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RESEARCH ARTICLE

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Two New Species of *Laccaria* (Agaricales, Basidiomycota) from Korea

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ABSTRACT

Species of *Laccaria* (Hydnangiaceae, Agaricales, and Basidiomycota) are well-known ectomycorrhizal symbionts of a broad range of hosts. *Laccaria* species are characterized by brown, orange, or purple colored basidiocarps, and globose or oblong, echinulate and multinucleate basidiospores. While some *Laccaria* species are easily identified at the species level using only the morphological characteristics, others are hard to distinguish at the species level due to small differences in morphology. Heretofore, ten *Laccaria* species have been reported in Korea. While studying the fungal diversity in the National Parks of Korea, two new *Laccaria* species were discovered. Species identification was done based on molecular analyses (ITS, 28S rDNA, *rpb*2, and *tef*1), then were confirmed by their corresponding morphologies. The two newly discovered *Laccaria* species are proposed here as *Laccaria macrobasidia* and *Laccaria griseolilacina*. The unique morphological characters of *L. macrobasidia* that distinguish it from its closely related species are orange-brown colored basidiocarp, long basidia and the absence of cheilocystidia. *L. griseolilacina* is characterized by a light grayish lavender-colored pileus and the absence of cheilocystidia. Two new species are described and illustrated in the present paper.

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1. Introduction

The genus *Laccaria* (Hydnangiaceae, Agaricales, and Basidiomycota) is a well-known ectomycorrhizal symbiont with a broad range of conifer and angiosperm hosts [1–4]. *Laccaria* is characterized by a brown, orange, or purple basidiocarp, and globose to oblong, echinulate, and multinucleate basidiospores [5–7]. *Laccaria* has a substantial number of species. Over 80 species have been recognized in the Index Fungorum website (www.indexfungorum.org, accessed on 25 March 2020). *Laccaria* species are observed in temperate and tropical areas, and they play an imperative role in alpine ecosystems [2,4,8–10].

While identification of some species of *Laccaria* is possible by their distinct morphological characters [2], accurate identification of most species using morphological traits alone is difficult because of insignificant morphological differences between them [2,11]. Molecular analysis has increased the accuracy of fungal identification [12–14]. Identification of *Laccaria* species based on molecular analysis has become possible as a result of the accumulation of sequence data in GenBank through active phylogenetic studies of *Laccaria* using the internal transcribed spacer (ITS) region, nuclear 28S rDNA (28S), RNA polymerase II subunit 2 (*rpb2*), and translation elongation factor 1-α (*tef1*) [4,10,11,15,16]. The accumulated sequence data also provide an advantage of promptly discovering new or rare species.

Previously we reported the presence of 10 *Laccaria* species in Korea based on sequence data [17]. During the survey of macrofungi in Korea National Parks, two unknown *Laccaria* species were newly discovered. Based on their morphological features and multigene analyses (ITS, 28S, *rpb2*, and *tef1*), we confirmed that the two species have yet been reported. Therefore, we propose names *Laccaria macrobasidia* sp. nov. and *Laccaria griseolilacina*. sp. nov., and present detailed descriptions and illustrations of these two new species.

2. Materials and methods

2.1. Sampling and morphological observations

The two new *Laccaria* basidiocarps were both collected in temperate forests. One (SFC20170822-59)

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was collected at Gayasan National Park, predominantly composed of *Pinus* and *Abies* species, and the other (SFC20190919-48) was collected at Taebaeksan National Park, in a *Larix* tree. The mushroom collection took place upon the permission of the Ministry of Environment.

Gross morphological features of fresh basidiocarps were compared with previous studies [7,17,18] and photographic illustrations (http://archive.fieldmuseum.org). The color names and alphanumeric codes follow the Methuen Handbook of Color [19]. Sections of dried basidiocarps were rehydrated in 3% KOH, subsequently stained in Congo red solution and Melzer's reagent [20], and then were observed under an 80i compound light microscope (Nikon, Tokyo, Japan) at either 400× or 1000× magnification. At least 40 basidiospores, 40 basidia, and 10 cystidia were measured per specimen. Measurements, rounded to the nearest integer or half a micrometer, indicate minimum to maximum length or width, excluding individual extremes or outliers, with the mean values provided in italics between the range sizes. For scanning electron microscope (SEM) imaging of basidiospores, dried pieces of lamellae with basidiospores were attached to aluminum stubs using double-sided adhesive tape, coated with platinum in a sputter coater (BalTec/SCD 005; Leica Microsystems, Wetzlar, Germany), and then were examined with SEM at 10000× magnification (SUPRA 55VP; Carl Zeiss, Oberkochen, Germany). Basidiospore size and echinulae were measured using SEM to the nearest 0.1 μm. "Q" refers to the length/width ratio of an individual basidiospore.

2.2. DNA extraction, PCR, and sequencing

A small piece of fungal tissue from each dried specimen was placed in a 1.5-mL tube containing cetyl trimethyl ammonium bromide (CTAB) buffer, and was grounded with a plastic pestle. Genomic DNA was extracted according to the modified CTAB extraction protocol [21].

The ITS region was amplified using primers ITS1F and ITS4B [13], the 28S region using LROR [22] and LR5 [23], *rpb2* using fRPB2-5f [24] and fRPB2-8.2R [25], and *tef1* using EF1-983F or EF1-altertative-3f and EF1-1567R or EF1-alternative-3r [26,27]. PCR amplifications were performed on a thermal cycler (C1000TM; Bio-Rad, Richmond, CA) using the AccuPower PCR premix (Bioneer, Daejeon, South Korea), following the instructions outlined in Park et al. [28]. PCR products were visualized on a 1% agarose gel and were purified using the Expin PCR purification kit (GeneAll Biotechnology, Seoul, South Korea). Sanger sequencing was performed at Macrogen (Seoul) on an automated DNA sequencer (ABI Prism 3730XL analyzer; Applied Biosystems, Foster City, CA) using the aforementioned PCR primers.

DNA sequences were proofread using MEGA version 5 [29] and then were deposited in GenBank. All sequences of four gene loci were individually aligned with *Laccaria* reference sequences from GenBank and UNITE using MAFFT [30]. Because *Laccaria* species in Southern Hemisphere form a grade and are clearly separate from the Northern Hemisphere lineages [16], we used *Laccaria* reference sequences only from the Northern Hemisphere lineages. Alignments were also checked thoroughly, and ambiguous positions were adjusted manually. Gaps were inserted in some sequences due to missing data.

Both ITS and multigene analyses were performed based on a maximum likelihood (ML) analysis in RAxML version 8.0.2 [31] implemented on the CIPRES Web portal [32], using a GTR-GAMMA model with 1000 bootstrap replicates [33]. In the multigene analysis, alignments of four genes were concatenated and then were partitioned by gene regions, codon positions, and intron regions. *Laccaria acanthospora*, a basal taxon of the Northern Hemisphere [16], was chosen as the outgroup in both ITS and multigene analyses.

3. Results

In total, eight new sequences of the ITS, 28S, *rpb2*, and *tef1* were generated from the two specimens (SFC20170822-59 and SFC20190919-48) and were deposited in GenBank under accession numbers MT322981–MT322984 and MT333266–MT333269 (see Taxonomy for a detailed information). We compared ITS sequences of the two species with 96 ITS sequences of 40 other *Laccaria* species reported in the Northern Hemisphere. For multigene analysis, 59 multigene sequences of 20 *Laccaria* species were downloaded and used. The adjusted alignments consisted of 662 bases for ITS, 902 for 28S, 589 for *rpb2*, and 604 for *tef1*. Phylogenetic analysis based on ITS and multigene showed similar results (Figures 1 and 2). One of the newly discovered *Laccaria* species (SFC20170822-59) formed a sister clade with *Laccaria laccata* (from Portugal, USA, and Russia), but was clearly demarcated from the *L. laccata* clade. The other *Laccaria* species (SFC20190919-48) was grouped with *Laccaria* sp. (KU685613, Japan) and *Laccaria* sp. (KM067827, USA) with high sequence similarities (<0.02% dissimilarity). These two references sequences of *Laccaria* sp. were indicated as *Laccaria* sp. JP4 by Wilson et al. [16] but have not been given a legitimate name.

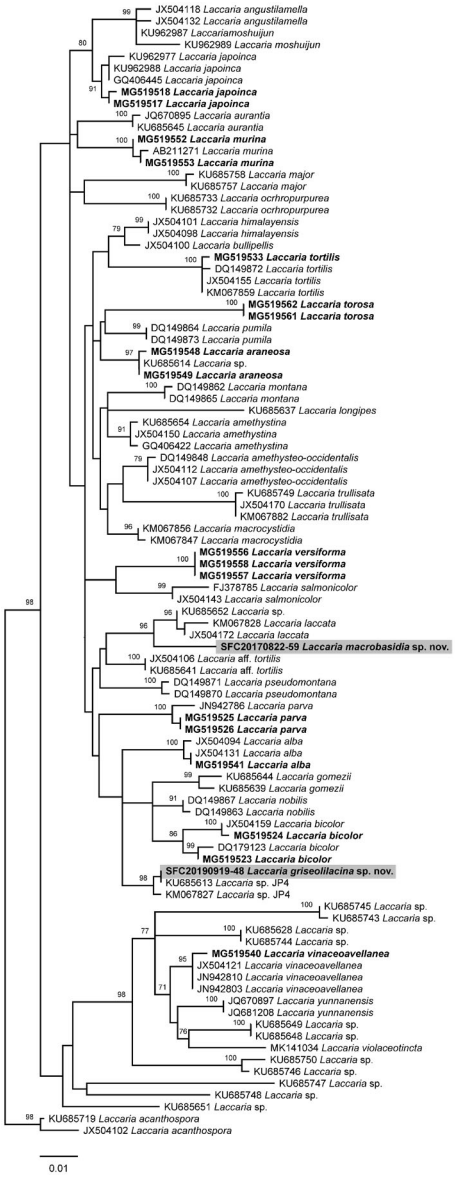

Figure 1. Phylogeny of *Laccaria* species based on ML analysis of ITS sequences. Bootstrap values >70% are stated at the nodes. The scale bar represents the number of expected nucleotide substitutions per site. *Laccaria* species reported in Korea are represented in bold and new species from this study are accented in gray shades.

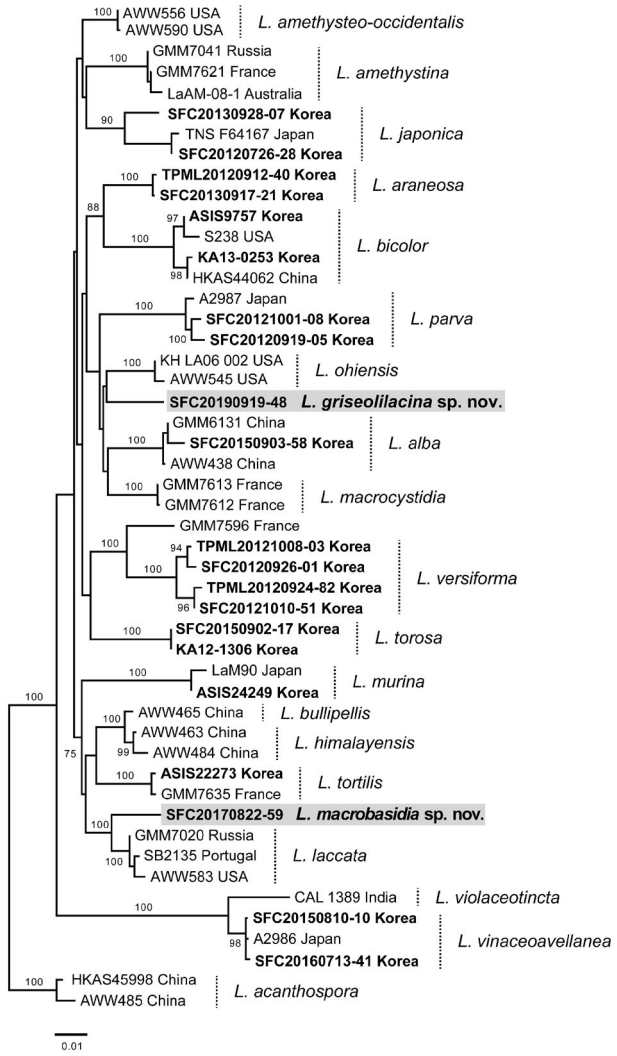

Figure 2. Phylogeny of *Laccaria* species based on ML analysis of a concatenated data set of ITS, 28S, *rpb*2, and *tef*1 sequences. Bootstrap values >70% are stated at the nodes. The scale bar represents the number of expected nucleotide substitutions per site. *Laccaria* species reported in Korea are represented in bold and new species from this study are accented in gray shades.

When comparing the size of basidia and basidiospore with other previously reported *Laccaria* species in Korea, the basidia of *Laccaria* sp. (SFC20170822-59) was the longest (mean value of 66 μm) but the size of the basidiospores was smaller than those of *L. tortilis*. In the case of *Laccaria* sp. (SFC20170822-48), the size of both basidia and basidiospore did not differ considerably from those of other species and had medium-sized basidia and basidiospore (Figure 3).

4. Taxonomy

L. macrobasidia H.J. Cho & Y.W. Lim, sp. nov. (Figure 4(A–D)).

MycoBank: MB835319

Typification: REPUBLIC OF KOREA. GYEONGSANGNAM-DO: Hapcheon-gun, Gayasan National Park, N 35°48′01″ E 128°05′47″, 626 m, 22 Aug 2017, H.J. Cho & K.H. Park (holotype SFC20170822-59). GenBank: ITS = MT322982; 28S = MT322984; *rpb*2 = MT333267; *tef*1 = MT333268.

Etymology: macro (Latin) means long. It is specified to the large size of the basidia.

Diagnosis: *L. macrobasidia* is characterized by an orange-brown colored pileus, long basidia, and the absence of cheilocystidia.

Description: Basidiocarps small. Pileus 15–45 mm diam., convex to plane, with a slight central depression, sometimes with papillae; orange-brown (5B5–7) or light-brown (5A5), hygrophanous, fading to pale orange buff (5C5); pectinate-striate inwards from the edge; margin involute to decurved, entire, finely crenate or wavy. Lamellae adnate to subdecurrent, thick, distant, light-brown (5A5), number

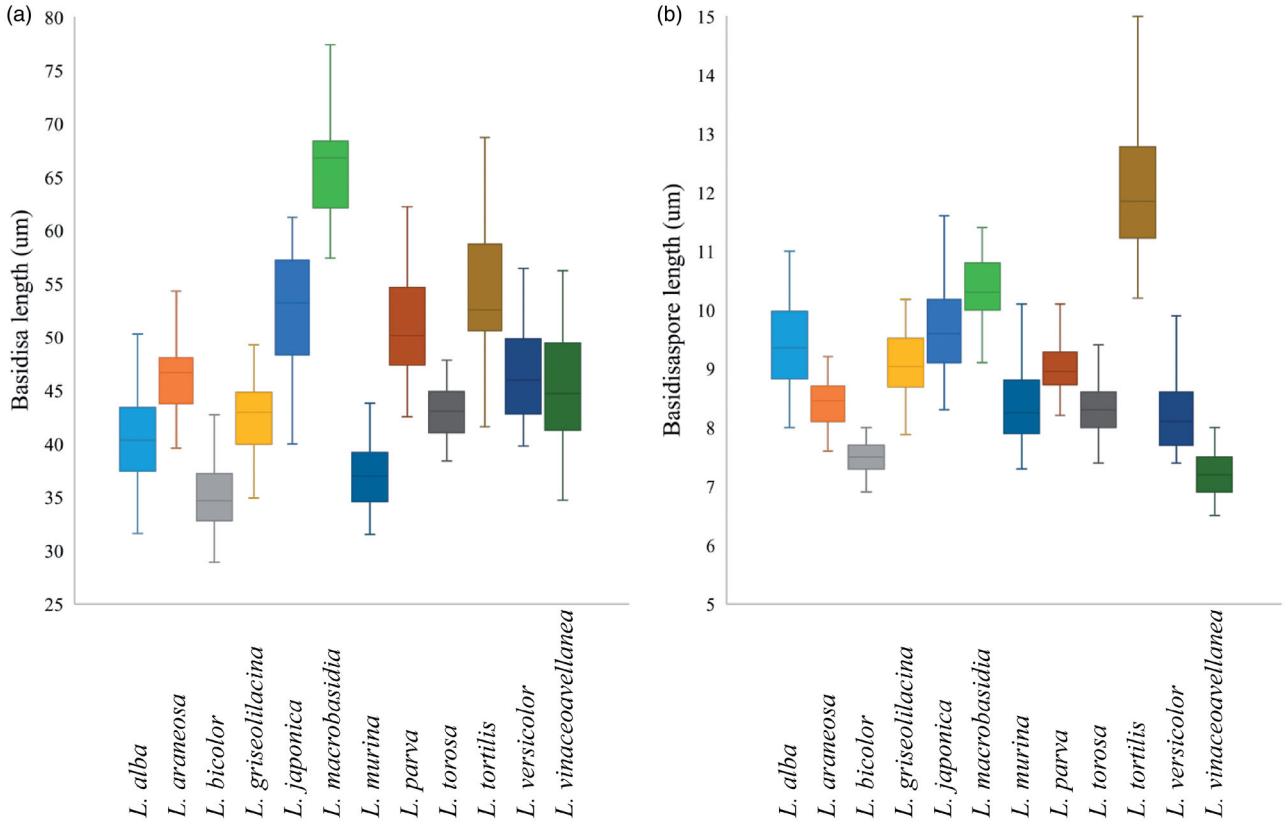

Figure 3. Length range of basidia (a) and basidiospore (b) of Korean *Laccaria*.

of complete lamellae (L) 20–28, number of lamellulae (l) 1–2. Stipe 10–50 × 0.5–1.0 mm, tapering toward the apex, solid, becoming hollow with age, minutely fibrillose the entire length; concolorous with pileus, sometimes darker. Context thin, concolorous with stipe.

Basidiospores 8.7–10.4–11.4 × 7.9–9.2–10.3 μm, $Q = 1.00–1.13–1.3$, globose to subglobose with spines (1.5–2.0 μm in length, 0.6–0.8 μm wide at base), hyaline. Basidia 52.0–66.2–80.4 × 11.2–12.8–14.6 μm, 4-spored, clavate, mature sterigmata up to 8–12 μm long, hyaline. Cheilocystidia absent. Pleurocystidia 19.1–27.4–40.1 × 4.3– *5.2*–6.8 μm, filamentous, hyaline. Stipitipellis composed of parallel, cylindrical, repent, hyaline hyphae. Caulocystidia absent. Pileipellis composed of interwoven, cylindrical, mostly repent, hyaline hyphae. Lamellar trama of subparallel to interwoven, cylindrical, repent, hyaline hyphae; subhymenium undifferentiated. All tissues inamyloid, clamp connections present.

Habitat and phenology: Scattered on ground in temperate forests dominated by *Pinus densiflora* and *Abies holophylla*, August.

Notes: *L. macrobasidia* is morphologically similar to *L. parva* and *L. torosa* in basidiocarp color and size. However, *L. macrobasidia* has longer basidia (52–80 μm) than those of *L. parva* (44–56 μm) and *L. torosa* (39–47 μm) [17]. *L. macrobasidia* lacks cheilocystidia while *L. parva* and *L. torosa* have filamentous to slenderly clavate cheilocystidia [17].

L. griseolilacina. H.J. Cho & Y.W. Lim, sp. nov. (Figure 4(E–G)).

MycoBank: MB835320

Typification: REPUBLIC OF KOREA. GANGWON-DO: Taebaek-si, Taebaeksan National Park, N 37°07′11″ E 128°54′30″, 907 m, 19 Sep 2019, M.S. Park & J.H. Park & S.N. Yoo (holotype SFC20190919-48). GenBank: ITS = MT322981; 28S = MT322983; *rpb2* = MT333266; *tef1* = MT333269.

Etymology: *griseolilacina* means grayish lilac or lavender (Latin), which refers to the color of the basidiocarp.

Diagnosis: *L. griseolilacina* is characterized by a light grayish lavender-colored pileus and the absence of cheilocystidia.

Description: Basidiocarps small. Pileus 20–35 mm diam., broadly convex to flat; light grayish lavender (15D3, 15C3) or orange-brown (6C5–7), hygrophanous, fading to pale orange buff (5A3); slightly pectinate-striate inwards from the edge when dried; margin involute to decurved, entire, sometimes crenate. Lamellae sinuate, thick, distant, concolorous with pileus, powdery, light grayish lavender (15D3) when fresh, orange-brown (5B5–7, 6C5–7) when dried, $L = 20–28$, $l = 1–2$. Stipe 40–60 × 0.3–0.8 mm, slightly tapering toward the apex, solid, becoming hollow in age, minutely fibrillose over entire length; concolorous with pileus, sometimes darker. Context thin, concolorous with stipe.

Figure 4. Images of *Laccaria macrobasidia* (A–D) and *Laccaria griseolilacina* (E–H). A & E, basidiocarps in their natural habitats; B & F, scanning electron micrograph (SEM) of basidiospores; C & G, basidia; D & H, pleurocystidia; Scale bars: A, $E = 10$ mm; B, $F = 2$ μm; C–D, G–$H = 2$ μm.

Basidiospores 8.0–9.2–10.8 × 8.2–9.5–10.9 μm, Q = 0.94–0.97–1.03, globose to subglobose with spines (1.3–1.5 μm in length, 0.7–0.9 μm wide at base), hyaline. Basidia 36.6–42.4–48.7 × 10.8–13.4–15.5 μm, 4-spored, clavate, mature sterigmata up to 6–10 μm long, hyaline. Cheilocystidia absent. Pleurocystidia 20.3–24.4–31.0 × 3.5–5.2–8.0 μm, filamentous, hyaline. Stipitipellis composed of parallel, cylindrical, repent, hyaline hyphae. Caulocystidia absent. Pileipellis composed of interwoven, cylindrical, mostly repent, hyaline. Lamellar trama of subparallel to interwoven, cylindrical, repent, hyaline hyphae; subhymenium undifferentiated. All tissues inamyloid, clamp connections present.

Habitat and phenology: Scattered on ground in temperate forests dominated by *Larix kaempferi*, September.

Notes: *L. griseolilacina* is morphologically similar to species with lavender-purple colored basidiocarps such as *L. japonica* and *L. vinaceoavellanea*, in which all of them are found in Korea. They can be differentiated by the color of basidiocarps and the presence of cheilocysitida. *L. griseolilacina* has a grayish lavender colored basidiocarp, and has no cheilocystidia while *L. japonica* and *L. vinaceoavellanea* have light purple to deep purple colored basidiocarps and cheilocystidia.

Key to Korean *Laccaria* species

1. Basidiocarps gray or purple 2
1′. Basidiocarps buff or orange-brown 5
2. Basidiocarps light gray to dark gray, basidia with 2-spored ..... *L. murina*
2′. Basidiocarps lavender-purple, basidia with 4-spored 3
3. Basidiocarps light grayish lavender, basidia with 4-spored ..... *L. griseolilacina*
3′. Basidiocarps lavender to deep purple, basidia with 4-spored 4

4. Pileus large at maturity (40–60 mm wide), basidiocarps lavender purple when fresh, grayish buff in age or dry; basidiospores globose ($Q = 1$) ..... *L. vinaceoavellanea*
4′. Pileus small at maturity (10–30 mm wide), basidiocarps deep purple when fresh, grayish brown in age or when dry; basidiospores subglobose ($Q > 1$) ..... *L. japonica*
5. Basidia with 2-spored ..... *L. tortilis*
5′. Basidia with 4-spored 6
6. Lamellae pinkish or light vinaceous 7
6′. Lamellae orange-brown 8
7. Basidia 32–42 µm long; basidiospores on average 7.5 × 7.4 µm ..... *L. bicolor*
7′. Basidia 41–56 µm long; basidiospores on average 8.2 × 8.3 µm ..... *L. versiforma*
8. Caulocystidia present 9
8′. Caulocystidia absent 10
9. Cheilocystidia present, pleurocystidia present ..... *L. torosa*
9′. Cheilocystidia present, pleurocystidia lacking ..... *L. alba*
10. Cheilocystidia present, pleurocystidia present ..... *L. parva*
10′. Cheilocystidia absent 11
11. Pleurocystidia present, basidia 52–80 µm long; basidiospores on average 10.4 × 9.2 µm ..... *L. macrobasidia*
11′. Pleurocystidia absent, basidia 42–52 µm long; basidiospores on average 8.4 × 8.2 µm ..... *L. araneosa*

4. Discussion

The discovery of new species in *Laccaria* is rapidly increasing by the means of molecular analyses [4,11,15–17,34,35] because before the accumulation of molecular data, only a few morphological and systematically informative characters of *Laccaria* species were available, and the color of the fruiting body varied in a broad range [2]. Therefore, the application of molecular methods is necessary for the classification and species identification of *Laccaria*. In this study, we determined two *Laccaria* species as new, initially through sequence analysis and then verified them anew by assessing the morphological features.

L. macrobasidia is characterized by an orange-brown colored basidiocarp with pecinate-striate pattern starting from the edge, thick and light-brown lamellae, brown and fibrillose stipe, globose to subglobose and echinulate basidiospore, and long basidia (52–80 µm). Macroscopic features of *L. macrobasidia* are similar to those of *L. parva*, *L. torosa*, and *Laccaria tortillis*, which are recently reported species with brown colored basidiocarps in Korea [17]. However, multi-gene phylogenetic analysis supported that *L. macrobasidia* forms a clearly distinct clade from them. A clear difference between species can be seen by comparing their microscopic characters. While *L. parva* and *L. torosa* have both pleurocystidia and cheilocystidia, *L. macrobasidia* only has pleurocystidia. Also, *L. macrobasidia* has a slightly bigger basidiospore (8.7–*10.4*–11.4 × 7.9–*9.2*–10.3 µm) than *L. parva* (8.0–*9.0*–10.0 × 8.5–*9.2*–10.0 µm) and *L. torosa* (8.0–*8.3*–9.0 × 8.0–*8.6*–9.5 µm) [17]. *L. tortillis* has by far been recognized to have the largest basidia and basidiospores in Korea. However, *L. macrobasidia* has larger basidia than *L. tortillis*, although the size of its basidiospore is still smaller (Figure 3).

Both the ITS and multi-gene analyses showed that *L. macrobasidia* forms a sister clade to "*L. laccata*", which originated from USA (KM067828), Russia (KU685652), and Portugal (JX504172) (Figure 2). *L. laccata* (Scopoli: Fries) Cooke var. *laccata* was originally distributed in Europe and USA, and its basidia are reported to be 27–55.5 × 6–13.5 µm in size [7,36]. *L. macrobasidia* appears to be phylogenetically close to the "*L. laccata*" clade in the phylogenetic tree, but the size of basidia makes these two species distinct.

L. griseolilacina is characterized by a grayish lavender-colored basidiocarp with slightly pecinate-striate pattern starting from the edge, thick and light grayish lavender lamellae concolorous with pileus, fibrillose stipe, globose to subglobose and echinulate basidiospore, and basidia (36–48 µm). Macroscopic features of *L. griseolilacina* are similar to those of *Laccaria* species with lavender colored basidiocarps, namely *L. amethystina*, *L. japonica*, *L. moshuijun*, and *L. vinaceoavellanea*. *L. griseolilacina* has a light grayish lavender colored basidiocarp while *L. amethystina* and *L. japonica* have deep purple colored basidiocarps when fresh [37]. *L. griseolilacina* and *L. vinaceoavellanea* have similar colors of basidiocarps when they are young. However, *L. griseolilacina* has a smaller size of basidiocarp than *L. vinaceoavellanea* (30–90 mm wide) [38]. *L. amethystina* is only distributed in Europe and not in Asia [37], so it is regionally distinct from *L. griseolilacina*. *L. griseolilacina* can be differentiated from *L. moshuijun*, which has a variable shape of cheilocystidia (clavate, flexuose dichotomy, or trichotomy), by the lack of cheilocystidia [37]. *L. moshuijun* has been reported from China but has not been reported from Korea. The fact that *L. griseolilacina* is a new species is well supported by molecular analysis (Figure 2).

Species in *Laccaria* are very similar in morphology, so ecological information such as host plant species and habitat can provide important clues for identification. Several species of *Laccaria* are known

as generalists with a broad host range [1,39–41], while other *Laccaria* species associate with a single or a limited group of hosts [2]. For example, *Laccaria amethystina* was reported to interact with a wide variety of hosts in forests [3] and in the *in vitro* test [42]. In contrast, some of the Southern Hemisphere *Laccaria* species were only found in *Nothofagus cunninghamii* forests [43], and the habitat of *Laccaria maritima* was restricted to sand dunes in Northern Europe [2,44]. Both *L. macrobasidia* and *L. griseolilacina* were collected from conifer forests. Since there is only one specimen of each species, however, it is too early to determine their host. Further studies are needed to document their fungal-host associations.

We found two new species based on a molecular-based approach, confirming that there are a total of 12 *Laccaria* species in Korea. Many previously collected specimens of the 12 *Laccaria* species have no associated information regarding their host plants and their distribution in Korea. Therefore, further studies on host specificity and distribution are required to elucidate the ecological role of *Laccaria* species in Korean forests.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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