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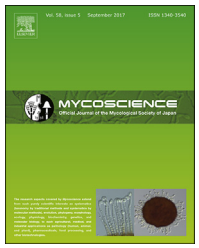

Full paper

Re-evaluation of the taxonomy and diversity of *Russula* section *Foetentinae* (Russulales, Basidiomycota) in Korea

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ABSTRACT

Russula section *Foetentinae* is a group of ectomycorrhizal symbiont fungi that are integral in maintaining biodiversity in diverse ecosystems. Identification and accurate classification of these fungi has proven challenging because of significant similarities in morphology, particularly among fruiting bodies. The objective of this study was to re-evaluate the diversity and taxonomy of *Russula* section *Foetentinae* in Korea using both phylogenetic analysis and morphological characteristics. A phylogenetic tree was constructed using internal transcribed spacer sequences and compared against key morphological characteristics. Our results reassigned several taxa and established important ecological relationships among closely related species. The phylogenetic analysis produced strong support for seven species, two of which were previously identified, one was a new record to Korea, and one was an undescribed species which we propose to name *R. catillus* sp. nov. *Russula* section *Foetentinae* separated into four clades when molecular and morphological data were combined. An important finding of the study was that several taxonomic assignments based on morphological characters were inconsistent with more reliable molecular data. This study highlights the need for a genetic database that can be easily accessed and used in conjunction with morphological data in order to better resolve the evolutionary history of this important fungal group.

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1. Introduction

The genus *Russula* is one of the most highly diverse groups of fungi and is found across a wide range of habitats from the tropics to polar ecosystems (Miller and Buyck 2002; Park et al. 2013). Approximately 750 *Russula* species have been recorded worldwide (Kirk et al. 2008). *Russula* species play an important role in sustaining the biodiversity of forests as ectomycorrhizal symbionts and are an important nutrient source for insects (Yamashita and Hijii 2007), animals (Fogel and Trappe 1978), including humans (Guo 1992; Hu and Zeng 1992).

The *Russula* section *Foetentinae* Melzer & Zvára is characterized by a fetid odor, tuberculate-striate pileus margin, and articulated and branched hair cuticles. Many *Foetentinae* species are well known in Europe and North America (Shaffer 1972; Romagnesi 1985; Sarnari 1998). Through the efforts of several studies, the members of the section *Foetentinae* have been classified into 27 species, 14 of which have been recorded in Asia: *Russula foetens* Pers. (the type species), *R. amoenolens* Romagn., *R. cerolens* Shaffer, *R. dubiana* K. Das et al., *R. fusogrisea* Petch, *R. grata* Britzelm. (= *R. laurocerasi* Melzer), *R. natarajanii* K. Das et al., *R. pectinata* Fr., *R. periglypta* Berk. & Broome, *R. pseudopectinatoides* G.J. Li & H.A. Wen, *R. punctipes* Singer, *R. senecis* S. Imai, *R. sororia* Fr., *R. tsokae* K. Das et al. (Singer 1935; Shaffer 1972; Hongo 1973; Romagnesi 1985; Pegler 1986; Imazeki and Hongo 1989; Woo 1989; Sarnari 1998; Kränzlin 2005; Das et al. 2006, 2010, 2013; Manimohan and Latha 2011; Li et al. 2015). Previously, individual species within the section *Foetentinae* were distinguished primarily by height and pattern of spore ornamentation, spore size, pileus color, taste, odor and presence of dermatocystidia on the pileus cuticle (Shaffer 1972; Sarnari 1998). Morphological identification at the species level, however, can be difficult due to extensive morphological plasticity and ambiguous descriptions (Miller and Buyck 2002).

The introduction of DNA based taxonomy has revolutionized systematics, and modern phylogenetic methods employing various genetic markers have become standard practice in tandem with traditional morphological character based classification. A dramatic increase in available genetic data has given rise to in-depth phylogenetic studies for inference of complex phylogenetic relationships and enabled species identification based on DNA sequences (Blaxter 2004). A primary tool in this effort is the use of DNA barcoding, which uses specific or more general DNA markers, depending on the depth of taxonomic inference desired (Savolainen et al. 2005). The use of DNA barcoding has already improved the resolution of several previously unclassified, incorrectly classified or unidentified taxa (Hebert et al. 2003). A primary objective is to generate a comprehensive database of DNA sequences against which sequences from any specimen, whether newly collected or archived, can be compared (Moritz and Cicero 2004). For species not easily delimitated morphologically, sequence-based analysis and subsequent morphological re-evaluation can be a more accurate and efficient method for identification (Jung et al. 2014).

Several molecular markers such as internal transcribed spacers (ITS), a universal fungal DNA barcode marker (Schoch et al. 2012), 28S ribosomal RNA (LSU), and RNA polymerase II subunit (RPB2), have been commonly used in the *Russula* phylogenetics (Eberhardt 2002; Miller and Buyck 2002; Shimono et al. 2004; Miller et al. 2006; Buyck et al. 2008; Park et al. 2013). However, as is common with other sections within the *Russula*, phylogenetic relationships within the section *Foetentinae* remain unresolved (Miller and Buyck 2002; Shimono et al. 2004; Li et al. 2015).

In Korea, six species in the section *Foetentinae* have been reported: *R. cerolens*, *R. foetens*, *R. grata*, *R. pectinata*, *R. senecis*, and *R. sororia* (Lee 1959; Lee and Cho 1976; Hong and Jung 1977; Kim et al. 1977; Lee and Lee 2014). As previous studies were based primarily on morphological characteristics of fruiting bodies, the true identity and diversity of this section in Korea is unclear. Recently, a Korean barcode of life project was organized by the National Institute of Biological Resources (http://www.nibr.go.kr) to promote the exploration of biodiversity and biological resources. As part of an ongoing effort to construct DNA barcodes for the *Russula* section *Foetentinae* in Korea, a total of 24 specimens from seven species were recognized based on ITS sequence analysis. By combining ITS sequence analysis and morphological characteristics, we re-evaluated the taxonomy of *Russula* section *Foetentinae* in Korea. The addition of ITS sequence analysis to taxonomic classifications that were formerly based exclusively on morphology successfully resolved the phylogenetic relationships of several members of the *Russula* section *Foetentinae*.

2. Materials and methods

2.1. *Collection of* Russula *section* Foetentinae *specimens and microscopic observation*

A total of 24 *Russula* specimens were examined in this study. Fruiting bodies were collected throughout South Korea between 1987 and 2015 (Table 1). Dried specimens were deposited in The Herbarium Conservation Center of The National Academy of Agricultural Sciences (HCCN) and the Seoul National University Fungus Collection (SFC). Each collection was assigned a species designation upon identification according to field guides (Hongo 1955; Romagnesi 1985; Kränzlin 2005; Park and Lee 2011) and a photographic illustration website (http://www.mtsn.tn.it/russulales-news/). The Methuen handbook of colour (Kornerup and Wanscher 1978) was used as the color standard for descriptions of the specimens.

In order to observe microscopic characteristics, mounts of specimens were made in 3% (w/v) KOH and 1% (w/v) phloxine (Largent 1977). Microscopy was performed using an Eclipse 80i light microscope (Nikon, Tokyo, Japan). To allow for comparisons with published data (i.e., Shaffer 1972), we measured basidia (n = 20/sample), cystidia (n = 20/sample), and spores (n = 40/sample) as length range × width range. Quotient (Q), which is the ratio of spore variation between mean length and width, was calculated for each specimen studied. For scanning electron microscope (SEM) analysis, pieces of dried lamellae with spores were attached to aluminum stubs using double sided adhesive tape, coated with platinum in a sputter coater (EM ACE200, Leica, Vienna, Austria), and then examined with a SEM (SUPRA 55VP, Carl Zeiss, Oberkochen, Germany).

Table 1 – List of specimens and GenBank accession number of sequences used in this study.

Species	First identification	Location	Collection date	Voucher	ITS Acc. No.
Russula insignis Quélet	*Russula sororia*	Seolcheon-myeon, Muju-gun, Jeollabuk-do	Jul 6, 2006	ASIS13717	KX574691
	R. sororia	Ssangyong-dong, Cheonan-si, Chungcheongnam-do	Jul 5, 2008	ASIS16471	KX574692
	R. sororia	Daehak-dong, Gwanak-gu, Seoul	Aug 27, 2012	SFC20120827-02	KX574693
R. aff. *pectinatoides* Peck	*R.* sp.	Seohwa-myeon, Inje-gun, Gangwon-do	Aug 7, 2002	ASIS10362	KX574694
	R. sp.	Sodo-dong, Taebaek-si, Gangwon-do	Jul 24, 2012	ASIS22692	KX574695
	R. grata	Daehak-dong, Gwanak-gu, Seoul	Sep 3, 2012	SFC20120903-05	KX574696
	R. pectinatoides	Songnisan-myeon, Boeun-gun, Chungcheongbuk-do	Jul 25, 2015	SFC20150725-13	KX574697
R. catillus Lee, Park & Lim	*R. adusta*	Cheongna-myeon, Boryeong-si, Chungcheongnam-do	Jul 25, 2012	SFC20120725-52	KX574685
	R. pectinata	Daehak-dong, Gwanak-gu, Seoul	Aug 27, 2012	SFC20120827-01	KX574686
	R. sororia	Daehak-dong, Gwanak-gu, Seoul	Sep 18, 2012	SFC20120918-04	KX574687
	R. foetens	Daehak-dong, Gwanak-gu, Seoul	Sep 19, 2012	SFC20120919-35	KX574688
	R. cf. *pectinata*	Hunjeong-dong, Jongno-gu, Seoul	Aug 12, 2015	SFC20150812-07	KX574689
R. cf. *sororia* (Fries) Romell	*R. sororia*	Seodun-dong, Gwonseon-gu, Suwon-si, Gyeonggi-do	Aug 1, 1987	2208ASI	KX574701
	R. sororia	–	Oct 7, 2007	ASIS15775	KX574702
	R. sp.	Jinbu-myeon, Pyeongchang-gun, Gangwon-do	Jul 22, 2012	ASIS22640	KX574703
R. aff. *cerolens* Shaffer	*R. adusta*	Seongju-myeon, Boryeong-si, Chungcheongnam-do	Jul 25, 2012	SFC20120725-02	KX574684
	R. alboareolata	Sagok-myeon, Gongju-si, Chungcheongnam-do	Aug 20, 2012	SFC20120820-41	KX574680
	R. sororia	Daehak-dong, Gwanak-gu, Seoul	Aug 31, 2012	SFC20120831-07	KX574681
	R. sororia	Yanghwa-dong, Yeongdeungpo-gu, Seoul	Sep 15, 2012	SFC20120915-20	KX574682
	R. cerolens	Donghyang-myeon, Jinan-gun, Jeollabuk-do	Jul 25, 2014	SFC20140725-09	KX574683
R. grata Britzelmayr	*R. foetens*	Dansan-myeon, Yeongju-si, Gyeongsangbuk-do	Jul 30, 2012	ASIS22807	KX574690
R. senecis S. Imai	*R. senecis*	Socho-myeon, Wonju-si, Gangwon-do	Sep 21, 2011	SFC20110921-18	KX574698
	R. senecis	Hagi-dong, Yuseong-gu, Daejeon	Jul 14, 2012	SFC20120714-13	KX574699
	R. senecis	Namwon-eup, Seogwipo-si, Jeju-do	Aug 7, 2012	SFC20120807-05	KX574700

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from sections of fresh or dried fruiting bodies using a modified CTAB extraction protocol of Rogers and Bendich (1994). The internal transcribed spacer (ITS) was amplified using forward primers ITS1F or ITS5 and reverse primers ITS4B or Russ3R (White et al. 1990; Gardes and Bruns 1993; Park et al. 2013). PCR amplification was performed as described by Park et al. (2013). The PCR products were electrophoresed through a 1% agarose gel stained with EcoDye DNA staining solution (SolGent Co., Daejeon, Korea) and purified with the Expin PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea) according to the manufacturer's instructions. Sequencing was done in both forward and reverse directions for each sample using the PCR primers. DNA Sanger sequencing was performed by Macrogen (Seoul, Korea) using an ABI3700 automated DNA sequencer.

2.3. Sequence analyses

Sequences were assembled and aligned using MEGA v 6 (Tamura et al. 2013) and deposited in GenBank (accession Nos. in Table 1). For phylogenetic analyses of *Russula* section *Foetentinae*, published ITS sequences were aligned with the generated data (Eberhardt 2002; Miller and Buyck 2002; Nara 2006; Palmer et al. 2008; Xie et al. 2010; Avis 2012; Osmundson et al. 2013; Li et al. 2015). In order to identify specimens to the species level, Korean data was combined with reference data from 14 species in the section *Foetentinae*.

Multiple sequence alignments were performed using MAFFT v 7 (Katoh and Standley 2013) with the default settings, checked by eye and adjusted manually. Sequence alignments were deposited in TreeBASE (http://treebase.org/treebase-web/; submission ID 19781). Neighbor-joining phylogenetic analyses were performed on ITS data with MEGA v 6 (Tamura et al. 2013) using the Kimura 2-parameter model with 1000 bootstrap replicates. Maximum likelihood phylogenetic analyses were performed with RAxML (Stamatakis 2006) using the GTR + G model of evolution with 1000 bootstrap replicates. Bayesian inference (BI) analyses were executed with MrBayes on XSEDE v 3.2.6 (Ronquist and Huelsenbeck 2003) using the HKY + G model. This model was chosen based on highest Bayesian Information Criteria (BIC) score after testing for the best fit in jModeltest v2.1.2 (Darriba et al. 2012).

3. Results

On the basis of phylogenetic analysis of the ITS sequences combined with morphological observation of 24 *Russula* specimens, we identified seven species in the section *Foetentinae* (Fig. 1). Intra-specific ITS variation varied from 0% (*R. insignis*, n = 3) to 0.96% (*R. senecis*, n = 3). Six species were confidently identified—*R.* aff. *cerolens*, *R. grata*, *R. insignis*, *R.* aff. *pectinatoides*, *R. senecis*, and *R.* cf. *sororia*. In the case of *R. senecis*, both morphological and molecular identification were identical in all samples; however, misidentification based on morphology in the six other species was frequent. For example, morphology based identification of eight specimens of *R. sororia* misidentified six specimens, while the ITS sequence analysis identified them as *R. catillus* (n = 1), *R.* aff. *cerolens* (n = 2) and *R. insignis* (n = 3) (Table 1). *Russula* aff. *cerolens* was also misidentified as *R. adusta* (n = 1), *R. alboareolata* (n = 1), *R. sororia* (n = 2) (Table 1).

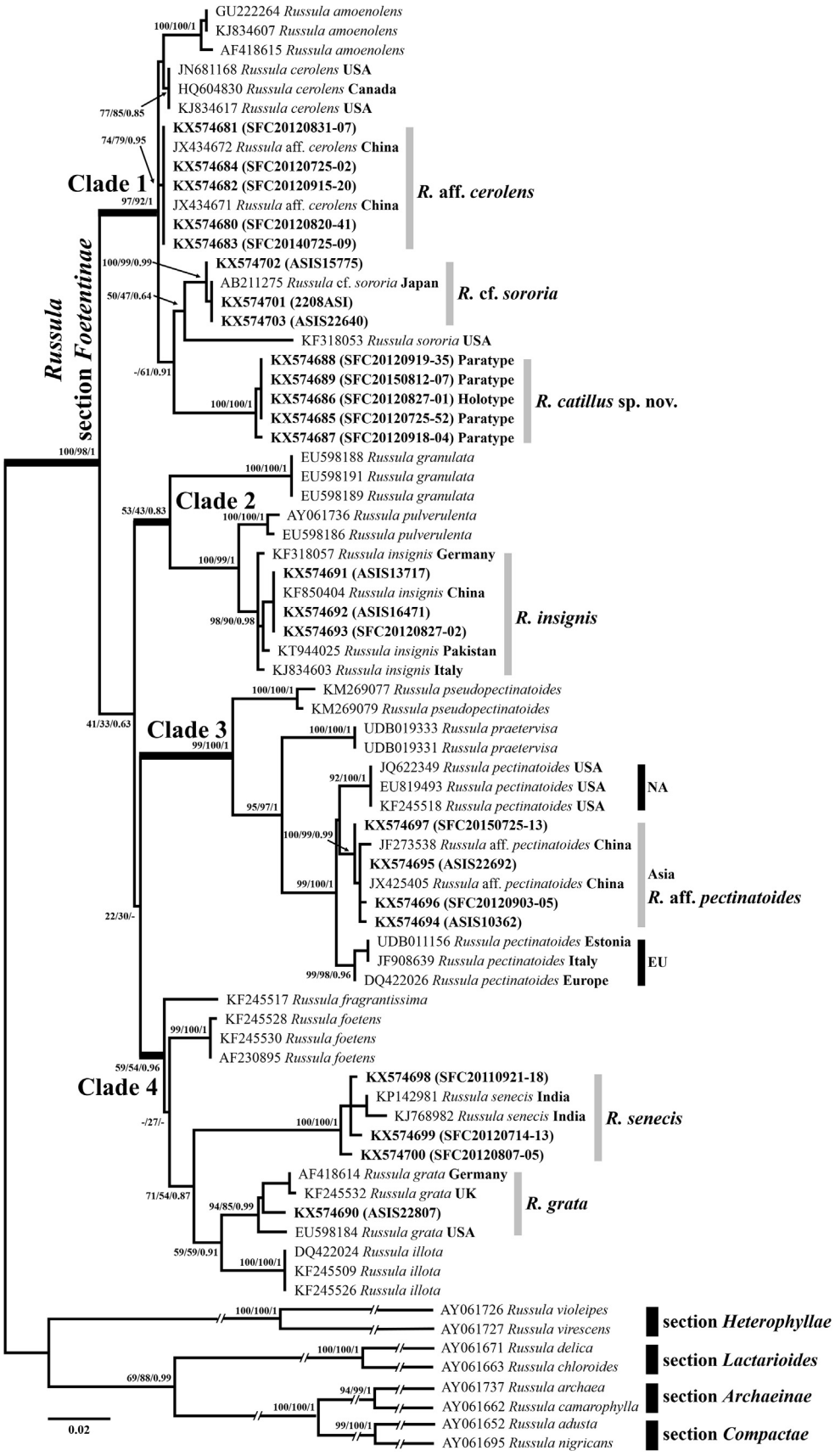

Fig. 1 – Bayesian phylogram inferred from the ITS dataset for *Russula* section *Foetentinae*. Support values (Neighbor Joining bootstrap/RAxML bootstrap/Bayesian posterior probability) are indicated above or below clade branches. The scale bar indicates the number of nucleotide substitutions per site.

Five specimens which had been erroneously identified as *R. adusta*, *R. foetens*, *R. pectinata* and *R. sororia* proved to the same species based on ITS analysis and they formed a monophyletic group with high statistical support (NJ bootstrap support/RAxML bootstrap support/Bayesian posterior probability = 100/100/1). Furthermore, this undescribed species had distinct morphological characteristics compared to the other *Russula* species within this section (Table 2). Based on the phylogenetic analysis and morphological observations we confirmed this species as a new taxon and propose the name *Russula catillus* sp. nov.

Analyses of 75 ITS sequences including 22 known taxa were conducted to infer phylogenetic relationships of species in *Russula* section *Foetentinae*. The topology of phylogenetic trees generated from NJ, ML and Bayesian analysis were nearly identical. The phylogenetic tree is presented in Fig. 1. Phylogenetic analysis showed that section *Foetentinae* is a well-supported monophyletic group (NJ/ML/BI = 100/98/1) and separates into four clades. Clades 1 and 3 were well supported by three analyses, while clades 2 and 4 were well supported by BI analysis alone. The relationship of clade 2 to clade 4 was unclear due to low resolution of basal branches in the tree (Fig. 1).

Four species, including a new taxon, belonged to clade 1, while clades 2 and 3 included three species each. In addition, five species belonged to clade 4 including the type species of *Russula* section *Foetentinae*; *R. foetens*. There were few clear morphological characteristics supporting each clade. These included size and shape of spore and ornamentation height. The spores of clades 1, 2 and 3 were more ellipsoidal and smaller than those of clade 4. In addition, spore ornamentation of members within clade 3 was small, quite irregular and wart-like shaped. The spores of clade 4 were much bigger in size and more globose than those of the other clades. Spore ornamentation of clade 4 had small, sharp ridges-often with a fragmentary reticulum and isolated warts (Fig. 2). *Russula grata* and *R. senecis* had the largest spores and significantly high spore ornamentation relative to other species in the section.

This paper reports an unrecorded species in Korea and a new taxon of *Russula* section *Foetentinae*. We provided a detailed description of a new species, *R. catillus* and a brief description of an unrecorded species in Korea including slight morphological variations, such as spore ornamentation height, compared with European descriptions.

3.1. *Taxonomy*

Russula insignis Quélet, Compt. Rend. Assoc. Franç. Avancem. Sci. 16(2): 588 (1888).

Specimens examined: KOREA, Seoul, Seoul National University, 37°27′26″ N, 126°56′59″ E, on the ground around oak trees, 27 Aug 2012, Hyun Lee, SFC20120827-02 (GenBank accession No. KX574693); on the ground of forest with *Quercus* spp.

Basidiomata medium-sized. Pileus 35–70 mm, greyish orange (6B3), to reddish grey (8B2) or brownish grey (8C2), becoming paler in age. Lamellae pale cream, close to crowded,

Table 2 – Macroscopic and microscopic features of species within *Russula* section *Foetentinae* in Korea.

Species	Pileus size (mm)	Stipe size (mm)	Spore size (μm)	Spore shape (Q value)	Spore ornamentation height (μm)	Basidia size (μm)	Pleurocystidia size (μm)
R. insignis[a]	40–80	30–70 × 10–15	6.6–7.3–7.6 × (5.6–)5.8–6.5–6.8(–7.2)	subglobose to broadly ellipsoidal (1.09–1.18–1.24)	up to 0.9	32.5–39.1 × 10.5–13.1	54.7–87.6 × 8.1–12.5
R. aff. *pectinatoides*	30–60	25–50 × 10–15	(6.2–)6.6–7.0–7.6 × (5.2–)5.4–5.6–6(–6.4)	broadly ellipsoidal (1.15–1.22–1.28)	up to 0.7	34–44.3 × 11.1–13.4	63–80.3 × 7.9–12.5
R. catillus[b]	40–90	40–100 × 10–25	5.6–6.2–6.6(–7.4) × 4.6–5.3–5.6(–5.8)	subglobose to broadly ellipsoidal (1.06–1.15–1.17)	up to 0.8	41.6–48.9 × 9.3–11.7	38.7–70.7 × 6.7–10.6
R. cf. *sororia*	–	–	6.8–7.2–7.6(–8.0) × 5.8–6.1–6.5(–7)	ellipsoidal (1.28–1.35–1.43)	up to 0.8	36–43.8 × 8.8–10.9	61.3–97.7 × 8.2–11.6
R. aff. *cerolens*	35–70	35–70 × 10–20	5–5.6–6(–6.2) × 4–4.4–5	broadly ellipsoidal to ellipsoidal (1.13–1.24–1.41)	up to 0.7	30–43.5 × 7.3–10	52.2–62 × 5.2–10
R. grata	–	–	(9.6–)9.7–9.9–10.6 × (7.6–)7.8–8.4–9.4	globose to subglobose (1.00–1.05–1.13)	up to 1.8	35–46.8 × 8.5–13.9	53.5–80.1 × 9.2–13.4
R. senecis	50–100	55–100 × 10–15	7.2–7.8–8.6(–9.4) × 6.2–7.2–7.6(–9.2)	globose to subglobose (1.02–1.10–1.15)	up to 1.6	45.1–51.1 × 8.3–13.1	51.9–79.9 × 7.5–9.4

[a] Species new to Korea.
[b] Species new to science.

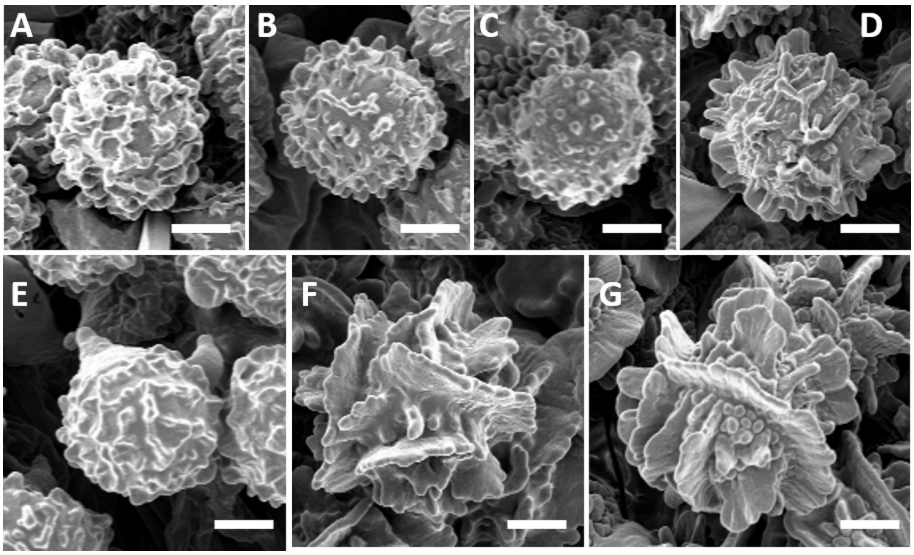

Fig. 2 – Basidiospores under SEM. A: *Russula* aff. *cerolens*. B: *R.* cf. *sororia*. C: *R. catillus*. D: *R. insignis*. E: *R.* aff. *pectinatoides*. F: *R. grata*. G: *R. senecis*. *Bars*: 2 μm.

lamellulae absent. Stipe 40–60 × 10–15 mm, cylindrical, becoming hollow in age.

Basidiospores 6.6–7.2–7.6 × (5.6–)5.8–6.4–6.8(–7.2) μm, Q = 1.09–1.18–1.24, subglobose to broadly ellipsoidal. Spore ornamentation up to 0.9 μm high. Basidia 32.5–39.1 × 10.5–13.4 μm, clavate. Cheilocystidia 30.8–54.0 × 4.6–9.9 μm, abundant. Pleurocystidia 37.5–70.5 × 7.1–10.1 μm, abundant. Pileipellis 180–240 μm thick, composed of apically tapered hyphae, 3–6 μm diam; dermatocystidia rare, containing some grayish contents, 2–3.5 μm diam.

Remarks: This species is distinguished by dark pileus color and by stipe orange-reddish discoloration with KOH. Spore ornamentation of Korean *Russula insignis* is taller than those of European species (Romagnesi 1985; Sarnari 1998).

Russula catillus H. Lee, M.S. Park & Y.W. Lim, sp. nov. Fig. 3. MycoBank no.: MB817870.

Diagnosis: Pileus hemispherical, then convex to applanate, in age center depressed; slightly viscous when wet; golden brown to brown at center, sometimes brownish grey. Lamellae adnate to adnexed, rarely forked; lamellulae absent. Stipe cylindrical, slightly rugulose longitudinally, hollow when mature. Basidiospores subglobose to broadly ellipsoidal, warty; ornamentation amyloid, mostly isolated. Basidia clavate, four spored. Pleurocystidia thin-walled, clavate, sometimes fusiform, apices acute. Pileipellis an ixotrichoderm; epipellis mostly erect hyphae, terminal cells cylindrical, with obtuse tips; dermatocystidia absent.

Specimens examined: KOREA, Seoul, Gwanak-gu, Seoul National University, 37°27′N, 126°57′E, alt. 104 m, 27 Aug 2012, H. Lee, M.S. Park, S.Y. Oh, Y.W. Lim (SFC20120827-01, holotype); same location, alt. 108 m, 18 Sep 2012, H. Lee, M.S. Park, Y.J. Min, Y.W. Lim (SFC20120918-04, paratype); same location, alt. 91 m, 19 Sep 2012, H. Lee, S.Y. Oh, W.D. Lee (SFC20120919-35, paratype); Seoul, Jongno-gu, Jongmyo Shrine, 37°34′N, 126°59′E, alt. 41 m, 12 Aug 2015, N.K. Kim, H. Y. Choi (SFC20150812-07, paratype); Chungcheongnam-do, Boryeong-si, Oseosan Natural Recreation Forest, 36°27′N, 126°40′E, alt. 314 m, 25 Jul 2012, H. Lee, P. E. Jung, Y. J. Min (SFC20150725-52, paratype).

Etymology: *catillus* (Latin), named so because it looks like a small bowl which holds soy sauce.

Basidiomata medium-sized. Pileus 40–90 mm diam, first hemispherical, then convex to applanate, in age depressed above the stipe, slightly viscous when wet, pale yellow to light yellow tinged, intermixed with light yellow fringe, golden brown (5D7) to brown (6E8) at center, sometimes brownish grey (6F8) when old and dry; margin rather thin, incurved first, horizontal to slightly funneled with age, prominently pectinate-striate 7–20 mm from the edge inwards, spilt in several pieces towards the center, yellowish white (2A2) to champagne (4A4), wax yellow (3B5) to khaki (4D5) when old and dry. Lamellae adnate to adnexed, 4–6 mm high, brittle, very rarely forked 1/3–1/2 from the stipe, sometimes slightly interveined near the pileus margin when wet, white to yellowish white, first white (-A1), pale yellow (2A3) when mature, sometimes stained light yellow to brownish orange tinge of pale yellow (2A3) topaz (5C5) when dry; edge even, slightly narrowing in height towards the edge, 8–11 pieces per cm in the pileus margin; lamellulae absent. Stipe central, 4–10 × 1–2.5 cm, cylindrical, slightly tapering towards the pileus, without annulus and volva, slightly rugulose longitudinally, whitish, partly turning pale grey, occasionally stained with tinge of topaz (5C5) to linoleum brown (5E7), hollow when mature. Context 2–3 mm thick from the lamellae attachment to the stipe, white (-A1) to milk white (1A2), unchanging or becoming slightly tinge with orange grey (5A2) very slowly.

Basidiospores [200/10/10] 5.6–6.2–6.6(–7.4) × 4.6–5.3–5.6(–5.8) μm, Q = 1.06–1.15–1.17, subglobose to broadly ellipsoidal, warty; ornamentation amyloid, composed of verrucous to conical warts up to 0.8 μm high, isolated, sometimes linked by fine lines to long ridges, partly reticulate.

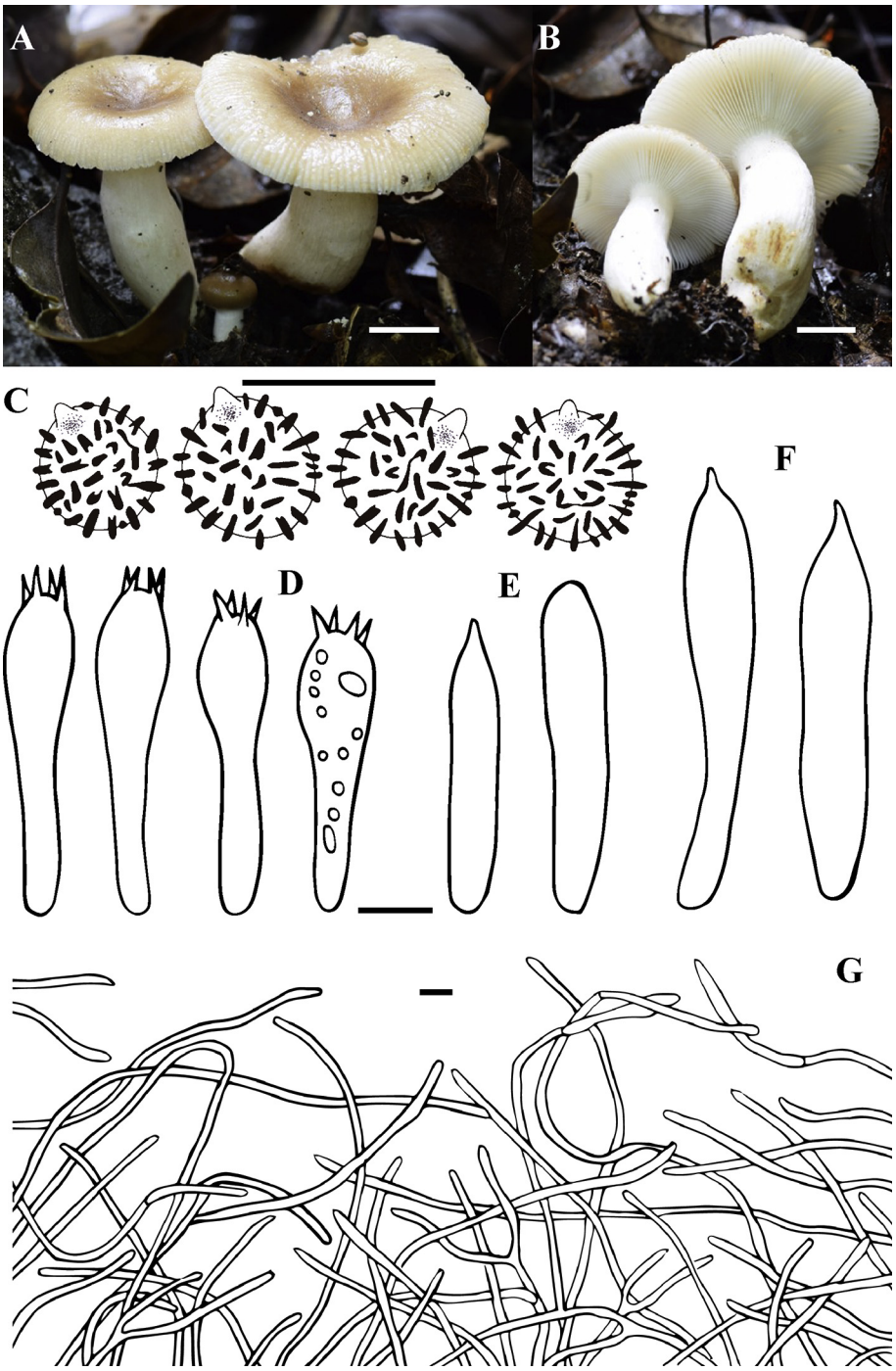

Fig. 3 – Morphological features of *Russula catillus* SFC20120827-01 (Holotype). A: Fruiting body. B: Lamellae and stipe. C: Basidiospores. D: Basidia. E: Pleurocystidia. F: Cheilocystidia. G: Pileipellis. *Bars*: A, B 2 cm; C–G 10 μm.

Basidia 41.6–48.9 × 9.3–11.7 μm, clavate, inflated in upper two to five, four-spored; sterigmata 3–5 μm, straight to slightly curved. Pleurocystidia 38.7–70.7 × 6.7–10.6 μm, originating from subhymenium, thin-walled, clavate, sometimes fusiform, apices acute, at times constricted, with a capitate adjunct. Cheilocystidia 43.2–59.7 × 6.9–11.2 μm; lamellar edge sterile. Pileipellis 110–210 μm thick, composed of an epipellis and a subpellis; epipellis an ixotrichoderm, 40–70 μm thick, mostly erect hyphae, terminal cells 41–72 × 3–7 μm, cylindrical, with obtuse tips; subapical cells sometimes branched; subpellis a cutis, 60–150 μm thick, composed of long, cylindrical hyphae 2–5 μm diam; dermatocystidia absent.

Habit and habitat: Mainly single or scattered in hardwood forests, with oaks, sometimes in mixed forests. Season: Jul to Sep.

Remarks: Closely related species of section *Foetentinae* can be distinguished from *R. catillus* as follows. *Russula catillus* can be distinguished from *R. amoenolens* from distinctively longer and thicker stipe and the size of pleurocystidia (Romagnesi

1985; Sarnari 1998). *Russula sororia* has conspicuously dark and greyish pilei compared to *R. catillus*. *Russula cerolens* has a reddish-brown stained stipe when bruised, whereas the stipe of *R. catillus* is not discolored (Shaffer 1972).

4. Discussion

We confirmed that seven species of *Russula* section *Foetentinae* exist in Korea. Only two species were previously recorded in Korea: *R. grata* and *R. senecis* (Lee and Cho 1976; Hong and Jung 1977), while *R. insignis* is previously unrecorded species in Korea. These species' macro- and microscopic features were nearly identical to previous descriptions (Imai 1938; Shaffer 1972; Romagnesi 1985; Sarnari 1998) (Table 2), however, spore ornamentation of Korean *Russula insignis* was taller than European descriptions (Table 2).

Recent studies have found that many Asian species share similar morphologies with different North American and European species (Le et al. 2007; Stubbe et al. 2008). In the case of *R. pectinatoides*, there was a high degree of geographic variation: an Asian subgroup, a North American subgroup and a European subgroup (Fig. 1). Further study of *R. pectinatoides* is needed to determine if this variation indicates a middle stage of speciation or a discrete species. This study provides evidence that Asian *R. cerolens* and North American *R. cerolens* are different species (Fig. 1). Accordingly, we renamed Asian *R. cerolens* as *R.* aff. *cerolens*. In the case of *R. sororia*, which was originally described from Europe, we confirmed Asian and North American taxa were different species. We were unable to compare Asian *R. sororia* to European *R. sororia* because of a lack of ITS sequences of European *R. sororia* in open databases. Thus, the name of Asian *R. sororia* remains *R.* cf. *sororia*.

Russula catillus had previously been identified using morphological characters as either *R. foetens*, *R. pectinata* or *R. sororia* based on European or North American descriptions; however, *R. catillus* can be distinguished from those species by their noticeably small spores and ITS sequence analysis.

Russula foetens and *R. pectinata*, two species previously recorded in Korea, were not found in this study. Three specimens that had been previously identified as *R. foetens* and *R. pectinata* were correctly identified as *R. catillus* and *R. grata* (Table 1). Additional study is needed in order to confirm the existence of *R. foetens* and *R. pectinata* in Korea. The length and width of the pleurocystidia seemed to be inappropriate keys for species-level identification because of small differences within each species. The patterns observed in the ITS tree suggests that spore ornamentation shape and height are good characters to distinguish species in this section. Overall, morphology based delimitation resulted in highly inaccurate identification of *Russula* species in the section *Foetentinae* (Table 1). When examining cases of misidentification, recurring errors are associated with the similar morphology among species of *Russula* section *Foetentinae*. Indeed, experts in this field are often confused when identifying species of *Foetentinae* species based on macro- and micro-morphology. DNA sequence analyses with morphological verification can reduce these misidentifications (Park et al. 2013; Van de Putte et al. 2016), and using this methodology we successfully identified members of *Russula* section *Foetentinae*.

ITS phylogenetic analysis revealed that all the species in *Russula* section *Foetentinae* formed a monophyletic group with high statistical support (Fig. 1). These taxa were divided into four clades, two of which (clades 1 and 3) had strong bootstrap support (100) and two (clades 2 and 4) that had moderate or weak bootstrap support (Fig. 1). Clade 1 shared small, mostly ellipsoidal spores (Shaffer 1972; Romagnesi 1985; Sarnari 1998). The species in Clade 2 were distinguished by thick stipes and comparatively hard fruiting bodies and nearly all species had firm and clavate stipes (Shaffer 1972; Romagnesi 1985; Sarnari 1998; Kränzlin 2005). Clade 3 shared more globose spores than those of clades 1 and 2, as well as wart-like spore ornamentation (Shaffer 1972; Romagnesi 1985; Sarnari 1998; Kränzlin 2005). The largest and most globose spores (Imai 1938; Shaffer 1972; Romagnesi 1985; Sarnari 1998; Kränzlin 2005) were found in Clade 4 and the species in this clade were easily distinguished from the species of other clades because of these distinctive characteristics.

Some general phenotypic patterns emerged from the ITS phylogenetic reconstruction of the *Russula* species examined in this study. While the evolutionary relationships among the four clades was not clarified by the ITS tree, phenotypic characters support the topology presented in Fig. 1. Clades 1 and 2 both have small spores relative to those of clades 3 and 4, and clades 1 and 2 share ellipsoidal spore shapes (high Q values) while those of clades 3 and 4 share the round spore phenotype (low Q values). There was a general trend for spores to increase in size and transition from ellipsoid to spherical descending down the tree from clade 1 (small spore and ellipsoid) to clade 4 (large spore and round shape). This difference in size of spores is obvious, with that of *R. grata* (clade 4) representing the largest and that of *R. cerolens* (clade 1) the smallest. Interestingly, we found that species that have smaller spores also have more ellipsoidal spores, with the exception of *R. sororia* (Fig. 2; Table 2). The largest species, *R. grata*, has most globose spores and the smallest species, *R. cerolens*, has the most ellipsoidal spores. It is unclear which shape represents the ancestral state, therefore additional species and out group comparisons are needed to confirm which characters are ancestral in this section.

ITS sequence enabled identification of *Russula* section *Foetentinae* to the species level and described some important phylogenetic relationships with strong statistical support; however, the basal relationships of each clade remain uncertain. In order to resolve this problem, further studies are needed using additional DNA markers as well as additional species of *Russula* section *Foetentinae*.

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