiospores (examined in Melzer's reagent), basidia, pleurocystidia, cheilocystidia and elements of the pileipellis and stipitipellis were measured, with at least 20 elements measured for each character. The abbreviation (n/m/p) = n spores from m basidiospores of p specimens. Basidiospore dimensions are shown as (a–) b–c (–d), with the range b–c including 90% of the measured values and the extreme values shown in parentheses. The spore quotient (Q) = spore length divided by spore width. **Q** (in bold) = average quotient value + standard deviation. Spore ornamentations and apiculi were not included in measurements. Further explanations of basidiospore data follow Yang (2000). Scanning electron images were also captured with an FEI Quanta 200 electron microscope. Abbreviations of herbarium names follow Holmgren et al. (1990). Specimens cited were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

For phylogenetic studies, genomic DNA was extracted from dried fruiting bodies briefly crushed with quartz sand in liquid nitrogen. DNA template for amplification was prepared using a BioTeke plant DNA rapid extraction kit. PCR was conducted with an Eastwin Dragon Black Vajra thermo cycler using ITS1 (5'-GTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (Shanghai Sangon, Beijing TransGen Biotech), EasyTaq DNA polymerase, dNTPs, and PCR buffer to amplify the ITS1+5.8S+ITS2 region. Amplification of a 50 μL mixture — 35.5 μl ddH_2O, 5 μl

PCR buffer, 4 µl dNTP (2.5 mmol/L), 1 µl DNA template, 2 µl (10 µmol/L) each of two primers, 0.5 µl (5u/µl) Taq DNA polymerase — followed a protocol of 5 min at 94°C (initial denaturation), 35 cycles of 1-min at 94°C (denaturation) + 1 min at 56°C (annealing) + 1 min at 72°C (extension), and a final 10-min extension at 72°C. The PCR products were electrophoresed in ethidium bromide-stained agarose gel and sequenced by SinoGenoMax Co. Ltd, using an ABI 3730XL Analyzer and ABI BigDye 3.1 Cycle Sequencing Kit. The sequences were deposited in GenBank.

Sequences were aligned and edited in ClustalX (Thompson et al. 1997) and Bioedit (Hall 1999). Phylogenetic analysis was performed with MEGA version 4.1 Beta (Tamura et al. 2007), and Minimum Parsimony trees were constructed with the test of inferred phylogeny a bootstrap of 1000 replications and a random seed (Eck & Dayhoff 1966, Rzhetsky & Nei 1992). The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree was drawn to scale, with branch lengths calculated using the average pathway method and in the units of the number of changes over the whole sequence (Nei & Kumar 2000). All positions containing gaps and missing data were eliminated from the data set (Complete Deletion option). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Of the 424 positions in the final dataset, 253 are parsimony informative. The ITS1-5.8S-ITS2 sequences of *Albatrellus ovinus* (Schaeff.) Kotl. & Pouzar and *Bondarzewia mesenterica* (Schaeff.) Kreisel (as *B. montana*) were used as outgroups (Wang et al. 2009).

Taxonomy

Russula jilinensis G.J. Li & H.A. Wen, **sp. nov.** Figs 1–4

MycoBank MB 563682

Differs from *Russula integra* by shorter basidia and pleurocystidia and smaller spores.

Etymology: named after the type locality.

Type: CHINA, Jilin Province, Antu County, Erdaobaihe, Changbai Mountains, 42°24′N 128°04′E, alt. 761 m, on ground in mixed coniferous–broadleaf forest dominated by *Pinus koraiensis* and *Picea*, 1 August 2008, M.X. Zhou & Y. Gafforov 08004 (**Holotype**, HMAS194253, GenBank GU966632).

Pileus 6.1–7.3 cm broad, first hemispheric, convex, then more or less convex to plano-convex, depressed weakly in the center when mature, colors Jasper Red to Coral Red (xiii3′–5′) when juvenile, Pompeian Red to Dragon's-blood Red (xiii3′i–5′i) with age, Nopal Red to Brazil Red (i3i–5i) when dried; center Vinaceous-Cinnamon to Pinkish Cinnamon (xxix13′′b—15′′b) when juvenile, Orange-Cinnamon to Cinnamon (xxix13′′–15′′) with age, Mikado Brown (xxix13′′i) when dried, Nopal Red (i3i) in SV, color unchanged in $FeSO_4$; viscid when wet, glabrous, smooth, non-pruinose when dried, pellis peeling 1/2 to the disc; margin obtuse, not or very slightly striate when old, never cracked. Context 1.0–1.5 cm thick from stipe top to pileus center, brittle, white (liii), Naples Yellow (xvi19′d) when old or dried, color unchanged when bruised;

Fig.1: *Russula jilinensis* (Holotype HMAS 194253).

Fig.2: *Russula jilinensis* (HMAS 262395). Scale bar = 1 cm.

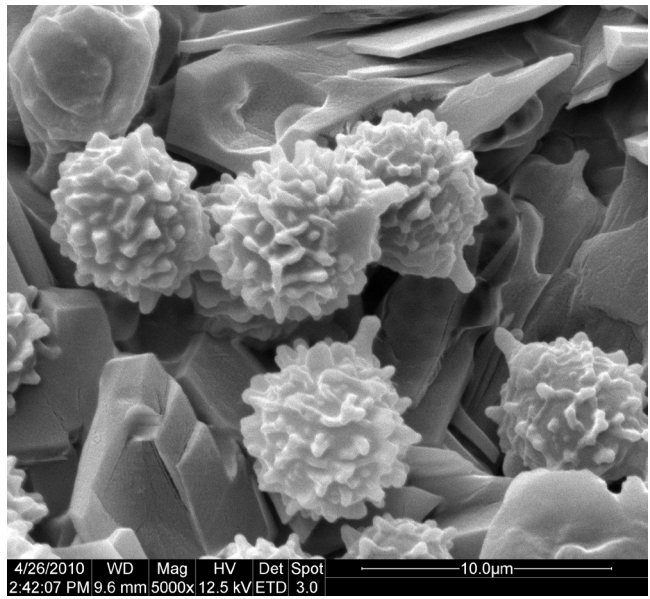

Fig. 3: *Russula jilinensis* (Holotype HMAS194253).
Scanning electron microscope (SEM) photo. Basidiospores. Scale bar = 10 µm.

taste mild; odor none or indistinct. Lamellae 4–7 mm broad, 9–12/cm at the edge, no lamellulae, decurrent to more or less adnate, not forked, fragile, Pale Yellow-Orange (III15f) first, Pale Orange-Yellow (III17f) when mature or dry, color unchanged in $FeSO_4$. Stipe clavate, enlarged towards the base, 9.2 × 1.8–2.1 cm, smooth, solid but irregularly hollowing when old, white (LIII) when juvenile, becoming Baryta Yellow, Wax Yellow, or Primuline Yellow (IV21f, XVI21′, XVI19′, respectively) towards the base when old or dry, Brazil Red (I5i) in SV, no color change in $FeSO_4$. Spore print dark yellow, ocher, IIIc.

Basidiospores (80/3/3) (6–)7–8(–8.5) × (5.5–)6–7 µm, average 7.5 × 6.3 µm, [Q= (1.02) 1.06–1.28 (1.31), **Q**= 1.18 ± 0.06], subglobose to ellipsoid, without oil droplet, plage indistinct and amyloid; ornamentation amyloid, mostly isolated, rarely linked by fine lines and not forming a mesh, warts conic to cylindrical, ≤0.4–1.0 µm tall. Basidia (33–)38–44 × 11–13(–14) µm, clavate to subclavate, broadly tapered towards the base, 4-spored, sometimes 2-spored, hyaline in KOH, occasionally containing a large droplet; mature basidia projecting ≤15–10 µm above the subhymenium; sterigmata 3–5 µm. Pleurocystidia (50–)53–56(–63) × (8–)9–10(–11) µm, rare, emergent, fusiform, clavate to subclavate, often with a subacute tip, sometimes with a frayed small appendage and dense crystal inclusions, projecting ≤35 µm above the subhymenium, color unchanged in $FeSO_4$, dark grey in SV reaction dark grey.

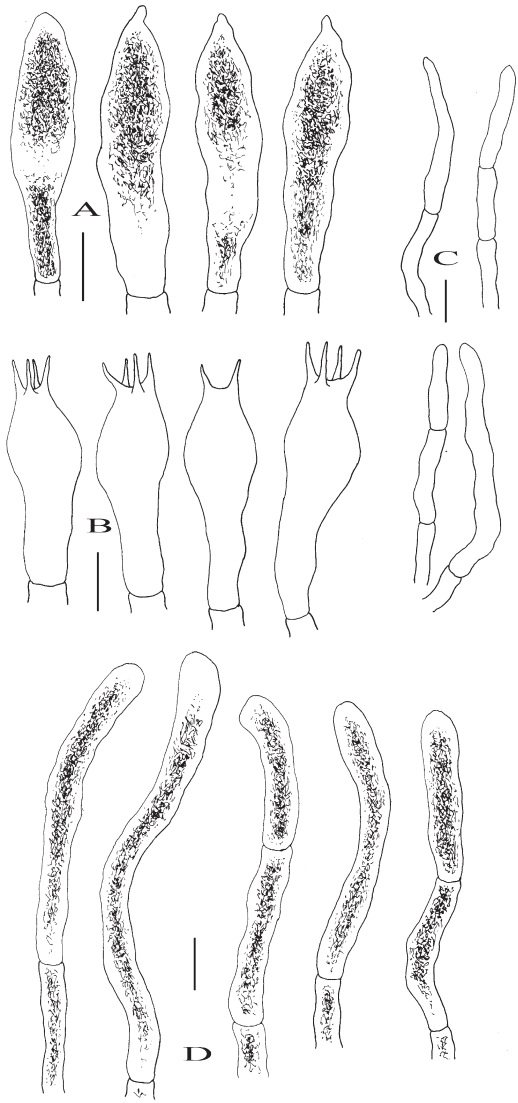

Fig. 4: *Russula jilinensis* (Holotype HMAS 194253).
A. Pleurocystidia; B. basidia; C. terminal elements of pileipellis; D. pileocystidia.
Scale bars = 10 μm.

Cheilocystidia mostly similar to pleurocystidia. Pileipellis two-layered, 100–125 μm thick, gelatinous; epicutis an ixotrichoderm, epicuticular hyphae 3–6 μm broad, septate, mostly unbranched, erect to suberect, tangled, hyaline

in KOH, terminal cells thin-walled, cylindrical, with obtuse, undifferentiated, often tapered ends; pileocystidia 48–64 × 5–7 μm, dispersed, numerous in surface, scattered, clavate, subclavate to cylindrical, with 0–2 septa, contents crystalline, granulate, slightly turning grey in SV; subcutis hyphae recumbent, filamentous, interwoven, branched, septate, 2.5–5 μm diam., hyaline to pale yellowish brown in KOH; trama composed of subspherical to angular 13–24 μm diam sphaerocysts and connective hyphae. Stipitipellis composed mostly of interwoven branched elongated 3–6 μm diam hyaline hyphae with inflated cells; caulocystidia absent. Clamps or laticiferous hyphae absent in all tissues.

Ecology: Solitary on soil in mixed coniferous–broadleaf forests, July–August. Known only from the Changbai Mountains. Edibility: unknown.

Additional specimens examined: **China**, **Jilin Province**, Antu County, Heping Forest Farm, 43°07′N 128°54′E, alt. 1014 m, in coniferous forest dominated by *Pinus*, 22 July 2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie 20100054 (HMAS262395, GenBank HQ693525); Changbai Mountains Forest Ecological System Research Center, 43°23′N 128°05′E, alt. 811 m, in mixed coniferous–broadleaf forest, 25 July 2010, X. Sun & G.J. Li 20100410 (HMAS262364, GenBank HQ693524).

Discussion

Russula jilinensis is macroscopically characterized by its bright red pileus, light-yellow lamellae, and a white stipe without red or purple tinge. It shows superficial affinities with subg. *Coccinula* sect. *Laetinae* Romagn., but its relatively shorter basidia and larger cystidia are more characteristic of subg. *Polychromidia* sect. *Integrinae* Maire (e.g., *R. integra* f. *gigas* Romagn.). The *R. jilinensis* ITS1-5.8S-ITS2 sequences cluster with *R. curtipes* F.H. Møller & Jul. Schäff. (Fig. 5), and a blasting with that of *R. curtipes* (AY061668, Miller & Buyck 2002) through NCBI databank shows a 93% max identification of 97% coverage, confirming that *R. jilinensis* is related to *R. integra*/*curtipes* group in *Russula* sect. *Integrinae*.

Russula integra (L.) Fr., which is common in Europe, has been reported many times from China and neighboring East Siberia (Teng 1964, Romagnesi 1967, Tai 1979, Xie et al. 1986, Ying et al. 1987, Knudsen & Stordal 1992, Mao & Zhuang 1997, Mao 2000, Bau et al. 2008). However, few specimens of *R. integra* have been recorded from northeast China. *Russula integra* and *R. jilinensis* are similar in spore ornamentation and cystidial shape, and both species are associated with *Pinus* and *Picea* in the subalpine zone (Romagnesi 1967, 1985). However, our microscopic observation followed by intensive comparison of descriptions of *R. integra* by Møller & Schäffer (1935) and Romagnesi (1967) indicates that *R. jilinensis* is a different species. The latter has shorter basidia and pleurocystidia and smaller spores than those of *R. integra* (with spores 8.2–10 × 7–9.2 μm, basidia 45–65 × 11–14.5 μm, cystidia 82–120 × 8.5–13 μm, in Romagnesi 1967).

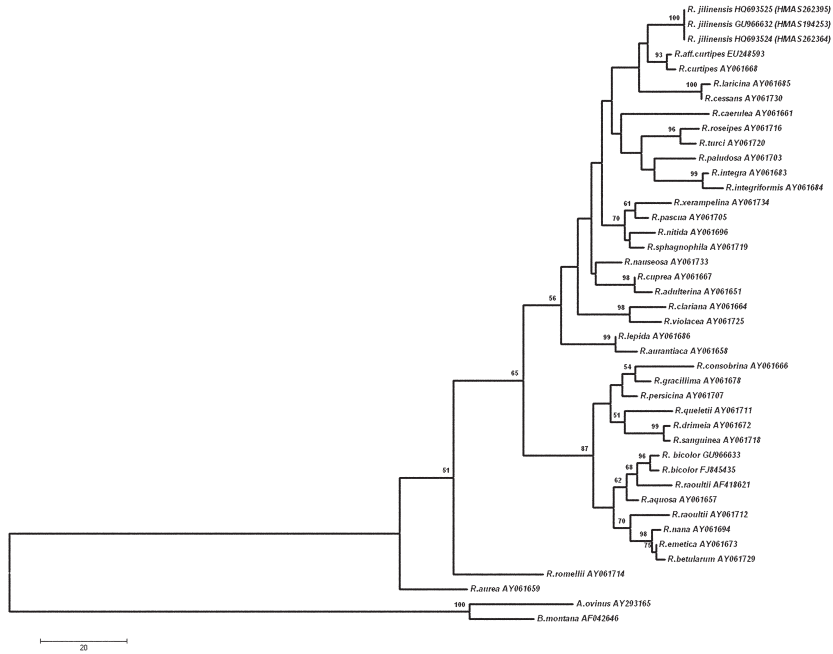

Fig. 5. One of 13 ITS1-5.8S-ITS2-based phylogenies of *Russula jilinensis* and related *Russula* species, using the Maximum Parsimony method. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches.

Another phylogenetically related species is *R. curtipes,* which shares with *R. jilinensis* the short basidia and firm and yellowing context of the *R. integra-curtipes* group, but they distinctly differ from each other in pileus color and habitat: *R. curtipes* has a dark wine-red, haematite red, to light russet vinaceous pileus and associates with beech in western Europe with a possible preference for limestone (Romagnesi 1967), while *R. jilinensis* has a brighter red pileus and grows in mixed forests dominated by conifers in efflorescent basalt soils. The reddish pileus of *R. jilinensis* distinguishes it from most of the typical violet- or purple-capped members of *R.* sect. *Integrinae* such as *R. romellii* Maire and *R. carpini* R. Girard & Heinem. Another species in *R.* sect. *Integrinae* with a dark-red pileus, *R. rubroalba* (Singer) Romagn., shares a more or less similar pileus color and spore ornamentation but is associated with a deciduous forest and has much longer cystidia than those of *R. jilinensis*.

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