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First Report of Eight Milkcap Species Belonging to *Lactarius* and *Lactifluus* in Korea

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ABSTRACT

Lactarius and *Lactifluus* are milkcaps that are characterized by the secretion of latex. These two genera are part of a globally distributed cosmopolitan group of ectomycorrhizal fungi that is an important food resource in various ecosystems. Recently, the taxonomy of *Lactarius* and *Lactifluus* has been revised based on molecular phylogenetics. Despite the importance of these taxa, Korean species of both genera are poorly understood. In an effort to describe milkcap species that are indigenous to Korea, a long-term study has been initiated. During a recent survey, eight species of milkcaps that were previously unrecorded in Korea were detected based on morphological observation and sequence analysis of the internal transcribed spacer region: five *Lactarius* species (*Lactarius atomarginatus*, *L. austrotorminosus*, *L. kesiyae*, *L. tabidus*, and *L. vietus*) and three *Lactifluus* species (*Lactifluus acicularis*, *Lf. pilosus*, and *Lf. pinguis*). Detailed morphological descriptions and phylogenetic relationships of these species are provided in this article.

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Lactarius; *Lactifluus*;
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1. Introduction

The term “milkcap” describes a genus of mushroom-forming fungi called *Lactarius* Pers. because of its latex production, and over 600 milkcap species have been reported worldwide [1]. *Lactarius* was one of the two major groups treated in the family Russulaceae (together with a larger genus called *Russula* Pers.) [2,3]. This genus is easily differentiated from its sister genus by the presence of latex and lactifer networks in subhymenium [4,5]. In lamellar trama, most *Lactarius* species have less complex sphaerocytes than those in *Russula* species [5]. *Lactarius* plays a critical ecological role in terrestrial ecosystems through ectomycorrhizal symbiotic relationships with various plants [6,7]. The basidiomata of many milkcap species as an important nutrient source for insects [8] and other animals including humans [9,10]. Some species of *Lactarius* have been studied in order to investigate the mechanisms for natural rubber synthesis [11].

In the last decade, the generic landscape of the family Russulaceae has changed. Four phylogenetic lineages have been found in this family and milkcap species appear to have evolved in three lineages [12]. Consequently, two additional genera have been defined: *Multifurca* Buyck & V. Hofst. and *Lactifluus* (Pers.) Roussel. Two species previously

placed in the *Lactarius* were actually shown to be representatives of the *Multifurca*: *M. furcata* [12] and *M. stenophylla* [13]. Species in another clade within the genus *Lactarius* (approximately 20% of species) were transferred to the genus *Lactifluus* [14–16]. The majority of species (approximately 80% of species) remain in the larger clade, *Lactarius* [17]. Although *Lactarius* can clearly be distinguished from *Multifurca* and *Lactifluus* using molecular sequence data, there are no absolute morphological characters to distinguish species in these genera. Some morphological characters seem common in all or at least two of the three genera. For example, the pileal surface of *Lactarius* can be viscid or lubricous when moist, often with distinctive zonation, and bearded at the pileus margins. Scrobiculate caps and stipes only occur in *Lactarius*. Many *Lactifluus* species generally have pileipellis and stipellis with thick-walled elements, as well as lamprocystidia and only few *Lactarius* species have these characteristics. A hymenophoral trama consists of sphaerocytes (as in the genus *Russula*) is general in the genus *Lactifluus* but is rarely found in *Lactarius* [18].

Although milkcap species are easy to recognize at the genus level because of their latex exuding fruiting bodies, identification at the species level is difficult due to extensive morphological variations and highly

similar morphological features among closely related species. Recently, DNA sequence analysis has improved the precision of species identification in milkcaps. The internal transcribed spacer (ITS) region [19] has been widely used in the identification of new species [20,21] and to delimitate among closely related species in Korea [21,22]. Recently, The National Institute of Biological Resources has organized a project that investigates diversity of Korean indigenous *Lactarius* species (NIBR, <http://www.nibr.go.kr>).

In this study, we were able to identify eight unrecorded milkcap species based on ITS sequence analysis. Here, we provide detailed morphological descriptions and phylogenetic support for each species.

2. Materials and methods

2.1. Sampling and morphological study

A total of 20 specimens were used in this study (Table 1). These samples have obtained from three

Table 1. Sequenced specimens used in this study, with GenBank accession numbers for the ITS.

Species	Voucher collection (Type)	Country	Accession No.
Genus <i>Lactarius</i>			
<i>Lactarius atromarginatus</i>	TMI18877	Japan	JQ446124
<i>Lactarius atromarginatus</i>	X. H. WANG 1983	China	JQ446086
<i>Lactarius atromarginatus</i>	LTH390	Thailand	EF560677
<i>Lactarius atromarginatus</i> ^a	HCCN369G	Korea	MG551722
<i>Lactarius atromarginatus</i> ^a	TPML110825-070	Korea	MG551723
<i>Lactarius austrotorminosus</i>	LTH111	Thailand	EF141531
<i>Lactarius austrotorminosus</i>	LTH130 (Holotype)	Thailand	EF141532
<i>Lactarius austrotorminosus</i>	LTH191	Thailand	EF141533
<i>Lactarius austrotorminosus</i> ^a	HCCN19723	Korea	MG551724
<i>Lactarius austrotorminosus</i> ^a	SFC20120907-14	Korea	MG551725
<i>Lactarius austrotorminosus</i> ^a	SFC20120907-18	Korea	MG551726
<i>Lactarius austrozonarius</i>	LTH129 (Holotype)	Thailand	EF141535
<i>Lactarius austrozonarius</i>	LTH299	Thailand	EF141536
<i>Lactarius cucurbitoides</i> ^a	SFC20130719-110 (Holotype)	Korea	KM371260
<i>Lactarius cucurbitoides</i> ^a	SFC20110818-21	Korea	KM371259
<i>Lactarius fulvissimus</i>	JN2001-088	Belgium	KT165245
<i>Lactarius fulvissimus</i>	WR2181	Belgium	KT165244
<i>Lactarius kesiyae</i>	KW224	Thailand	KR025615
<i>Lactarius kesiyae</i>	KW032 (Holotype)	Vietnam	KR025619
<i>Lactarius kesiyae</i> ^a	SFC20140702-11	Korea	MG551727
<i>Lactarius kesiyae</i> ^a	SFC20140702-13	Korea	MG551728
<i>Lactarius kesiyae</i> ^a	SFC20150701-62	Korea	MG551729
<i>Lactarius kesiyae</i> ^a	SFC20150701-99	Korea	MG551730
<i>Lactarius tabidus</i>	AV13-001	Canada	KT165307
<i>Lactarius tabidus</i>	noordeloos9386	Sweden	KT165310
<i>Lactarius tabidus</i> ^a	TPML110928-062	Korea	MG551731
<i>Lactarius torminosus</i>	RW3183	Czech Republic	KF133281
<i>Lactarius torminosus</i>	LVL2002-013	Belgium	AY336959
<i>Lactarius vietus</i>	2006_09_12_3	UK	JQ888186
<i>Lactarius vietus</i>	UE11.19.2004-1	Sweden	KF133267
<i>Lactarius vietus</i> ^a	HCCN10890	Korea	MG551732
<i>Lactarius vietus</i> ^a	HCCN20878	Korea	MG551733
Genus <i>Lactifluus</i>			
<i>Lactifluus acicularis</i>	KVP08-029 (Holotype)	Thailand	HQ318239
<i>Lactifluus acicularis</i>	KVP08-033	Thailand	HQ318242
<i>Lactifluus acicularis</i>	KVP08-007	Thailand	HQ318230
<i>Lactifluus acicularis</i> ^a	SFC20140828-37	Korea	MG551734
<i>Lactifluus acicularis</i> ^a	SFC20160726-19	Korea	MG551735
<i>Lactifluus distantifolius</i>	DS07-461 (Holotype)	Thailand	HQ318223
<i>Lactifluus distantifolius</i>	LTH288	Thailand	HQ318274
<i>Lactifluus foetens</i>	ADK3688	Benin	KR364022
<i>Lactifluus foetens</i>	ADK2840 (Holotype)	Benin	KR364023
<i>Lactifluus pilosus</i>	LTH205 (Holotype)	Thailand	KR364006
<i>Lactifluus pilosus</i> ^a	HCCN10487	Korea	MG551736
<i>Lactifluus pilosus</i> ^a	HCCN12316-1	Korea	MG551737
<i>Lactifluus pilosus</i> ^a	HCCN12410	Korea	MG551738
<i>Lactifluus pilosus</i> ^a	HCCN14809	Korea	MG551739
<i>Lactifluus pilosus</i> ^a	SFC20120814-43	Korea	MG551740
<i>Lactifluus pinguis</i>	LTH117 (Holotype)	Thailand	HG318211
<i>Lactifluus pinguis</i>	LTH255	Thailand	HQ318263
<i>Lactifluus pinguis</i>	LTH278	Thailand	HQ318268
<i>Lactifluus pinguis</i> ^a	SFC20120907-20	Korea	MG551741
<i>Lactifluus subvellereus</i>	AV05-210	USA	KR364010
<i>Lactifluus vitellinus</i>	KVP08-024 (Holotype)	Thailand	HQ318236
<i>Lactifluus vitellinus</i>	LTH348	Thailand	HQ318251
<i>Lactifluus xerampelinus</i>	MH201176	Mozambique	KR364099
<i>Lactifluus xerampelinus</i>	TS1116 (Holotype)	Tanzania	KR364039
Genus <i>Multifurca</i>			
<i>Multifurca furcata</i>	REH7804	Costa Rica	DQ421995
<i>Multifurca stenophylla</i>	JET956	Australia	JX266631

^aKorean samples used in this study.

herbaria in South Korea: the Seoul National University Fungus Collection (SFC) herbarium, Kangwon National University (TPML), and the National Institute of Agricultural Sciences (HCCN). Fruiting bodies in these herbaria were collected from various regions across the Korean peninsula and annexed islands from 1993 to 2016. Specimen information, such as collection date, site, composition of forest, and photographs were obtained from each herbarium. Specimens were putatively identified using field guides [3,5,23] and the Russulales News website (<http://www.mtsn.tn.it/russulales-news/>). For terminology of macro-morphology, we followed Verbeke and Walley [24]. The Methuen Handbook of Color [25] was used as the color standard for description of specimens.

In order to observe microscopic features, specimens were rehydrated in 5% (w/v) KOH and stained with 1% (w/v) Congo red. Basidiospores were observed and measured in Melzer's reagent. All measurements of basidiospores and hymenial elements (e.g., Basidia, cystidia, and marginal cells) were performed using a Nikon Eclipse 80i optical microscope (Nikon, Japan) at 40X magnification. Forty basidiospores were measured for each collection and 95% limits were calculated. The height of basidiospore ornamentation was measured separately. The ratio of basidiospore (length/width) was calculated which inferred Q-values. Both basidiospore sizes and Q-value were calculated with 0.1 precision. Basidiospore ornamentation of all collections was observed using the same microscope at 100X magnification. For scanning electron microscope (SEM) analysis, an EM ACE200 platinum coater (Leica, Austria) was used to preserve a piece of lamellae on the surface of an SEM sample stub. All SEM photos were produced using a SUPRA 55VP scanning electron microscope (Carl Zeiss, Germany) at 5000× or 10,000× magnification.

2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from sections of fresh or dried fruiting bodies by using a modified version of the CTAB DNA extraction method described by Rogers and Bendich [26]. ITS regions were amplified using several combinations of primer sets: forward primers ITS1F or ITS5 [27] and reverse primers ITS4B or Russ3R [23,28]. PCR amplification of the ITS was performed as previously described by Park and Lee [23]. PCR products were purified with the Expin PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea) according to the user's manual. Finally, DNA sequencing was performed by Macrogen (Seoul, South Korea), using the same set

of primers for each locus, on an ABI3730 automated DNA Sequencer.

2.3. Sequence alignment and phylogenetic analyses

The ITS sequences were assembled and edited with MEGA version 6 (Pennsylvania State University, State College, PA) [29]. For phylogenetic analyses of *Lactarius* and *Lactifluus*, the generated ITS sequences were aligned with those of 17 taxa of *Lactarius* and *Lactifluus* from published data. Two representatives of *Multifurca* were used as an out-group. All ITS sequences generated in this study were deposited in GenBank (Table 1). Sequence alignment was performed separately using the online MAFFT version 7 program (CBRC, Tokyo, Japan) [30] with the default settings and edited manually using MEGA version 6 (Pennsylvania State University, State College, PA). Maximum likelihood (ML) analyses were performed with RAxML version 7.03 (Bioinformatics Institute, Singapore) [31] using the GTR + G model and 1000 bootstrap replicates. jModelTest version 2.1.7 (National Academy of Sciences, Washington, DC) [32] was used to decide the model of character evolution. Bayesian inference (BI) analyses were executed with MrBayes on XSEDE version 3.2.6 [33] using the HKY + I + G model. The analysis was performed with four independent runs, each with four chains. Each run had 20 million generations and the sampling frequency was set to 100. All phylogenetic analyses were performed on the CIPRES Science Gateway [34].

3. Results

3.1. Phylogenetic analyses

The dataset used in this study contained 58 Russulaceae collections, of which 32 and 24 belonged to the genus *Lactarius* and *Lactifluus*, respectively. The ITS dataset consisted of a 770 bp alignment. Tree topology obtained from ML and BI analyses were similar, with slight variation in nodal support (support: ML > 70, BI > 0.95) (Figure 1). The genera *Lactarius* (99/1) and *Lactifluus* (91/0.98) were well supported. Twenty specimens were conspecific to five and three species of *Lactarius* and *Lactifluus*, respectively. ITS sequences of each species had more than 98.2% sequence similarity with reference sequences on Genbank. Each species was well supported by both ML bootstrap values and BI posterior probabilities.

For *Lactarius*, two specimens (HCCN369G and TPML110825-070) grouped into a well-supported clade (98/1) with Chinese, Japanese, and Thai samples that were denoted as *Lactarius atromarginatus*

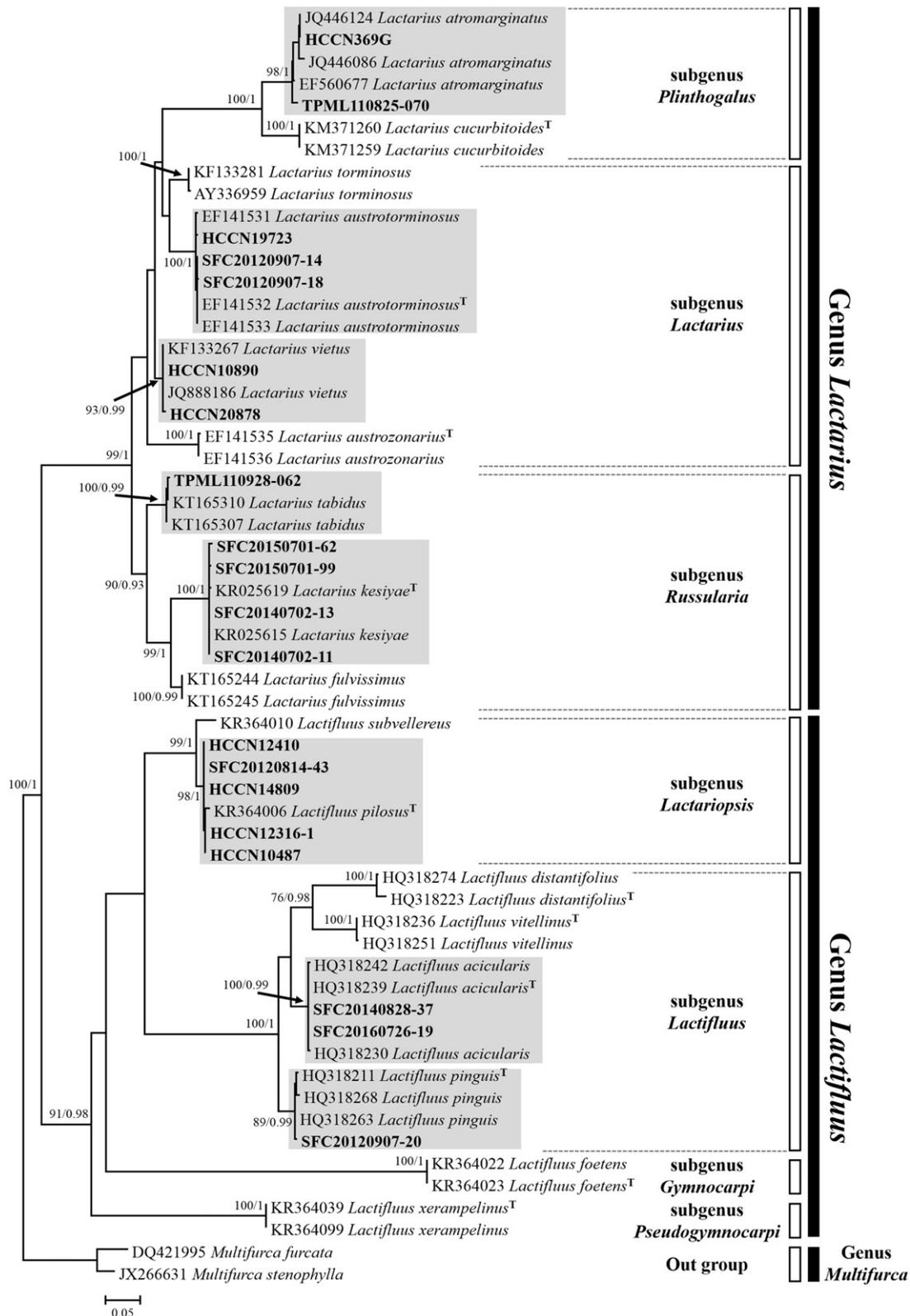


Figure 1. Phylogram generated from maximum likelihood analysis based on ITS sequence data of milkcaps. Branch support values are given as maximum likelihood bootstrap values >70 and Bayesian Inference posterior probabilities >0.95 are shown. The scale bar indicates the number of nucleotide substitution per site. Superscript T indicates the holotype specimen of each species.

in *L.* subg. *Plinthogalus*. Three samples (HCCN19723, SFC20120907-14, and SFC20120907-18) and two collections (HCCN10890 and HCCN20878) matched with *L. austrotorminosus* (100/1.0) and European *L. vietus* (93/0.99), respectively. Five specimens grouped

with *L. fulvissimus*, *L. kesiyae*, and *L. tabidus* within *L.* subg. *Russularia*. Four of these (SFC20140702-11, SFC20140702-13, SFC20150701-62, and SFC20150701-99) formed a monophyletic group with *L. kesiyae* from Vietnam and Thailand (100/1.0). TPML110928-062



Figure 2. Fruiting bodies of eight milkcap species. (A) *Lactarius atromarginatus*, (B) *L. austrominosus*. (C) *L. kesiyae*, (D) *L. tabidus*, (E) *L. vietus*, (F) *Lactifluus acicularis*, (G) *Lf. pilosus*, and (H) *Lf. pinguis*. Scale bar = 1 cm.

was conspecific with Swedish and Canadian specimens identified as *L. tabidus* (100/0.99).

For *Lactifluus*, eight samples were conspecific with three Thai *Lactifluus* species, *Lf. acicularis*, *Lf. pilosus*, and *Lf. pinguis*. Five specimens (HCCN10487, HCCN12316-1, HCCN12410, HCCN14809, and SFC20140702-13) matched to the type specimen of *Lf. pilosus* (LTH205) (98/1) which belongs to *Lf.* subg. *Lactariopsis*. Two specimens (SFC20140828-37 and SFC20160726-19) grouped with the holotype of *Lf. acicularis* (KVP08-029) (100/0.99). One specimen (SFC20120907-20) was conspecific with *Lf. pinguis* (89/0.99). These two species are members of *Lf.* subg. *Lactifluus*.

3.2. Taxonomy

***Lactarius atromarginatus* Verbeken & E. Horak**, Aust. Syst. Bot. 13(5): 688 (2000) (Figures 2(A), 3(A) and 4(A))

Pileus 25–50 mm, planoconvex when young, later applanate and depressed in the center to infundibuliform with central umbo; surface dry, rugose, not viscid when moist, dark brown to yellowish brown, and paler toward margin. Lamellae adnate to subdecurrent, rather close, white to pale yellow, with abundant lamellulae, and staining purplish grey when bruised; with abundant lamellulae. Stipe 30–60 × 5–8 mm, cylindrical, yellowish brown to pale brown, and paler toward base. Latex watery white, abundant, and turning pale lilac. Basidiospores 7.2–10.6 × 7.2–10.0 μm, $Q = 1.0$ –1.1, and globose to subglobose; ornamentation mostly up to 1.2 μm high, consisting of amyloid ridges which form a complete reticulum. Basidia 46.8–65.0 × 11.0–14.8 μm and subclavate. Pleuromacrocystidia absent. Cheiloleptocystidia and pseudocystidia abundant.

Specimens examined: Korea, Chungcheongbuk-do, Boeun-gun, Mt. Songni. 36°29'28" N, 127°48'16" E, August 25 2011, Nam Kyu Kim, TPML110825-070; on the ground of *Quercus* forest. Korea, Gyeonggi-do, Yangpyeong-gun, Mt. Yongmun. 37°33'15" N, 127°34'10" E, July 27 1993, Soon Ja Seok, HCCN369G; on the ground of mixed forest.

Remarks: *L. atromarginatus* seems to be a widely distributed species in Asia. It was first described from Papua New Guinea [35] and later in China, Japan, and Thailand [16,36]. According to the original description of the species [35], the umber brown pileus with wrinkled surface, pinkish, and lilac discoloration of context and latex and subreticulate basidiospores allow for characterizing of this species. This is the only species with lilac discoloration of latex belonging to the subgenus *Plinthogalus* in Korea.

***Lactarius austrotorminosus* H.T. Le & Verbeken**, Fungal Diversity 24: 198 (2007) (Figures 2(B), 3(B) and 4(B))

Pileus 25–70 mm, convex when young, later applanate and depressed in the center to almost infundibuliform; margin incurved with numerous hairs; surface scaly, zonate, with rings of hairs, greyish orange to pink, greyish brown. Lamellae cream to pale yellow, subdecurrent, crowded, with abundant with abundant lamellulae. Stipe 25–65 × 5–15 mm, cylindrical, white, pale orange to brown toward the base, discoloring orange to brown if bruised, becoming hollow in age, and sometimes scrobiculate. Latex white and unchanging. Basidiospores 6.3–8.0 × 4.5–7.1 μm, $Q = 1.05$ –1.25, subglobose to broadly ellipsoid; ornamentation amyloid, mostly up to 1.2 μm high, consisting of ridges which form incomplete reticulum. Basidia 36.1–62.3 × 7.8–9.6 μm and cylindrical to slightly clavate. Pleuromacrocysti-

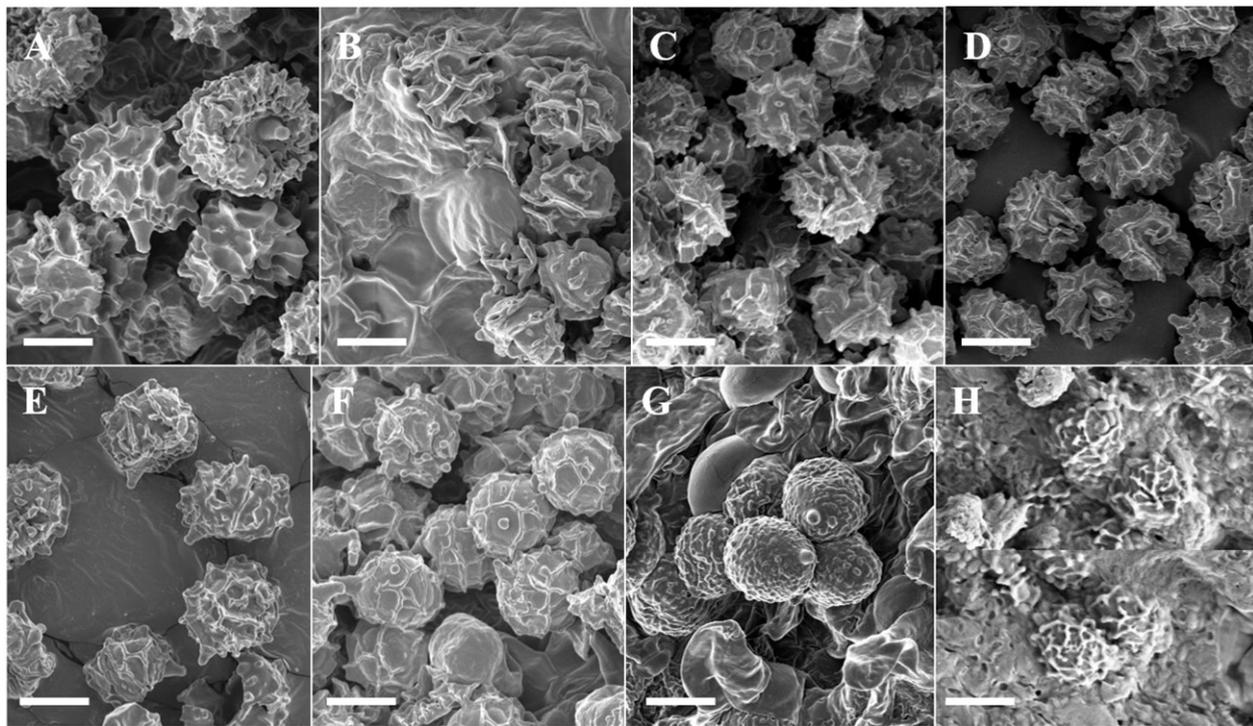


Figure 3. SEM photos of basidiospores. (A) *Lactarius atromarginatus*, (B) *L. austrotorminosus*, (C) *L. kesiyae*, (D) *L. tabidus*, (E) *L. vietus*, (F) *Lactifluus acicularis*, (G) *Lf. pilosus*, and (H) *Lf. pinguis*. Scale bar = 5 μ m.

dia 34.9–71.4 \times 6.9–11.2 μ m, fusiform, apically constricted, and abundant. Cheilomacrocytidia absent. Pseudocystidia abundant.

Specimens examined: Korea, Jeju-do, Seogwipo-si, Donnaeko campsite. 33°18'01" N, 126°35'03" E, September 7 2012, Hyun Lee, SFC20120907-14; on the ground of evergreen *Quercus* and *Castanopsis* forest. Korea, Jeju-do, Seogwipo-si, Donnaeko campsite. 33°18'03" N, 126°35'04" E, September 7 2012, Hyun Lee, SFC20120907-18; on the ground of evergreen *Quercus* and *Castanopsis* forest. Korea, Jeju-do, Seogwipo-si, Namwon-eup. 33°20'09" N, 126°36'40" E, July 14 2010, Soon Ja Seok, HCCN19723; On the ground of broad-leaved forest.

Remarks: *L. austrotorminosus* was originally described in broad-leaf forests in Thailand that were dominated by Fagaceae trees with few *Dipterocarpus* and *Pinus* trees [37]. To date, this species was only found on Jeju Island. This species is easily recognized in the field because of it has pinkish orange colors, strikingly hairy, and zonate pileus, and involute margin. Under the microscope, incompletely reticulate basidiospores and the absence of cheilocystidia are useful characteristics to recognize this species.

***Lactarius kesiyae* Verbeken & K.D. Hyde**, Phytotaxa. 207(3): 215–241 (2015) (Figures 2(C), 3(C) and 4(C))

Pileus 25–50 mm diameter, convex when young, later applanate and depressed in the center to infundibuliform; margin pectinated with age; surface smooth, slightly viscid when moist, and dark blond

to pale orange or pale greyish brown. Lamellae subdecurrent, ivory to cream, apricot when bruised, crowded, and with abundant lamellulae. Stipe 35–60 \times 5–10 mm, cylindrical, cream to pale apricot, and hollow. Latex watery white, abundant, and unchanging on exposure. Basidiospores 6.0–7.8 \times 5.4–7.0 μ m, Q = 1.0–1.2, globose to broadly ellipsoid; ornamentation amyloid, mostly up to 1.2 μ m high, consisting of isolated warts and ridges which form an incomplete reticulum. Basidia 30.7–45.0 \times 6.7–10.7 μ m, clavate. Pleuromacrocytidia 42.1–73.8 \times 4.1–7.9 μ m and subulate. Lamellar edge fertile. Cheilomacrocytidia 40.9–50.4 \times 5.7–7.5 μ m and fusiform to subulate. Pseudocystidia abundant.

Specimens examined: Korea, Jeju-do, Seogwipo-si, Andeok valley. 33°15'25" N, 126°21'08" E, July 2 2014, Hyun Lee, SFC20140702-11; on the ground of evergreen trees and *Pinus densiflora* forest. Korea, Jeju-do, Seogwipo-si, Andeok valley. 33°15'25" N, 126°21'12" E, July 2 2014, Hyun Lee, SFC20140702-13; on the ground of *Pinus densiflora* forest. Korea, Jeju-do, Dongbaekdongsan. 33°30'51" N, 126°32'08" E, July 1 2015, Hyun Lee, SFC20150701-62; on the ground of evergreen trees and *Pinus densiflora* forest. Korea, Jeju-do, Dongbaekdongsan. 33°30'57" N, 126°42'50" E, July 1 2015, Hyun Lee, SFC20150701-99; On the ground of evergreen trees and *Pinus densiflora* forest.

Remarks: *Lactarius kesiyae* is distinguished by its sticky and glossy appearance in moist conditions, its pale brownish grey to pale brownish orange color and the pectinate pileus margin. The latex is watery

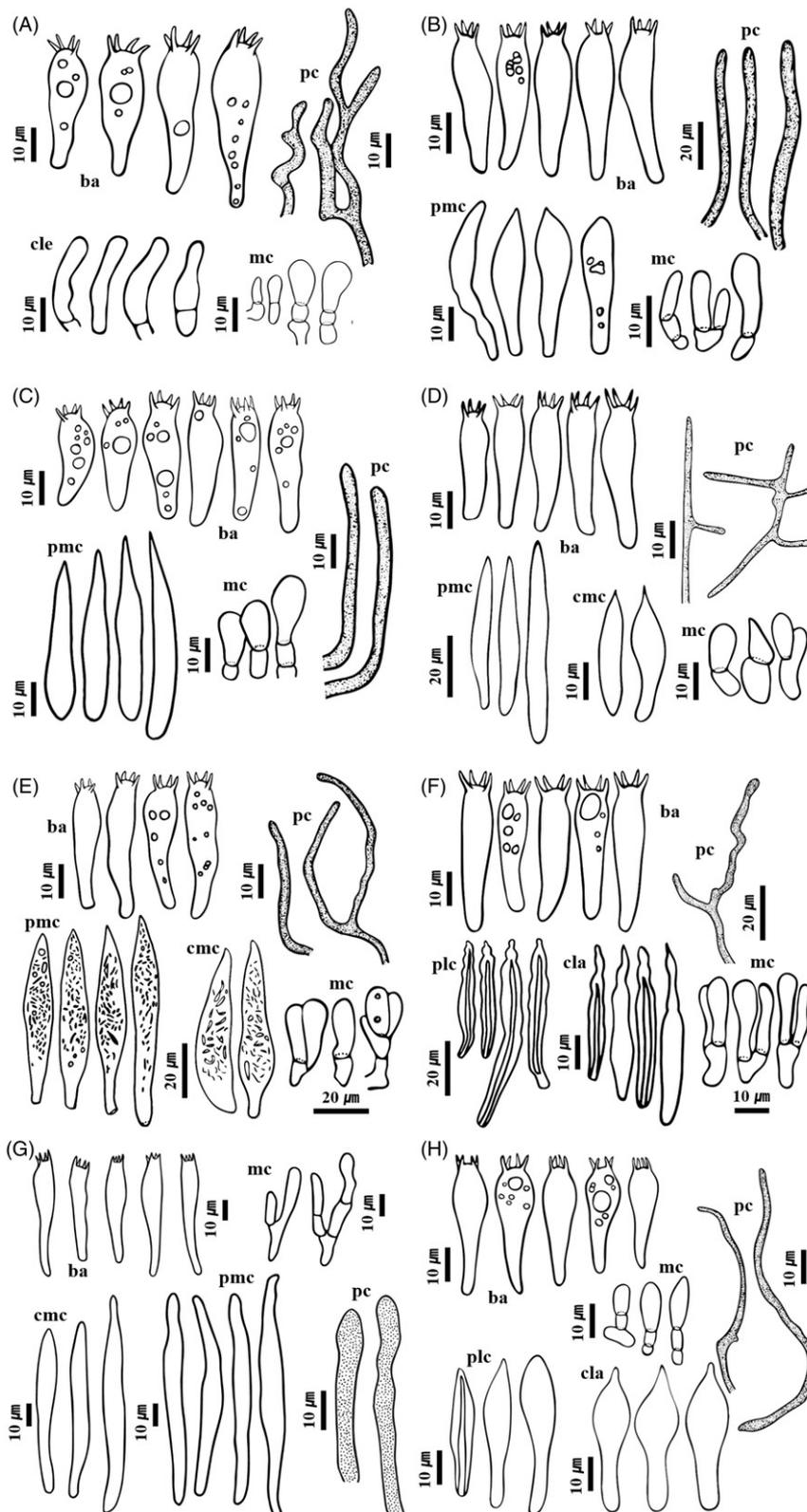


Figure 4. Microscopic features of eight milkcap species. (A) *Lactarius atomarginatus*, (B) *L. austrotorminosus*, (C) *L. kesiyae*, (D) *L. tabidus*, (E) *L. vietus*, (F) *Lactifluus acicularis*, (G) *Lf. pilosus*, and (H) *Lf. pinguis*. Abbreviation in figure: ba: basidia; cla: cheilamprocystidia; cle: cheiloleptocystidia; cmc: cheilomacrocystidia; mc: marginal cells; pc: pseudocystidia; plc: pleurolamprocystidia; pmc: pleuromacrocystidia.

white and turns yellow on white tissue. Microscopically, pleuromacrocystidia are very long (up to 100 μm long in the original description), protruding from the hymenium. This species was originally described growing with *Pinus kesiya* in

Thailand and Vietnam [38]. However, specimens collected in Korea were found in mixed forests where evergreen broad-leaved trees and *Pinus densiflora* grow together. This species was found only on Jeju Island in Korea.

***Lactarius tabidus* Fr.**, Epicr. syst. mycol. (Upsaliae): 346 (1838) [1836–1838] (Figures 2(D), 3(D) and 4(D))

Pileus 15–50 mm diameter, convex when young, later appanate and depressed in the center, sometimes with central umbo; surface even when young, slightly venose toward the center in age, sometimes with wrinkled margin, yellowish brown to greyish orange brown, and sometimes hygrophanous. Lamellae adnexed, rather crowded, cream to pale pink, sometimes forked, and with abundant lamellulae. Stipe 20–70 × 5–10 mm, cylindrical, sometimes clavate, and clay pink to greyish orange brown. Latex white to cream and slightly turning pale yellow. Basidiospores 6.0–7.8 × 5.4–7.0 μm, Q = 1.0–1.2, globose to broadly ellipsoid; ornamentation mostly up to 1.3 μm high, consisting of isolated amyloid warts and ridges which form an incomplete reticulum. Basidia 30.7–45.0 × 6.7–10.7 and clavate. Pleuromacrocytidia 34.3–84.2 × 5.6–10.4 μm, fusiform, and rare. Cheilomacrocytidia 27.3–52.0 × 4.7–8.7 μm, fusiform to subulate, and abundant.

Specimens examined: Korea, Gangwon-do, Taebaek-si, Mt. Hambaek, 37°09'42" N, 128°55'03" E, September 29 2011, Nam Kyu Kim, TPML110928-062; on the ground of *Betula* and *Sorbus* forest.

Remarks: The most distinctive character of *Lactarius tabidus* is a hygrophanous cap margin when moist. Basidiospores with acute warts were observed in European specimens [5]; however, basidiospores of Korean samples have more interconnection in ornamentation (Figure 3). The fruiting body of *L. tabidus*, with its dull brownish colors, is easily confused with *L. subdulcis*. The unchanging latex and the presence with *Fagus* trees can help to discriminate this species from *L. tabidus*.

***Lactarius vietus* (Fr.) Fr.**, Epicr. syst. mycol. (Upsaliae): 344 (1838) [1836–1838] (Figures 2(E), 3(E) and 4(E))

Pileus 20–75 mm diameter, planoconvex when young, later appanate and depressed in the center to infundibuliform; azonate to indistinctly greyish zonate near cap margin, surface finely rugulose, and slightly lubricous when moist. Lamellae adnate to subdecurrent, white to cream, rather crowded, sometimes forked, and with abundant lamellulae. Stipe 22–65 × 7–18 mm, cylindrical; surface even, often a narrow pale zone under the gills, and pale cream to olivaceous buff; context hollow in age. Latex white and drying greenish grey. Basidiospores 6.4–9.2 × 5.4–7.6 μm, Q = 1.0–1.3, globose to broadly ellipsoid; ornamentation mostly up to 1.5 μm high, consisting of some isolated amyloid warts and ridges which forms a nearly complete reticulum. Basidia 38.8–50.3 × 8.6–11.4 μm, and clavate. Pleuromacrocytidia 58.8–108.3 × 8.9–13.4 μm,

fusiform, and abundant. Cheilomacrocytidia 41.8–55.3 × 9.1–14.2 μm, fusiform, and abundant.

Specimens examined: Korea, Gangwon-do, Inje-gun, Baekdam-sa temple, 38°09'53" N, 128°22'26" E, October 14 2010, Soon Ja Seok, HCCN20878; on the ground of *Betula* and *Quercus* forest. Korea, Gangwon-do, Wonju-si, Mt. Chiak, 37°23'41" N, 128°03'07" E, October 10 2002, Soon Ja Seok, HCCN10890; on the ground of *Betula*, *Pinus* and *Quercus* forest.

Remarks: The pale greyish brown cap, the milk, which turns greenish grey, pinkish buff gills, pale zone at the top of stipe and subreticulate basidiospores are useful in delimitation of this species. *L. glyciosmus* also has a greyish brown cap and pinkish buff gills but this species has unchanging latex, smaller basidiospores than *L. vietus* and non-gelatinous cap surface.

***Lactifluus acicularis* (Van de Putte & Verbeken) Van de Putte**, Mycotaxon 120: 444 (2012) (Figures 2(F), 3(F) and 4(F))

Pileus 40–70 mm, convex when young, later appanate and depressed in the center to infundibuliform; surface dry, rugose, pruinose, yellowish brown to reddish brown, paler toward the margin. Lamellae subdecurrent to decurrent, rather crowded, and staining brown to greyish brown when bruised; lamellulae of different lengths abundant. Stipe 46–75 × 5–12 mm, cylindrical to subclavate; surface dry, pruinose, longitudinally rugulose, and concolorous with pileus. Latex white, sticky, and slowly changing brownish within few minutes to half an hour. Basidiospores 7.0–9.0 × 6.5–8.0 μm, Q = 1.0–1.2, globose to subglobose; ornamentation amyloid, mostly up to 1.3 μm high, consisting of some isolated warts and ridges which form a nearly complete reticulum. Basidia 41.8–62.0 × 9.2–11.8 μm, clavate. Pleurolamprocystidia 43.7–91.4 × 5.8–10.8 μm, fusiform, and abundant. Lamella edge mixed. Cheilolamprocystidia 25.1–63.6 × 4.2–7.8 μm, fusiform, and abundant.

Specimens examined: Korea, Chungcheongnam-do, Cheonan-si, Mt. Gwangdeok, 36°40'06" N, 127°01'34" E, August 28 2014, Young Woon Lim, SFC20140828-37; on the ground of *Acer*, *Pinus densiflora* and *Quercus* forest. Korea, Incheon-si, Ongjin-gun, Jangbong island, 37°32'21" N, 126°20'12" E, July 26 2016, Jae Young Park, SFC20160726-19; on the ground of *Pinus rigida* and *Quercus* forest.

Remarks: *Lactifluus acicularis* and *Lf. volemus* shared some characteristics, such as the very long pileipellis hairs, the brown to reddish-brown pileus color and basidiospores with an almost complete reticulum [39]. Korean collections were often identified as *Lactifluus volemus*. However, they can be distinguished from *Lf. volemus* by the average size of

basidiospores. Basidiospores of *Lactifluus volemus* (9.0–10.0 × 8.4–9.3 μm) are much larger than that of *Lf. acicularis* [40].

***Lactifluus pilosus* (Verbeken, H.T. Le & Lumyong) Verbeken**, Mycotaxon 102: 287 (2007) (Figures 2(G), 3(G) and 4(G))

Pileus 80–170 mm, convex when young, later infundibuliform and deeply depressed in the center, sometimes with wavy margin; surface dry, velvety, often rugulose near the margin, whitish to pale yellow, and with orange to brownish spots in age. Lamellae decurrent, distant, cream to greyish cream, turning orange brown when bruised, and with abundant lamellulae of different lengths. Stipe 10–45 × 10–25 mm, cylindrical to slightly tapering downwards, central, sometimes eccentric; surface dry, velvety, and concolorous with pileus. Latex white which changes to pale yellow when dried. Basidiospores 7.0–8.2 × 5.6–7.2 μm, Q = 1.0–1.3, globose to ellipsoidal; ornamentation amyloid, mostly up to 0.7 μm high, and consisting of ridges which form an incomplete reticulum; sometimes isolated ridges present; isolated warts present. Basidia 55.3–71.4 × 8.0–11.3 μm and subclavate. Pleuromacrocytidia 91.1–105.2 × 7.2–9.8 μm, subfusiform to clavate, and abundant. Lamella edge sterile. Cheilomacrocytidia 63.7–74.9 × 5.5–7.8 μm, narrowly clavate, and abundant.

Specimens examined: Korea, Gyeongsangbuk-do, Ulleung-gun, Ulleung island, Mt. Seonginbong, 37°30'35" N, 130°51'36" E, August 14 2012, Young Woon Lim, SFC20120814-43; On the ground of *Pinus densiflora* and *Acer okamotoanum* forest. Korea, Gangwon-do, Inje-gun, Baekdam-sa temple, 38°12'03" N, 128°19'21" E, August 16 2002, Soon Ja Seok, HCCN10487; on the ground of *Pinus densiflora* and *Quercus* forest. Korea, Gangwon-do, Pyeong Chang-gun, Woljeong-sa temple, 37°43'37" N, 128°35'47" E, August 30 2004, Soon Ja Seok, HCCN12316-1; on the ground of *Abies holophylla*, *Pinus densiflora* and *Acer pseudosieboldianum* forest. Korea, Gangwon-do, Pyeong Chang-gun, Sangwon-sa temple, 37°47'06" N, 128°34'24" E, August 31 2004, Soon Ja Seok, HCCN12410; on the ground of *Abies holophylla* and *Pinus densifolia* forest. Korea, Gangwon-do, Pyeong Chang-gun, Woljeong-sa temple, 37°43'24" N, 128°35'49" E, September 20 2006, Soon Ja Seok, HCCN14809; on the ground of mixed forest.

Remarks: These Korean specimens were initially misidentified as *Lactifluus vellereus*, however, *Lf. vellereus* differs from *Lf. pilosus* by having unchanging latex, medium crowded gills, and larger basidiospores with very low ridges (0.2 μm) forming a complete reticulum [41]. The other European species, *Lf. bertillonii* is also similar but this species can be distinguished by the unchanging latex, medium

crowded lamellae, and larger basidiospores with incomplete reticulum [5].

***Lactifluus pinguis* (Van de Putte & Verbeken) Van de Putte**, Mycotaxon 120: 444 (2012) (Figures 2(H), 3(H) and 4(H))

Pileus 40–80 mm, convex when young, later appanate and deeply depressed in the center, sometimes with margin wavy in age; surface dry, rugulose when young, pale yellow to wheat straw, and partly pale pink or pale orange when young. Lamellae decurrent, crowded, white to cream, staining pale brown when bruised, and with abundant lamellulae of different lengths. Stipe 45–90 × 8–12 mm, cylindrical to slightly tapered toward pileus, sometimes eccentric; surface dry, and concolorous with pileus. Latex white, sticky, and slowly changing pale brownish. Basidiospores 8.0–9.7 × 7.5–9.2 μm, Q = 1.0–1.15, globose to subglobose; ornamentation amyloid, mostly up to 2.0 μm high, consisting of ridges which form a complete reticulum; sometimes isolated ridges present; isolated warts rare. Basidia 42.2–63.0 × 11.0–13.6 μm and subclavate. Pleurolamprocystidia 55.2–75.3 × 8.1–12.9 μm, fusiform to clavate, and abundant. Lamella edge mixed. Cheilolamprocystidia 35.2–61.0 × 9.5–12.1 μm, clavate to subclavate, and abundant.

Specimens examined: Korea, Jeju-do, Seogwiposi, Donnaeko campsite, 33°18'01" N, 126°35'03" E, September 7 2012, Hyun Lee, SFC20120907-20; On the ground of evergreen broad-leaved forest.

Remarks: Like *Lf. acicularis*, this species was previously misidentified as *Lactifluus volemus* in Korea. However, they can be distinguished from *Lf. volemus* by the height of basidiospore ornamentation. The height of ornamentation in *Lf. volemus* (up to 1.5 μm) is lower than that of *Lf. pinguis* [40]. Furthermore, pleurolamprocystidia cell wall thickness of *Lf. pinguis* (3–7 μm) is much thicker than that of *Lf. volemus* (1.5–4.5 μm) [39,40].

4. Discussion

To date, 66 milkcap species have been reported in South Korea [42]. These specimens were identified based solely on morphology and many European and North American names were used [23,43,44]. For this reason, it is probable that the number of Korean milkcap species is considerably different from what is currently reported, and DNA sequence analysis can be used for more accurate species identification [21,45]. In this study, eight species in the family Russulaceae were identified as new records to South Korea. All presented species were identified using molecular data from ITS sequences and morphological characteristics. Five species were shown to belong to three subgenera of *Lactarius* and three species belonged to two subgenera of *Lactifluus*.

Recent molecular studies showed that the majority of Asian milkcap species were not conspecific to mycoflora found in other continents, such as Europe and North America [16,20,46]. Intercontinental conspecificity of milkcaps can occur infrequently in boreal and alpine forests, as some species were found in both North America and Europe [47,48], and some in both in Europe and Asia [49,50]. In this study, we found that some Korean specimens were conspecific with two European species, *Lactarius tabidus* and *L. vietus*. These species have also been reported in North America and Europe [4,5,47]. The wide distribution of *L. tabidus* has recently been confirmed using ITS sequence data [50]. The remaining six species were originally described in Southeast Asia. Korean collections of *Lactifluus acicularis* and *Lf. pinguis* were previously misidentified as European *Lf. volemus* because of their similar morphological characteristics. Previous studies revealed that there were many species hidden in the *Lactifluus volemus* species complex and indeed *Lf. volemus* has not been found in Asia [39,40]. Furthermore, Korean collections of *Lactifluus pilosus* have been misidentified as *Lf. vellereus*, which was first described from Europe. Thus, a thorough study of the Korean collections designated as *Lf. vellereus* is needed. *L. austrotorminosus*, *L. kesiyae* and *Lf. pinguis* were first described in Thailand [37–39]. Interestingly, these species were found only on Jeju Island. This observation suggests that climate change has affected fungal distribution in Korea. Beginning in the last decade, tropical and subtropical marine organisms [51,52] and plants [53,54] have frequently been reported on Jeju Island, the southernmost part of Korea. Thus, Jeju Island may represent a climate change hot spot. In addition, a recent study showed that the distribution pattern of ectomycorrhizal fungi was affected by climate change, inducing both host migration and host modification [55]. Three other species, *Lactarius atromarginatus*, *Lf. acicularis* and *Lf. pinguis*, were also reported from tropical and subtropical Asia. *Lactarius atromarginatus* was reported initially in Papua New Guinea [35], and then reported in Thailand, Japan, and southern China. These species were also collected in central Korea. Further studies are needed to establish any relationship between the fruiting of these species in Korea and climate change.

Various tree species serve as hosts for milkcaps through ectomycorrhizal symbiosis. Some species are specific to host trees [56]. For example, *Lactarius tabidus* and *L. vietus* both use *Betula* as their ectomycorrhizal symbiont [5]. The distribution of *Betula* is seems to be restricted to some regions in Korea, as these species were only collected from high mountain forests mixed with *Betula* and *Quercus* trees. *Lactarius kesiyae* is an ectomycorrhizal

symbiont of *Pinus kesiyae* [38]; however, *P. kesiyae* is not found in Korea, and in this study, specimens of *L. kesiyae* were collected near *P. densiflora*. Therefore, the host of *L. kesiyae* likely includes two *Pinus* species in *Pinus* sect. *Pinus* subsec. *Pinus* [57]. However, further molecular data obtained from ectomycorrhizal rootlets and fruiting bodies is needed to establish the symbiont host of Korean species.

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