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Russula atroaeruginea and *R. sichuanensis* spp. nov. from southwest China

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Abstract — Two new species of *Russula* are described from southwestern China based on morphology and ITS1-5.8S-ITS2 rDNA sequence analysis. *Russula atroaeruginea* (sect. *Griseinae*) is characterized by a glabrous dark-green and radially yellowish tinged pileus, slightly yellowish context, spores ornamented by low warts linked by fine lines, and numerous pileocystidia with crystalline contents blackening in sulfovanillin. *Russula sichuanensis*, a semi-sequestrate taxon closely related to sect. *Laricinae*, forms russuloid to secotioid basidiocarps with yellowish to orange sublamellate gleba and large basidiospores with warts linked as ridges. The rDNA ITS-based phylogenetic trees fully support these new species.

Key words — taxonomy, *Macowanites*, *Russulales, Russulaceae, Basidiomycota*

Introduction

Russula Pers. is a globally distributed genus of macrofungi with colorful fruit bodies (Bills et al. 1986, Singer 1986, Miller & Buyck 2002, Kirk et al. 2008). As ectomycorrhizal symbionts, they play an important beneficial role in forest ecosystems (Richardson 1970, Bills et al. 1986, Villeneuve et al. 1991, Claridge & May 1994, Buyck et al. 1996, Gardes & Bruns 1996). China has a long history of using *Russula* mushrooms as traditional food and medicine. Twenty-two medicinal species and 82 edible species have been reported in the country (Ying et al. 1982, 1987; Wang & Liu 2002; Yang 2002; Wang et al. 2004; Dai et al. 2009; Li et al. 2010a), and their ectomycorrhizal capacity, population genetics, and mycochemistry have been studied in recent years (Gao et al. 2001;

Tan et al. 2001, 2004; Liang et al. 2004; Liu 2004, 2005; Li et al. 2010b; Zhao et al. 2010). So far, only 14 new species and three new varieties have been reported from China (Singer 1935; Chiu 1945; Ying 1983, 1989; Bi & Li 1986; Zang & Yuan 1999; Wen & Ying 2001; Wang et al. 2009; Li et al. 2011, 2012). Although southwestern China represents one of the world's biodiversity "hotspots" and has a high diversity of macrofungi (Yang & Zang 2003; Dai et al. 2004, 2007; Dai & Yuan 2008), only a few russulacean species have been reported in this region and the adjacent Himalayas (Wang & Liu 2009, 2010; Das et al. 2010; Buyck & Atri 2011; Das & Verbeken 2011; Wang et al. 2012). During a systematic survey of *Russula* species in southwestern China from 2004 to 2009, two interesting species were found, which we propose here as new species.

Materials & methods

Specimens were photographed, and the macroscopic characteristics of fresh mature fruit bodies were recorded under daylight in the field. Specimens were tested chemically in sulfovanillin (SV) solution. They were then kept 50–60 °C until completely dried and were subsequently deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). Color names and codes were assigned based on Ridgway (1912). Tissues of dried specimens were immersed in 5% KOH for 10–20 seconds to prepare for microscopic observation in Congo Red using a Nikon E80i microscope with a 100× oil immersion objective lens. Basidiospores were observed and measured in Melzer's reagent. Spore measurements do not include apiculus and ornamentation, and sterigma lengths were excluded from basidium measurements. Sections from the pileipellis were anticlinally cut through the pileus centre to the margin. Cystidia with incrustations were observed in distilled water. Scanning electron microscopic images were captured with an FEI Quanta 200 microscope. Microscopic observation and statistical citation of measure technicalities follow Yang (2000) and Wang et al. (2009).

Protocols for DNA extraction, PCR, and sequencing followed those in Li et al. (2012). The internal transcribed spacer (ITS) regions were amplified with the primer pairs ITS1/ITS4 (Gardes & Bruns 1993). PCR products were purified with the Bioteke DNA Purification Kit (Bioteke, Beijing, China). The ITS regions were sequenced with the ABI 3730 DNA analyzer and ABI Bigdye 3.1 terminator cycle sequencing Kit (BGI, Beijing, China), and new sequences were deposited in GenBank (see Fig. 5 for accession numbers). Sequences of representative and closely related *Russula* taxa, with an emphasis on samples of the russuloid to secotioid forms (Eberhardt 2002, Miller & Buyck 2002, Lebel & Tonkin 2007), were selected from GenBank and included in this analysis. Assembly and editing of sequences of each region were performed with Clustal X and BioEdit (Thompson et al. 1997, Hall 1999) and were manually adjusted when necessary. Some poorly aligned terminal sites were excluded from further analysis.

Based on previous results (Lebel & Tonkin 2007), *Albatrellus ovinus* (Schaeff.) Kotl. & Pouzar was chosen as outgroup (Ryman et al. 2003). Sequences of datasets including the ITS1-5.8S-ITS2 region were analyzed with maximum parsimony (MP) and Bayesian analysis (BA) methods.

MP analysis of the phylogenetic relationships were performed in PAUP* v.4.01 (Swofford 2004). Gaps in alignment were treated as missing data, and all sites were treated as unordered and unweighted. The tree bisection-reconstruction (TBR) algorithm was performed using with the heuristic search option, and bootstrap analysis was conducted with 1000 replicates (Felsenstein 1985). Consistency index (CI), retention index (RI), and tree length (TL) were also calculated. Trees were displayed with Treeview 1.6.6 (Page 1996).

Bayesian analysis was carried out in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). The best-fit model of Bayesian posterior probabilities (PP) were calculated with the Markov Chain Monte Carlo (MCMC) algorithm (Larget & Simon 1999). One cold and three heated Markov chains were run for 2,000,000 generations, the trees were sampled every 100th generation, and the run was terminated once the average standard deviation of split frequencies fell below 0.01. A discard of 25% trees was set in the Burn-in option (Hall 2004).

Taxonomy

Russula atroaeruginea G.J. Li, Q. Zhao & H.A. Wen, **sp. nov.** Figs 1–2

MycoBank MB804644

Differs from *Russula aeruginea* by its non-fading green pileus lacking scattered rusty spots and whitish to pale cream spore print.

Type: China. Sichuan Province, Daofu County, 25.VII.2007, Z.W. Ge 1540 (**Holotype**, HKAS53626, GenBank JX391970)

Etymology: *atroaeruginea* (Latin) = dark green. The epithet refers to the dark-green basidioma pileus.

Basidiomata small to medium sized. Pileus 3–7 cm in diam., first hemispherical, then convex to applanate, sometimes slightly depressed above the stipe, blackish-green tinged, intermixed with radial greenish yellow fringe, Dull Blackish Green (XLI29′′′m) at centre, Sepia (XXIX17′′m) when dry, never viscous; margin incurved first, straight when mature, sometimes undulate, not striate, Meadow Green (VI35k) to Antique Green (VI33m) with yellowish tinge of Oil Yellow (V25i) towards the margin, Verona Brown (XXIX13′′k) when dry. Lamellae 10–15/cm at the edge, ≤7 mm wide, adnate to almost free, forked around the stipe, occasionally near the pileus margin, not interveined, cream to yellowish, Cream Color (XVI19′f), brittle; edge even, slightly narrowing towards the pileus margin; lamellulae occasional. Stipe 4–6 × 1–2 cm, central to slightly eccentric, smooth, not pruinose, cylindrical, ventricose toward the base, without annulus, whitish, often with pale-greenish tinge, part turning pale yellow when aged, sometimes with rusty spots, first unchanging, stuffed, hollow when mature. Context 3–5 mm thick from the lamellae attachment to the stipe, compact under pileus, white to whitish, no color change when bruised, turning slightly pale yellowish in age; taste mild; no distinct odor. Spore print whitish to pale cream (Romagnesi Ib–IIa).

edge sterile. Subhymenium 15–20 µm thick, composed of slender cylindrical cells 4–7 µm wide under basidia and inflated cells (15–)20–35(–45) µm in diam. next to trama. Pileipellis a trichoderm 200–300 µm thick, composed of 4–6 µm thick, slender hyaline hyphae without intracellular pigmentation; pileocystidia numerous, 40–70 µm long, sometimes inflating up to 7–10 µm wide, fusiform, with crystal contents but no septa, sharply blackened in SV. Stipitipellis not well developed, a cutis composed of thin–walled, septate, cylindrical hyphae 3–6 µm wide; caulocystidia absent. Clamp connections absent in all tissues.

Ecology & distribution: Single or gregarious in conifer forest (*Picea* spp.) in July and August in China (Sichuan, Xizang, and Yunnan).

Additional specimens examined: **China**, **Sichuan Province**, Daofu County, 25.VII.2007, Z.W. Ge 1532 (HKAS53618); **Yunnan Province**, Yulong, Laojunshan, 9.VIII.2008, Q. Zhao 8238 (HKAS55220); **Xizang Autonomous Region**, Changdu County, Zhuge Village, 31°04′N, 96°58′E, alt. 4200 m, 7.VIII.2003, Z.L. Yang 4305 (HKAS45684).

Comments: *Russula atroaeruginea* is characterized by its dark-green intermixed with radially yellowish-tinged (striate) pileus, whitish to pale-cream spore print, and basidiospores with thin low fine ornamentations (<1.0 µm tall). The dark-green pileus contrasting with the whitish (often green-tinged) stipe is a good field character, although it is sometimes difficult to distinguish this species against from the green forest surroundings. This species should be placed in the *Russula* sect. *Griseinae* (Jul. Schäff.) Romagn. based on its mild-flavored context, pileipellis lacking primordial hyphae and red pigments but with typical multiseptate (often subulate) hyphal extremities, and unicellular pileocystidia that blacken in SV.

Romagnesi (1967) recognized 5 greenish tinged species in sect. *Griseinae*: *R. pseudoaeruginea* (Romagn.) Kuyper & Vuure, *R. aeruginea* Fr., *R. medullata* Romagn., *R. stenotricha* Romagn., and *R. anatina* Romagn. The morphologically similar *R. aeruginea*, *R. anatina*, *R. cyanoxantha* f. *peltereaui* Singer, *R. medullata*, and *R. pseudoaeruginea* have been reported in eastern Asia (Tai 1979, Ying et al. 1982, Imazeki & Hongo 1989, Ying 1989, Shimono et al. 2004). *Russula pseudoaeruginea*, however, has a gray-tinged green olive or somewhat cream and pale-brown intermixed pileus and an ochreous-cream spore print (Romagnesi 1967, Sarnari 1998); it often grows in temperate to boreal deciduous forests dominated by *Tilia* and *Quercus* spp. (Romagnesi 1967, Hansen & Knudsen 1992), while *R. atroaeruginea* grows in conifer forests dominated by *Picea* spp. in alpine subtropical regions.

Another taxon superficially resembling *R. atroaeruginea* is *R. aeruginea*, a very common species originally described from Europe which grows in subalpine coniferous to mixed forests containing *Betula*, *Pinus*, and *Picea*. However, *R. aeruginea* has a green-tinged pileus with a darker pileus centre, whitish

Fig. 1: *Russula atroaeruginea*: a. HKAS 45684; b. HKAS 53626 (Holotype)

stipe with rusty spots, a slightly acrid taste, cream-colored spore deposits, and spores with many fine lines formed by connected low warts (Schaeffer 1952, Romagnesi 1967, Roger 1981, Woo 1989, Hansen & Knudsen 1992, Sarnari

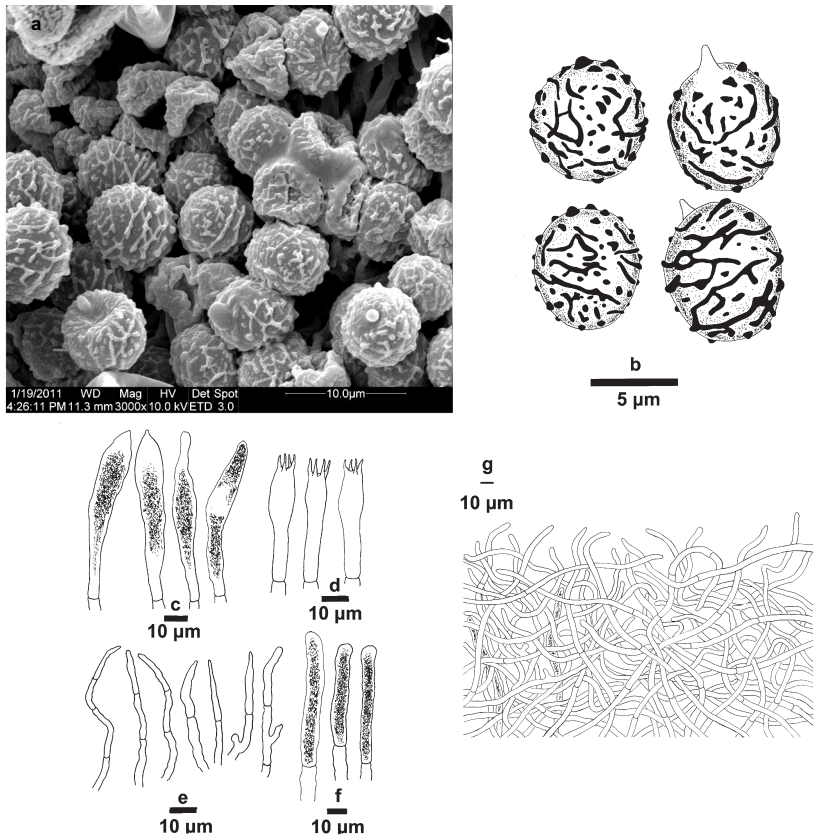

Fig. 2: *Russula atroaeruginea* (HKAS 53626): a. Basidiospores (SEM); b. Basidiospores; c. Pleurocystidia; d. Basidia; e. Hyphal extremities in pileipellis; f. Pileocystidia; g. Pileipellis

Basidiospores [80/4/3] (6.3–) 6.8–8.1 (–9.0) × (5.9–) 6.1–7.4 (–7.8) µm, Q = (1.01–)1.04–1.23(–1.32) (**Q** =1.15 ± 0.07), subglobose to broadly ellipsoid or (sometimes) ellipsoid, verrucose; ornamentation amyloid, composed of warts mostly fused into fine lines, cristulate to subreticulate, with some isolated verrucae, <1.0 µm high; plage not or indistinctly amyloid. Basidia 40–48 × 9–11 µm, clavate, inflated towards upper half, 4–(rarely 2–) spored, projecting 7–13 µm beyond hymenium, hyaline in KOH; sterigmata 3–5 µm, pointed, straight to slightly tortuous. Pleurocystidia numerous, 72–115 × 9–13 µm, originating in subhymenium, projecting 35–50 µm beyond hymenium, thin–walled, clavate to subfusiform, sometimes with refractive contents; apices subacute to acute, at times with a moniliform appendage. Cheilocystidia not observed; lamellar

1998). *Russula aeruginea* can be distinguished from *R. atroaeruginea* by its more or less discolored pileus, which is often spotted with scattered, rusty spots.

Russula atroaeruginea is also easily distinguished from three other species originally described from France: *R. medullata* grows under deciduous trees of *Corylus*, *Populus*, *Fraxinus*, *Betula*, *Fagus*, *Castanea*, and *Quercus*, has an ochre (III a, b–c) spore print, spores with isolated warts, and rare pileocystidia (Romagnesi 1967, Hansen & Knudsen 1992); *R. stenotricha* has a grayish-green pileus, slightly spicy lamellae, short obtuse pileipellis terminal cells, and short wide pileocystidia 25–50 × 5–9 (12) µm (Romagnesi 1967, Hansen & Knudsen 1992); and *R. anatina* has long cystidia 60–130 × 6.7–12 µm (Romagnesi 1967).

Also similar to *R. atroaeruginea* is the green form of *R. cyanoxantha* (Schaeff.) Fr. (= *R. cyanoxantha* f. *peltereaui*), which differs in its elastic lamellae, white (Ia) spore mass, basidiospores with scattered low warts that are never connected with fine lines, and slender cystidia 65–100 × 5.7–7 µm (Romagnesi 1967, Roger 1981, Hansen & Knudsen 1992).

Among the *Russula* species originally described from China, the morphologically related *R. viridirubrolimbata* J.Z. Ying differs from *R. atroaeruginea* by its finely rimose pileus, unchanging white context, isolated spore ornamentations, hyaline pleurocystidia, and a pileipellis without pileocystidia (Ying 1983).

Russula sichuanensis G.J. Li & H.A. Wen, **sp. nov.** Figs 3-4

MycoBank MB804645

Differs from *Macowanites yunnanensis* by its larger gastrocarp, dirty white to pale tinged pileus, cream to yellowish lamellae, and larger basidiospores with larger ornamentations.

Type: China. Sichuan Province, from Rangtang County to Seda County, banks of Duke River, 5.VIII.2007, Z.W. Ge1707 (**Holotype**, HKAS53792, GenBank JX391969)

Etymology: Referring to the type locality region.

Basidiomata semi-hypogeous to epigeous, russuloid to secotioid, small. Pileus 3.1–4.4 cm in diam., irregularly globose to subglobose when young, hemispheric, barely expanded, sometimes pulvinate when mature, centre slightly depressed with age, whitish, dirty-white to pale-pinkish tinge of Pale Salmon Color (XIV9′f) to Pale Flesh Color (XIV7′f), rarely with greenish tinge of Dark Olive Buff (XL21′′′), intermixed with brown to olivaceous brown tinge of Wood Brown (XL17′′′), Brussels Brown (III15m) when dry; margin whitish to cream, White (LIII) to Cream color (XVI19′f), often glabrous, dry, slightly viscid when moist, strongly incurved and indented around the stipe, sometimes slightly radially rugose, with slightly pinkish tinge of Pale Flesh Color (XIV7′f), not striate. Context ≤3 mm at the disc, whitish, fragile; odor not distinctive; taste mild. Lamellae/Gleba 3–6 mm high, sublamellate to lamellate, composed of

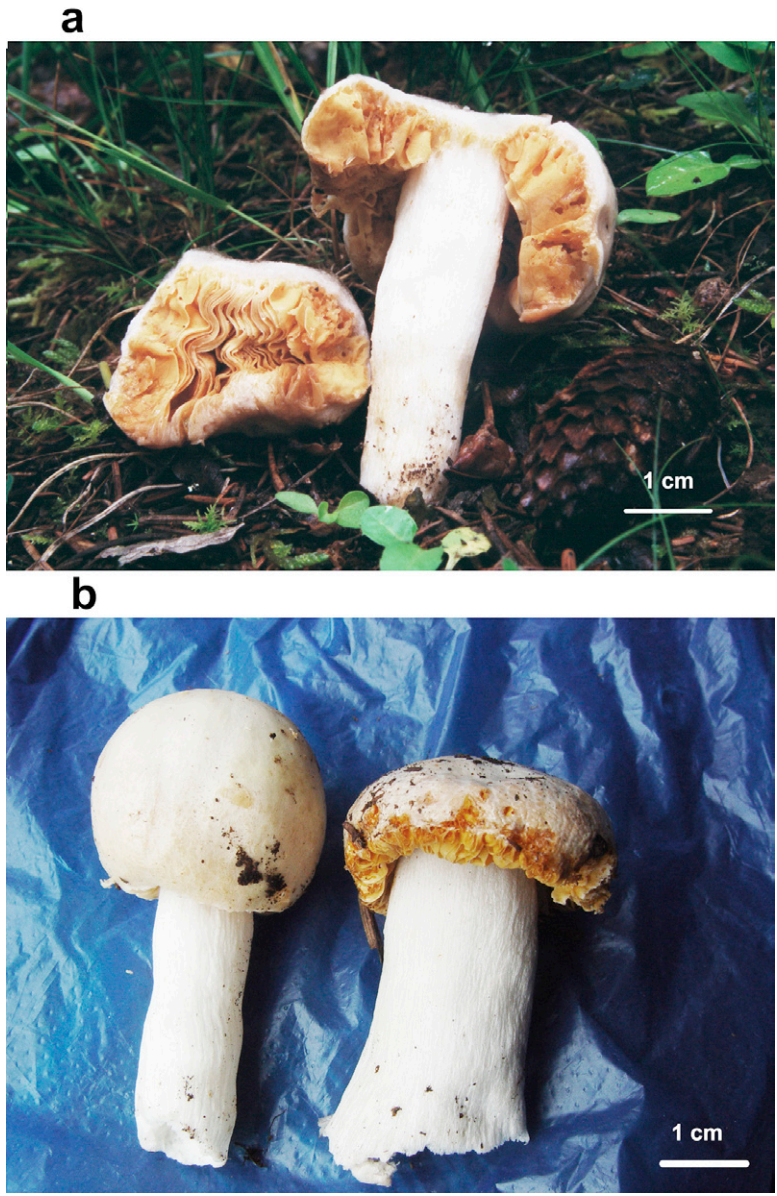

Fig. 3: *Russula sichuanensis*: a. HKAS 45645; b. HKAS 53792 (Holotype)

sinuate, contorted, very crowded, convoluted lamellae, often exposed near the stipe, cream to yellowish tinge of Pale Orange-Yellow (III15f) when juvenile,

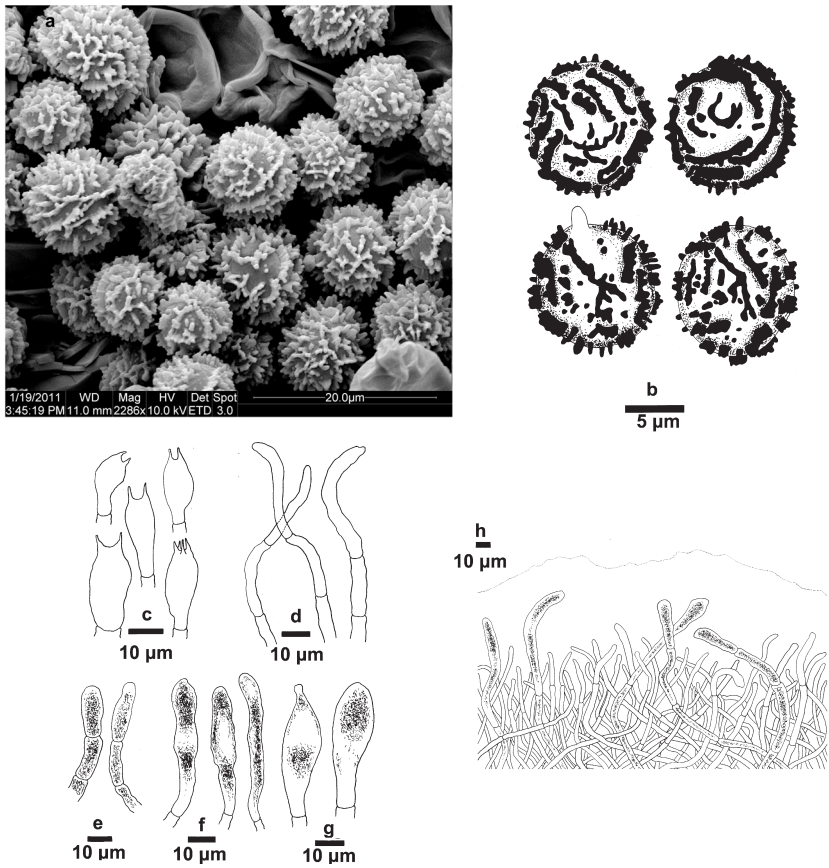

Fig. 4: *Russula sichuanensis* (Holotype HKAS 53792): a. Basidiospores (SEM); b. Basidiospores; c. Basidia; d. Hyphal extremities in pileipellis; e. Pileocystidia; f. Caulocystidia; g. Pleurocystidia.

with pale orange tinge of Orange Buff (III15d) to Light Orange-Yellow (III17d) when mature. Stipe 4.0–6.0 × 1.0–1.5 cm, subcylindrical, surface dry, rugulose longitudinally, dull, without annulus, slightly attenuate upwards, whitish, ≤3 mm at pileus diameter, stuffed first, becoming hollow when old. Spore print ocher to yellow (Romagnesi IIIc–IVa).

Basidiospores [100/3/2] (8.8–)9.4–14.1(–15.5) × (7.6–)7.9–12.8(–13.1) µm, Q = (1.01–)1.02–1.26(–1.32), (**Q** = 1.11 ± 0.08), globose to subglobose, slightly yellowish; ornamentation cristulate to subreticulate, composed of amyloid warts linked as small crests and ridges, forming a partial to nearly complete network, rarely intermixed with isolated verrucae, warts often >1 µm high (but no more than 1.5 µm); suprahilar plage not well defined. Basidia

24–35 × 11–15 μm, mostly with two sterigmata 4–7 μm long, rarely 4–spored, claviform to clavate, short and rather voluminous, rarely cylindrical, hyaline, or sometimes yellow in KOH. Pleurocystidia rare, 45–72 × 10–15 μm, distinctly projecting 10–30 μm beyond the basidia, ventricose, sometimes clavate to sphaeropedunculate, thin-walled, rarely thick-walled, often empty, at times with refringent contents, no reaction in SV. Subhymenium a 20–35 μm thick cellular layer composed of inflated cells 7–13 μm in diam., hyaline, sometimes pale yellowish in KOH. Pileipellis an ixotrichoderm 125–150 μm thick, slightly gelatinized, composed of thin–walled hyphae 3–6 μm wide, cylindrical, hyaline; pileocystidia septate, clavate to cylindrical, 6–10 μm wide, apex sometimes inflated, with refractive contents. Stipitipellis filamentous hyphae 3–6 μm in diam., intermixed with inflated cells, hyaline, some hyphae yellowish to pale ocher in KOH; caulocystidia infrequent, 35–44 × 8–11 μm, cylindrical, septate, with round apex. Clamp connections and laticiferous hyphae absent from all tissues.

Ecology & distribution: Single or gregarious in conifer forests (*Picea* spp.) at altitude of 3300-3900m during July and August, China (Xizang and Sichuan).

Other specimens examined: **China**, **Xizang Autonomous Region**, Jiangda County, Tongpu Township, 31°35′N, 98°23′E, alt. 3300 m, 2.VIII.2004, Z.L. Yang 4266 (HKAS45645); Riwoche County, Sangduo Township, 31°05′N, 96°29′E, alt. 3900 m, 11.VIII.2004, Z.W. Ge335 (HKAS46115); Zuogong County, Wangda Township, alt. 3700 m, 18.VII.2009, Z.L. Yang 5285 (HKAS 57828); **Sichuan Province**, Aba County, Anqiang Township, Ping' an Village, 11.VIII.2007, Z.W. Ge 1800 (HKAS53885, GenBank JX391971).

Comments: Until *Russula* species were emended by Lebel & Tonkin (2007), *Russula sichuanensis* was treated in *Macowanites* Kalchbr., a widespread sequestrate genus containing diverse species phylogenetically close to *Russula* (Miller et al. 2001, Lebel & Castellano 2002, Eberhardt & Verbeken 2004). That very little knowledge is available concerning *Macowanites* in China may result partly from its semi-hypogeous habit; only *M. yunnanensis* M. Zang has been described, based on materials collected from Zixi Mountain, Chuxiong, Yunnan Province (Zang & Yuan 1999). Its morphology conspicuously differs from that of *R. sichuanensis: M. yunnanensis* has a smaller gastrocarp (0.7–1.2 cm broad), a pinkish- to reddish-tinged pileus surface, whitish lamellae, and smaller basidiospores (10–12.5 × 6–7 μm) with 0.3–0.5 μm ornamentations (Zang & Yuan 1999).

Russula sichuanensis resembles a medium-sized, distorted or aborted agaricoid *Russula*, because it has a curly pileus, a white percurrent stipe often covered by the pileus, and tightly contorted sinuate cream to yellow lamellae. Other characters include a cream-tinged pileus (often olivaceous brown at the centre), a yellowish to orange sublamellate gleba, and stipitate basidiomata.

The following microscopical characters are distinctive: the partially reticulate globose to subglobose basidiospores, short voluminous basidia, very rare pleurocystidia, and septate pileocystidia with crystal contents. In addition, the association of *R. sichuanensis* with *Picea* spp. in coniferous forests of the eastern Qinghai–Tibet plateau is a distinctive ecological and biogeographical feature.

A number of species have a similar sublamellate gleba and percurrent stipe: *Macowanites citrinus* Singer & A.H. Sm., *M. chlorinosmus* A.H. Sm. & Trappe, *M. luteolus* A.H. Sm. & Trappe, *M. subolivaceus* A.H. Sm., *Russula galbana* T. Lebel, *R. kermesina* T. Lebel, *R. luteirosea* (Bougher) T. Lebel, and *M. arenicola* S.L. Mill. & D. Mitch. However, *M. citrinus*, originally described from Idaho (in the northwestern U.S.A.), differs from *R. sichuanensis* in having smaller subglobose to short ellipsoid basidiospores ornamented by conical spines and short flattened ridges and with a small indistinct suprahilar plage (Pegler & Young 1979), and *M. chlorinosmus* (from Oregon, U.S.A.) has a rimose pileus cutis, a strong chlorine odor, a very unpleasant taste, and basidiospores with low warts (Smith 1963). *Macowanites luteolus* (also from Oregon) differs by its short thin stipe, smaller basidiospores with scattered to close 0.3–0.7 μm ornamentations, subhymenium cells that are hyaline in KOH, and numerous macrocystidia (Smith 1963). *Macowanites subolivaceus* (from Idaho) has a lobed to very irregular pileus margin, a very fragile thin context, basidiospores 7–9 μm in diam., and a peridial epicutis consisting of a turf of gelatinous branched hyphae with 60–200 μm long pseudocystidia (Smith 1963). *Russula galbana* (from Queensland, northeastern Australia) has smaller basidiomata, a short slender stipe, and common, dimorphic hymenophoral cystidia with obtuse or mucronate apices (Lebel & Tonkin 2007); *R. kermesina*, (from Nelson, New Zealand) is characterized by its carmine red to dark-red-tinged pileus, chalk-white gleba, large cystidia not or only slightly projecting beyond the basidia, and pileipellis without pileocystidia (Label & Castellano 2002). *Russula luteirosa* (from western Australia) has a pileus with intermixed cream to pale yellowish and rosaceous colors, large basidia, and an association with *Allocasuarina*, *Acacia*, and *Eucalyptus* (Bougher 1997). *Macowanites arenicola* (from northwestern Florida, U.S.A.) has a yogurt- or soy sauce-type odor, smaller broadly elliptical to elliptical spores with low ornamentation (8.8–10.4 × 6.8–7.2 μm, Q = 1.37, verrucae 0.2–0.4 μm high), long slender cystidia (80–120 × 6–7.5 μm), and grows in white quartzite sands of costal scrub forests (Miller 2004).

Russula sichuanensis also shows some superficial affinities with *Russula* sect. *Laricinae* Romagn. for its small-sized basidiocarps, ocher to yellow spore print, short basidia, rare pleurocystidia, and pluriseptate dermatocystidia (Romagnesi 1985), but its russuloid to secotioid basidiocarps and large basidiospores obviously distinguish it from the agaricoid sect. *Laricinae*.

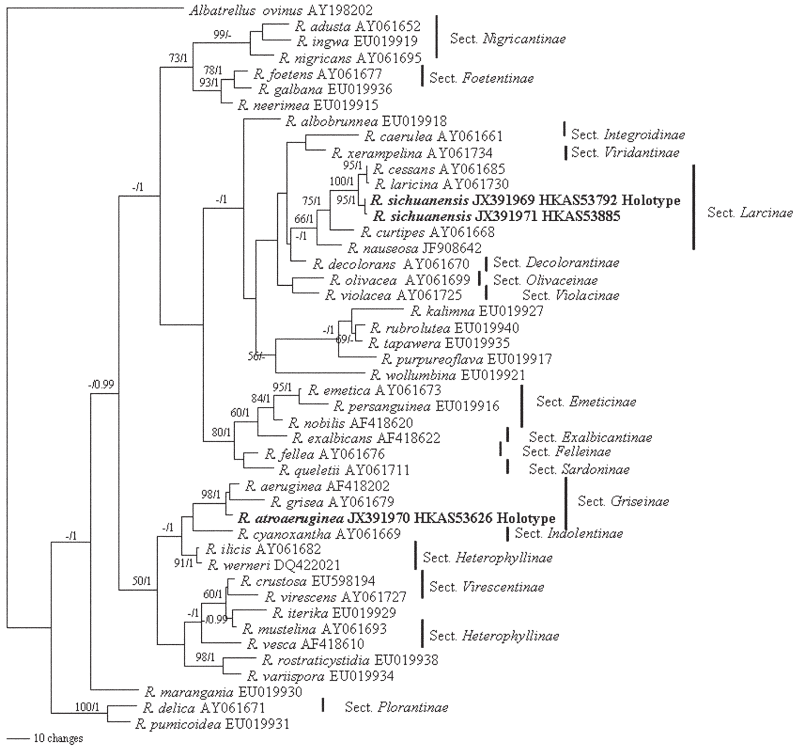

Fig. 5. Phylogenetic relationship tree of *Russula* species inferred from the dataset of ITS1-5.8S-ITS2 sequences. Consistency index (CI) = 0.461, retention index (RI) = 0.665, and tree length (TL) = 1030. MP bootstrap support values (>50%) and PP (>0.94) from Bayesian Analysis are indicated above or below the branches as MP BS/BA PP. Sectional names are from Romagnesi (1987).

Phylogenetic results

The ITS1-5.8S-ITS2 dataset included sequences representing 44 taxa representing nine *Russula* subgenera. The aligned sequence comprised 471 nucleotide sites. In the MP analysis, 195 characters were constant, 69 variable characters were parsimony-informative, and 207 characters were parsimony-uninformative. In the ITS phylogenetic tree (Fig. 5), *R. atroaeruginea* clustered with *R. aeruginea* and *R. grisea* with strong statistical support (98% boot strap, 1.0 posterior probability). A Blast comparison of *R. atroaeruginea* (JX391970) and *R. aeruginea* (AF418612) through the NCBI databank shows a 93% max identification and 96% coverage. Another ITS blast with *R. atroaeruginea* (JX391970) and *R. grisea* (AY061679) shows a 90% max identification of 99% coverage, confirming that *R. atroaeruginea* is related to *R. grisea* / *aeruginea* group in *Russula* sect. *Griseinae*. The separation between *R. atroaeruginea* and

R. cyanoxantha is also well supported. These phylogenetic results suggest that *R. atroaeruginea* and *R. aeruginea* are closely related but different species.

The clade in which *R. sichuanensis* is located is highly supported (Fig. 5). Members of *Russula* subgen. *Tenellula* sect. *Larcinae* cluster together, suggesting that *R. sichuanensis* is closely related to the *R. laricina* / *cessans* group. However, given the lack of stability in the topologies due to GenBank's limited number of ITS sequences from closely related secotioid *Russula* taxa, we hesitate making a definitive statement about the phylogenetic position of *R. sichuanensis*. Broader collections from East Asia, particularly of russuloid to secotioid *Russula* taxa, are required in future work.

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