




## Taxonomic Revision of the Genus *Lactifluus* (Russulales, Basidiomycota) of South Korea

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### ABSTRACT

*Lactifluus* (Pers.) Roussel is an ectomycorrhizal genus that was recently recognized to be distinct from the genus *Lactarius*. To date, 226 *Lactifluus* species have been reported worldwide. Misidentification of *Lactifluus* species is common because of intraspecific morphological variation, cryptic diversity, and the limited number of taxonomic keys available. Molecular data are indispensable for species delimitation; a multilocus phylogenetic analysis showed that most Asian *Lactifluus* species are not conspecific with morphologically similar species present on other continents. In particular, Korea has misused European and North American *Lactifluus* names. In this study, we evaluated the taxonomy of *Lactifluus* in Korea using both morphological and multilocus molecular (ITS, nrLSU, *rpb1*, and *rpb2*) data. We examined 199 *Lactifluus* specimens collected between 1980 and 2016, and a total of 24 species across the four *Lactifluus* subgenera were identified. All Korean species are distinct and clearly separated from European and North American species. Five taxa corresponded to previously described species from Asia and the remaining 19 taxa are confirmed as new species. Herein, we provide keys to the Korean *Lactifluus* species within their subgenera, molecular phylogenies, a summary of diversity, and detailed description of the new species.

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## 1. Introduction

The ectomycorrhizal genus *Lactifluus* (Pers.) Roussel was recently recognized as a distinct genus of milkcaps [1–3]. Previously, *Lactifluus* was considered a part of *Lactarius*, likely because species in both genera exude latex when the fruiting body is bruised. Molecular data showed that the original genus *Lactarius* was not monophyletic and warranted the creation of the new milkcap genera *Lactifluus* and *Multifurca* (the latter sometimes without latex). Upon closer inspection, *Lactifluus* and *Lactarius* have some key morphological differences. Although no synapomorphic characteristic has been found to consistently distinguish both genera, the distinction can usually be made on a combination of characters and trends. First, *Lactifluus* species, relative to *Lactarius*, are more likely have dry pilei with a velvety to pruinose texture. Also, *Lactifluus* species never have zonate, hairy, viscid, or scrobiculate pilei. The genus *Lactifluus* contains many species with

veiled velvety pilei, as well as all known pleurotoid milkcap species [1,4]. So far, no sequestrate species are known within the genus *Lactifluus*. Microscopically, species of *Lactifluus* are characterized by having spherical cells (sphaerocytes) in the hymenophoral trama and very often cells with thickened cell walls in the pileipellis structure and/or in the hymenium [5]. In many cases, a clear distinction between *Lactifluus* and *Lactarius* relies on genetic data.

To date, 226 *Lactifluus* species have been described worldwide [6]. The species richness of *Lactifluus* is higher in tropical compared to temperate regions, with the highest known diversity in Africa [7] and Asia [8–12]. Recent studies indicate that *Lactifluus* is also well represented in South America [13–20]. In Korea, 21 species of *Lactifluus* have been recorded, with the first record being *Lactifluus volemus* (first identified as *Lactarius volemus*) in 1936 [21–24]. *Lactifluus volemus* is a typical example of a name that has been used in several

continents, but covers a very rich species complex [10,25,26].

*Lactifluus* species are an important resource for human use. Most *Lactifluus* species are edible and are important food sources in the Northern Hemisphere (e.g., *Lf. piperatus* and *Lf. volemus*). Some species are sold commercially in markets of Europe [27,28], Central & North America [29,30], and Asia [27,31–33]. Also, some species, such as *Lf. Vellereus*, are the source of useful bioactive secondary metabolites, such as sesquiterpenes with lactarane or marasmane skeletons [34]. *Lactifluus volemus* (Fr.) Kuntze has a large amount of low-molecular-weight natural rubber in its latex that was used for revealing the mechanism of natural rubber synthesis [35,36].

Inaccurate description and misidentification of *Lactifluus* species is common in the literature due to intraspecific morphological variation and the occurrence of cryptic diversity. Verbeken and Nuytinck [4] predict that cryptic species are more common in *Lactifluus*, a genus with a very large genetic diversity [20], than in *Lactarius*. Identification of species is complicated by the limited number of taxonomic keys available. To counter these problems, mycologists have utilized DNA sequence data for species identification and phylogenetic studies. The most commonly used genes for identification of milkcaps (genera *Lactifluus* and *Lactarius* in the family Rusulaceae) are the internal transcribed spacer (ITS), the large subunit 28S region (nrLSU), and the region between the conserved domain 6 and 7 of the second largest subunit of RNA polymerase II (*rpb2*) [1,37,38]. Several studies suggested incorporation of the region between domains A and C of the largest subunit of RNA polymerase II (*rpb1*) and *rpb2* sequences for clearer understanding in infrageneric relationships [5,39,40]. However, DNA data have uncovered new problems—some *Lactifluus* species have been identified to be species complexes and remain difficult to identify [9,10,25,26,41,42].

As for many other groups of macrofungi, *Lactifluus* research has primarily been conducted in Europe and North America, where there is a long history of mycology. In the twentieth century, the taxonomy of *Lactifluus* was well studied under the name of *Lactarius* sensu lato in Europe and North America [43,44]. Subsequently, mycologists studying Asian fungal diversity used the scientific names originating from Europe and North America to refer to morphologically similar individuals (e.g., Pegler & Fiard [45], Singer et al. [46]). Recent molecular studies, however, showed that most Asian *Lactifluus* species are not conspecific with morphologically similar species present on other continents [8,10,11,25,42]. Recent work on Korean milkcaps

revealed new species and raised doubt of previous identifications based on morphological characters [23,24,47]. Such findings warrant a comprehensive reevaluation of Korean *Lactifluus*. In this study, we examine all available *Lactifluus* specimens from Korean herbaria to delineate species using morphological and multilocus molecular (ITS, nrLSU, *rpb1*, and *rpb2*) data.

## 2. Materials and methods

### 2.1. Sampling

A total of 199 *Lactifluus* specimens were obtained from four herbaria in South Korea: Kangwon National University (TPML), Korea National Arboretum (KA), National Institute of Agricultural Sciences (HCCN), and Seoul National University (SFC). These specimens were collected across South Korea from 1980 to 2016, with the initial identification based on morphological characters. When available, the collection information of each specimen was obtained from each herbarium.

### 2.2. Morphological approaches

Morphological characters were recorded from fresh materials, such as size and color of basidiomata, latex features, taste and smell. Specimen colors were recorded during daylight hours with color standard codes from Korneup and Wanscher [48]. For macromorphological characters, we follow the terminology in Verbeken and Walley [7]. Most collections were photographed in the field.

All microscopic characters were observed in dried specimens, using a Nikon Eclipse 80i microscope (Nikon, Japan), with the aid of the NIS element BR v3.2 (Nikon, Japan). Measurements and statistics follow Lee et al. [47], and were based on at least 20 spores and 10 hymenial structures (e.g., basidia, cystidia, marginal cells and pseudocystidia). Spore size and ornamentation were measured and observed in Melzer's reagent. The spores were measured in lateral view and the height of ornamentation was examined separately. The Q value represents the length/width ratio of the spore, referring to its shape. The spore ornamentation was photographed using a scanning electron microscope (SEM) at 5000 $\times$  and 10,000 $\times$  magnification (SUPRA 55VP, Carl Zeiss, Germany). Before SEM photography, dried pieces of lamellae were attached onto aluminum stubs, coated with platinum using a sputter coater (EM ACE200, Leica, Austria). Hymenial and pileal elements were mounted in 5% (w/v) KOH and observed in 5% (w/v) Congo red. The width and contents of pseudocystidia were recorded. For the features of hymenial and pileal cystidia, we

recorded size, contents, abundance, and thickness of the cell wall. We documented the following pileipellis characters: overall structure, depth, delimitation, stratification, and features of dermatocystidia, if present.

### 2.3. Molecular approaches

We used a modified CTAB method [49] to extract genomic DNA from dried tissue. Four loci were amplified and sequenced: ITS, nrLSU, *rpb1*, and *rpb2*. Molecular approaches were performed in two steps. First, we attempted to amplify and sequence ITS for all samples. Next, we provisionally identified the species based on morphological characters and the ITS-based phylogeny. For each provisional species, one to five representative specimens were selected (more recently collected) for additional amplification and sequencing of nrLSU, *rpb1*, and *rpb2*.

We used a C1000 thermal cycler (Bio-Rad, Hercules, CA, USA) for PCR amplifications using AccuPower<sup>®</sup> PCR premix (Bioneer Co., Daejeon, Korea). The primer sets and PCR conditions used to amplify ITS, nrLSU, and *rpb2* region followed Lee et al. [47]. For *rpb1*, we developed six *Lactifluus*-specific primers using the Primer3 program [50]: *rpb1*-Lac1F (TTC GGG CAC ATT GAA CTG GCC), *rpb1*-Lac2F (CAT TGA ACT GGC CAG ACC RG), *rpb1*-Lac3F (TCC ATC CAG GTG CGT GGC), *rpb1*-Lac1R (GTC CAT RTA AGT CGC CAC GT), *rpb1*-Lac2R (RTA AGT CGC CAC GTG GAA CTG), and *rpb1*-Lac3R (ACC TGR AGR AGT TGY TCR T). The partial *rpb1* locus was amplified using two steps. The first amplification of the *rpb1* locus was conducted using the primer set *rpb1*-Lac1F/*rpb1*-Lac1R under the same conditions of the first *rpb2* amplification in Lee et al. [47]. The primer set *rpb1*-Lac2F/*rpb1*-Lac2R or *rpb1*-Lac3F/*rpb1*-Lac3R was used in the second amplification of *rpb1* with the same amplification conditions previously described for nrLSU in Lee et al. [47].

PCR products were visualized on a 1% agarose gel stained with EcoDye (SolGent Co., Daejeon, Korea) and purified with the Expin PCR purification kit (GeneAll Biotechnology, Seoul, Korea), according to the manufacturer's instructions. All successfully amplified products were sequenced using the respective PCR primers on an ABI3730 automated DNA sequencer by Macrogen (Seoul, Korea). Obtained sequences were checked and manually edited using the software FinchTV v1.4 (Geospiza Inc.) and assembled in MEGA 6 [51]. All consensus sequences generated in this study were deposited in GenBank (Table 1).

### 2.4. Phylogenetic analyses

Four datasets (ITS, nrLSU, *rpb1*, and *rpb2*) were generated from the representative specimens of each species and used for phylogenetic analyses. Data for relevant species were downloaded from GenBank (shown in Figures 1–4), with the majority being from De Crop et al. [5]. Six *Lactarius* species were chosen as outgroups: *L. fuliginosus*, *L. hatsudake*, *L. miniatescens*, *L. olympianus*, *L. scrobiculatus*, and *L. tenellus* [5]. All DNA datasets were aligned using the online version of MAFFT v7 [52], using the E-INS-I strategy, then trimmed or edited in MEGA 6. Species delimitation was first examined using single locus phylogenies. When significant conflict was not observed among the single locus phylogenies, we concatenated all single locus alignments using MEGA 6. A total of 52 concatenated sequences were generated in this study, representing species in six sections/four subgenera: *Lf.* subg. *Gymnocarpi* sect. *Luteoli*, *Lf.* subg. *Lactariopsis* sect. *Albati*, three sections belonging to *Lf.* subg. *Lactifluus* (sect. *Gerardii*, *Lactifluus*, and *Piperati*), and *Lf.* subg. *Pseudogymnocarpi* sect. *Pseudogymnocarpi*.

All phylogenetic analyses were performed in the CIPRES Science Gateway [53]. Maximum likelihood (ML) analyses were done using RAxML 8.2.10 [54]. jModeltest 2.1.7 [55] was used to find best substitution model for each gene in the concatenated dataset. Models found by jModeltest 2.1.7 under Bayesian information criterion (BIC) were: K80+I+G (ITS and nrLSU), HKY+I+G (*rpb1*), and SYM+I+G (*rpb2*). Four parallel runs of Bayesian Inference (BI), each containing one cold and three heated chains, were carried out using MrBayes v.3.2.6 [56]. Each analysis was run for 20 million generations, sampling every 1000<sup>th</sup> generation. The convergence and burn-in value were visualized in Tracer 1.6 [57].

## 3. Results

### 3.1. Diversity of *Lactifluus* in South Korea and multigene analysis

Of the 199 Korean *Lactifluus* specimens, ITS sequences were successfully obtained from 143 specimens. The 56 specimens without sequence data were excluded from the study. A total of 24 putative taxa were identified based on morphological characters and the ITS-based phylogenetic tree. To confirm species identity and to infer the phylogenetic relationships of Korean *Lactifluus*, three additional loci (nrLSU, *rpb1*, and *rpb2*) were amplified and sequenced from the 52 representative specimens of the 24 taxa. The final phylogenetic analyses were performed with datasets of four loci from 134

**Table 1.** GenBank accession numbers for DNA sequences of four loci, species names, voucher and potential host trees of studied specimens in this study.

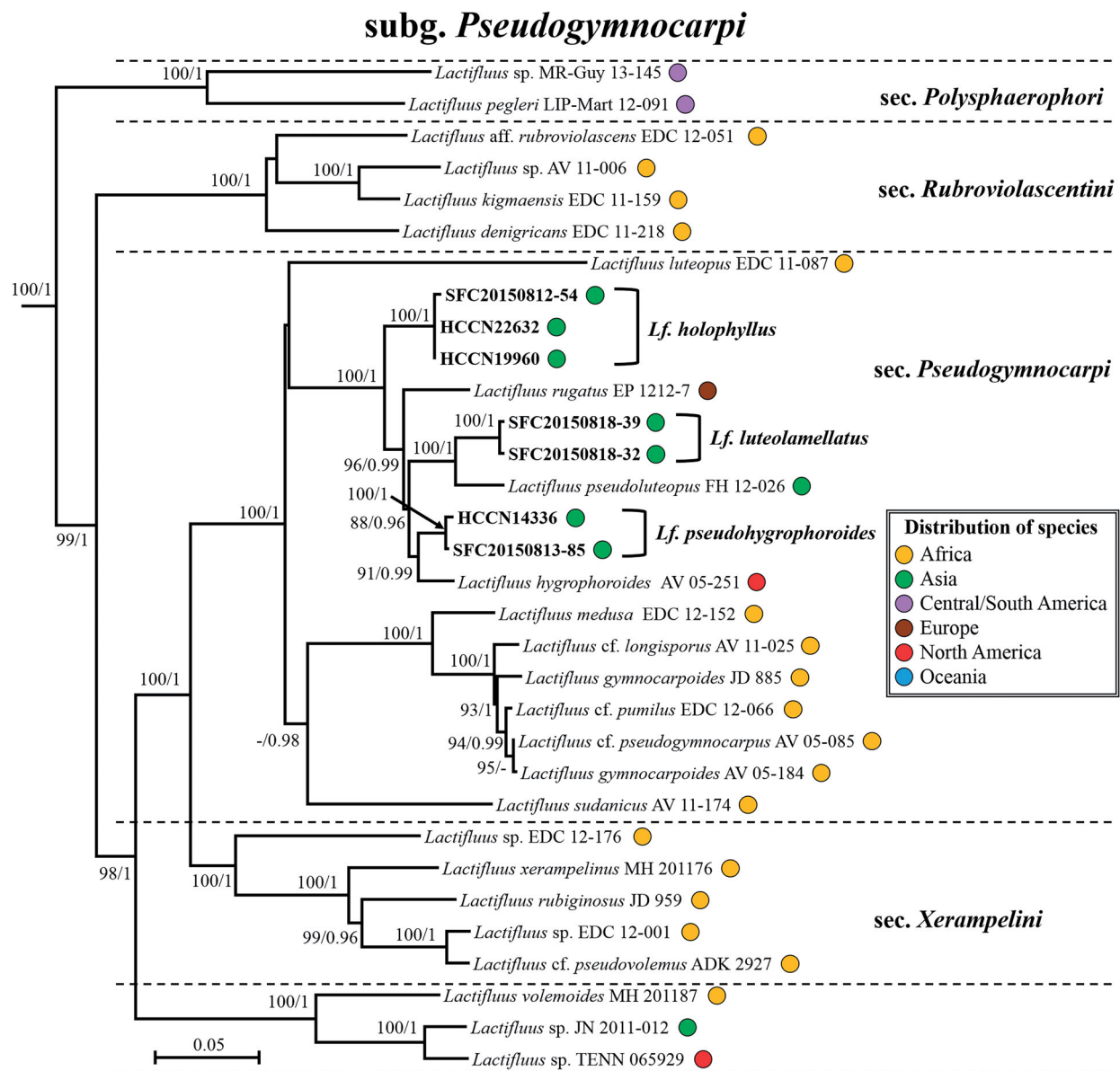
Species	Voucher <sup>a</sup>	Type	Accession no.				Potential host tree
			ITS	nrLSU	<i>rpb2</i>	<i>rpb1</i>	
<b><i>Lactifluus</i> subg. <i>Pseudogymnocarpi</i></b>							
<b>sect. <i>Pseudogymnocarpi</i></b>							
<i>Lactifluus holophyllus</i>	HCCN19960		MF611684	MF611659	MF617786	MN212852	<i>Abies</i>
	HCCN22632		MF611685	MF611660	MF617787	MN212853	<i>Abies</i>
	SFC20150812-54		MN215360	MN215311	MN212802	MN212854	<i>Abies</i>
<i>Lactifluus luteolamellatus</i>	SFC20150818-32		MN215361	MN215312	MN212803	MN212855	<i>Quercus</i>
	SFC20150818-39	Holotype	MF611680	MF611655	MN212804	MN212856	<i>Quercus</i>
<i>Lactifluus pseudohygrophoroides</i>	HCCN14336		MN215362	MN215313	MN212805	MN212857	<i>Pinus, Quercus</i>
	SFC20150813-85		MN215363	MN215314	MN212806	MN212858	<i>Pinus, Quercus</i>
<b><i>Lactifluus</i> subg. <i>Lactariopsis</i></b>							
<b>sect. <i>Albati</i></b>							
<i>Lactifluus multiseparatus</i>	SFC20130809-37		MN215364	MN215315	MN212807	MN212859	<i>Betula</i>
	SFC20150902-83		MN215365	MN215316	MN212808	MN212860	<i>Betula</i>
	SFC20150902-104	Holotype	MN215366	MN215317	MN212809	MN212861	<i>Betula</i>
<i>Lactifluus orientivellereus</i>	KA12-1562		MN215367	MN215318	MN212810	MN212862	<i>Abies</i>
	TPML130812-058		MN215368	MN215319	MN212811	MN212863	<i>Abies</i>
	TPML150909-054	Holotype	MN215369	MN215320	MN212812	MN212864	<i>Abies</i>
<i>Lactifluus pilosus</i>	HCCN10487		MG551736	MN215321	MN212813	MN212865	<i>Pinus</i>
	HCCN12410		MG551738	MN215322	MN212814	MN212866	<i>Pinus</i>
	SFC20120814-43		MG551740	MN215323	MN212815	MN212867	<i>Pinus</i>
<b><i>Lactifluus</i> subg. <i>Gymnocarpi</i></b>							
<b>sect. <i>Luteoli</i></b>							
<i>Lactifluus albidopectinatus</i>	SFC20160726-09	Holotype	MN215370	MN215324	MN212816	MN212868	<i>Quercus</i>
<i>Lactifluus pallidotestaceus</i>	SFC20150818-02	Holotype	MN215371	MN215325	MN212817	MN212869	<i>Quercus</i>
	SFC20150818-33		MN215372	MN215326	MN212818	MN212870	<i>Quercus</i>
	SFC20150820-29		MN215373	MN215327	MN212819	MN212871	<i>Quercus</i>
<b><i>Lactifluus</i> subg. <i>Lactifluus</i></b>							
<b>sect. <i>Gerardii</i></b>							
<i>Lactifluus betulicola</i>	SFC20150902-72	Holotype	MN215374	MN215328	MN212820	MN212872	<i>Betula</i>
<i>Lactifluus luminosus</i>	SFC20150701-53	Holotype	MN215375	MN215329	MN212821	MN212873	<i>Quercus</i>
<i>Lactifluus stellatus</i>	SFC20140724-70	Holotype	MN215376	MN215330	MN212822	MN212874	<i>Pinus, Quercus</i>
<b>sect. <i>Lactifluus</i></b>							
<i>Lactifluus acicularis</i>	SFC20140828-37		MG551734	MN215331	MN212823	MN212875	<i>Quercus</i>
	SFC20160726-19		MG551735	MN215332	MN212824	MN212876	<i>Quercus</i>
<i>Lactifluus koreanus</i>	SFC20120807-03	Holotype	MN215377	MN215333	MN212825	MN212877	<i>Quercus</i>
	TPML130812-025		MN215378	MN215334	MN212826	MN212878	<i>Quercus</i>
	TPML150817-038		MN215379	MN215335	MN212827	MN212879	<i>Quercus</i>
<i>Lactifluus longistipes</i>	SFC20140822-50	Holotype	MN215380	MN215336	MN212828	MN212880	<i>Quercus</i>
	TPML130730-025		MN215381	MN215337	MN212829	MN212881	<i>Quercus</i>
	TPML130813-004		MN215382	MN215338	MN212830	MN212882	<i>Quercus</i>
<i>Lactifluus orientivolemus</i>	TPML110803-018	Holotype	MN215383	MN215339	MN212831	MN212883	<i>Pinus, Quercus</i>
<i>Lactifluus porphyreus</i>	SFC20160726-60	Holotype	MN215384	MN215340	MN212832	MN212884	<i>Pinus</i>
	TPML130729-005		MN215385	MN215341	MN212833	MN212885	<i>Pinus</i>
	TPML150817-059		MN215386	MN215342	MN212834	MN212886	<i>Pinus</i>
<i>Lactifluus rugiformis</i>	SFC20150818-14	Holotype	MN215387	MN215343	MN212835	MN212887	<i>Quercus</i>
<b>sect. <i>Piperati</i></b>							
<i>Lactifluus involutus</i>	SFC20150818-15		MN215388	MN215344	MN212836	MN212888	<i>Quercus</i>
	SFC20160726-95	Holotype	MN215389	MN215345	MN212837	MN212889	<i>Quercus</i>
<i>Lactifluus quercicola</i>	SFC20130719-29	Holotype	MN215390	MN215346	MN212838	MN212890	<i>Quercus</i>
	TPML130729-023		MN215391	MN215347	MN212839	MN212891	<i>Quercus</i>
	TPML130729-035		MN215392	MN215348	MN212840	MN212892	<i>Quercus</i>
<i>Lactifluus subquercicola</i>	HCCN10587		MN215393	MN215349	MN212841	MN212893	<i>Quercus</i>
	TPML110818-058		MN215394	MN215350	MN212842	MN212894	<i>Quercus</i>
	TPML120730-006	Holotype	MN215395	MN215351	MN212843	MN212895	<i>Quercus</i>
<i>Lactifluus subviridilacteus</i>	SFC20140827-34		MN215396	MN215352	MN212844	MN212896	<i>Quercus</i>
	SFC20140828-19	Holotype	MN215397	MN215353	MN212845	MN212897	<i>Quercus</i>
<i>Lactifluus undulatus</i>	TPML110803-086		MN215398	MN215354	MN212846	MN212898	<i>Pinus</i>
	TPML130729-041	Holotype	MN215399	MN215355	MN212847	MN212899	<i>Pinus</i>
<i>Lactifluus viridilacteus</i>	HCCN12866		MN215400	MN215356	MN212848	MN212900	<i>Quercus</i>
	SFC20130808-37		MN215401	MN215357	MN212849	MN212901	<i>Quercus</i>
	SFC20150819-08	Holotype	MN215402	MN215358	MN212850	MN212902	<i>Quercus</i>
<i>Lactifluus</i> sp. 1	TPML120808-056		MN215403	MN215359	MN212851	MN212903	<i>Quercus</i>

The type specimens are indicated as "Holotype".

<sup>a</sup>Abbreviations of voucher collections. HCCN: National Institute of Agricultural Sciences; KA: Korea National Arboretum; SFC: Seoul National University; and TMPL: Kangwon National University.

*Lactifluus* samples and six *Lactarius* species as outgroups. The concatenated dataset contained 3,519 bp. The ML and BI analyses generated nearly identical tree topologies with minimal variation in statistical support values. A simplified tree detailing

the relationships between the subgenera of *Lactifluus* is shown in Figure 5. In line with previous studies, the monophyly of the genus *Lactifluus* is well-supported (ML: 100, BI: 1.0), with four well-supported subgenera: *Lf.* subg. *Pseudogymnocarpi*



**Figure 1.** Maximum Likelihood (ML) phylogenetic tree of *Lactifluus* subg. *Pseudogymnocarpi* based on concatenated ITS, nrLSU, *rpb2* and *rpb1* sequence data. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated. The color of circles after the specimen voucher name indicates the continental origin of each species; Africa (orange), Asia (green), Australasia (blue), Europe (brown), Central/South America (violet), North America (red).

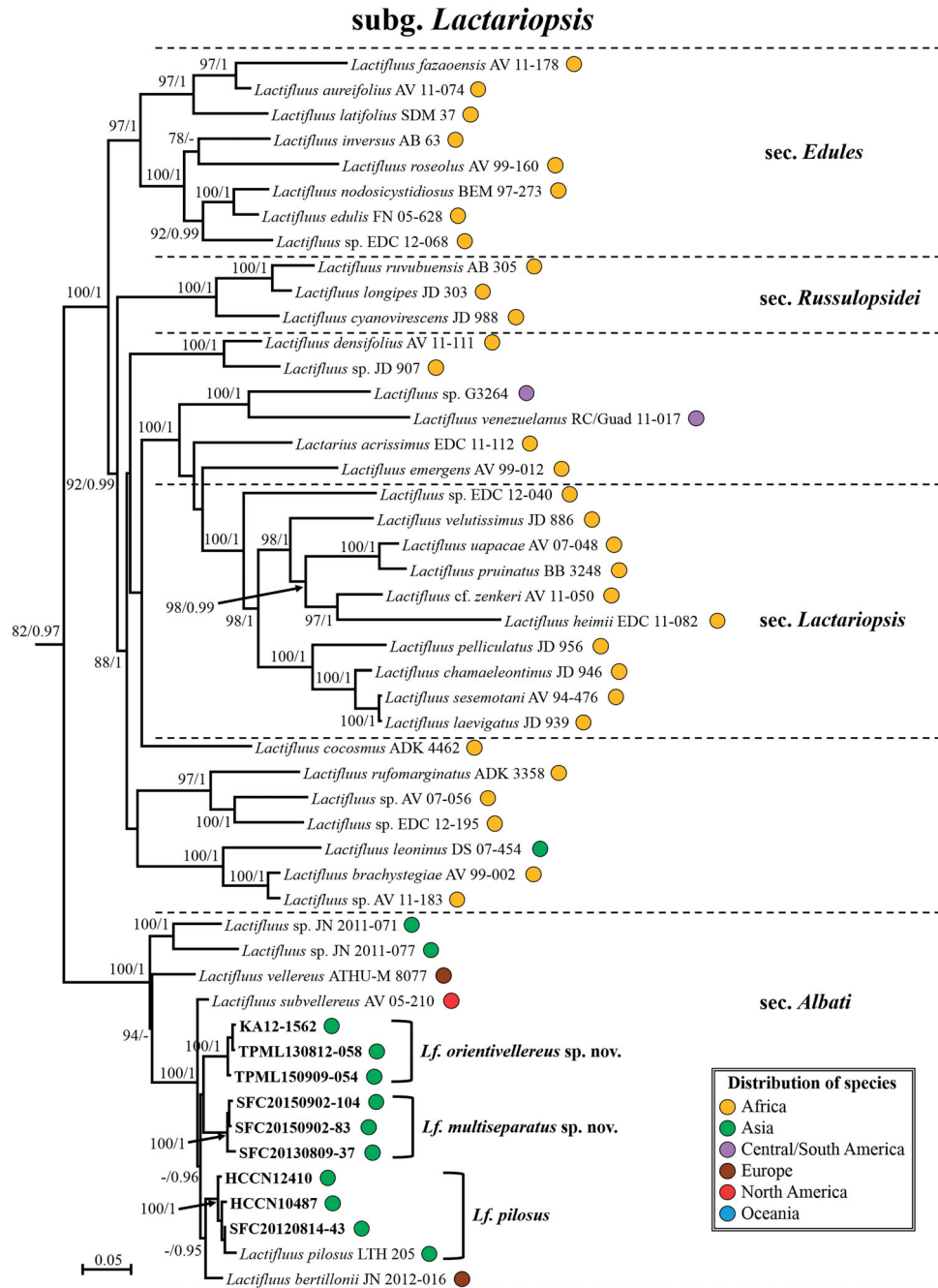
(ML: 100, BI: 1.0), *Lf.* subg. *Lactariopsis* (ML: 82, BI: 0.97), *Lf.* subg. *Gymnocarpi* (ML: 100, BI: 1), and *Lf.* subg. *Lactifluus* (ML: 100, BI: 1). Each subgenus can be further divided into several sections, which is described below with their known morphological characteristics.

Of the 24 putative taxa, five correspond to previously described species: *Lf. acicularis*, *Lf. holophyllus*, *Lf. luteolamellatus*, *Lf. pilosus*, and *Lf. pseudohygrophoroides*. The other 19 taxa did not correspond to any known species. One of these taxa lacks micromorphological data because of immature basiomata and thus remains unnamed until additional data are collected. The other 18 taxa are confirmed as new species after morphological comparison with phylogenetically closely related species. The 24 Korean

species identified in this study are distributed across the four *Lactifluus* subgenera: 3 species in *Lf.* subg. *Pseudogymnocarpi*, 3 in *Lf.* subg. *Lactariopsis*, 2 in *Lf.* subg. *Gymnocarpi*, and 16 in *Lf.* subg. *Lactifluus* (Figure 5).

### 3.2. Classification and morphological characterization of Korean *Lactifluus* species

*Lactifluus* subg. *Gymnocarpi*, originating from Africa and Central-South America, includes four well-supported sections and two unnamed clades (Figure 3). The two Korean species found in this study (*Lf. albidopectinatus* sp. nov. and *Lf. pallidotestaceus* sp. nov.) belonging to *Lf.* sect. *Luteoli*. This section contains species from all continents except Antarctica



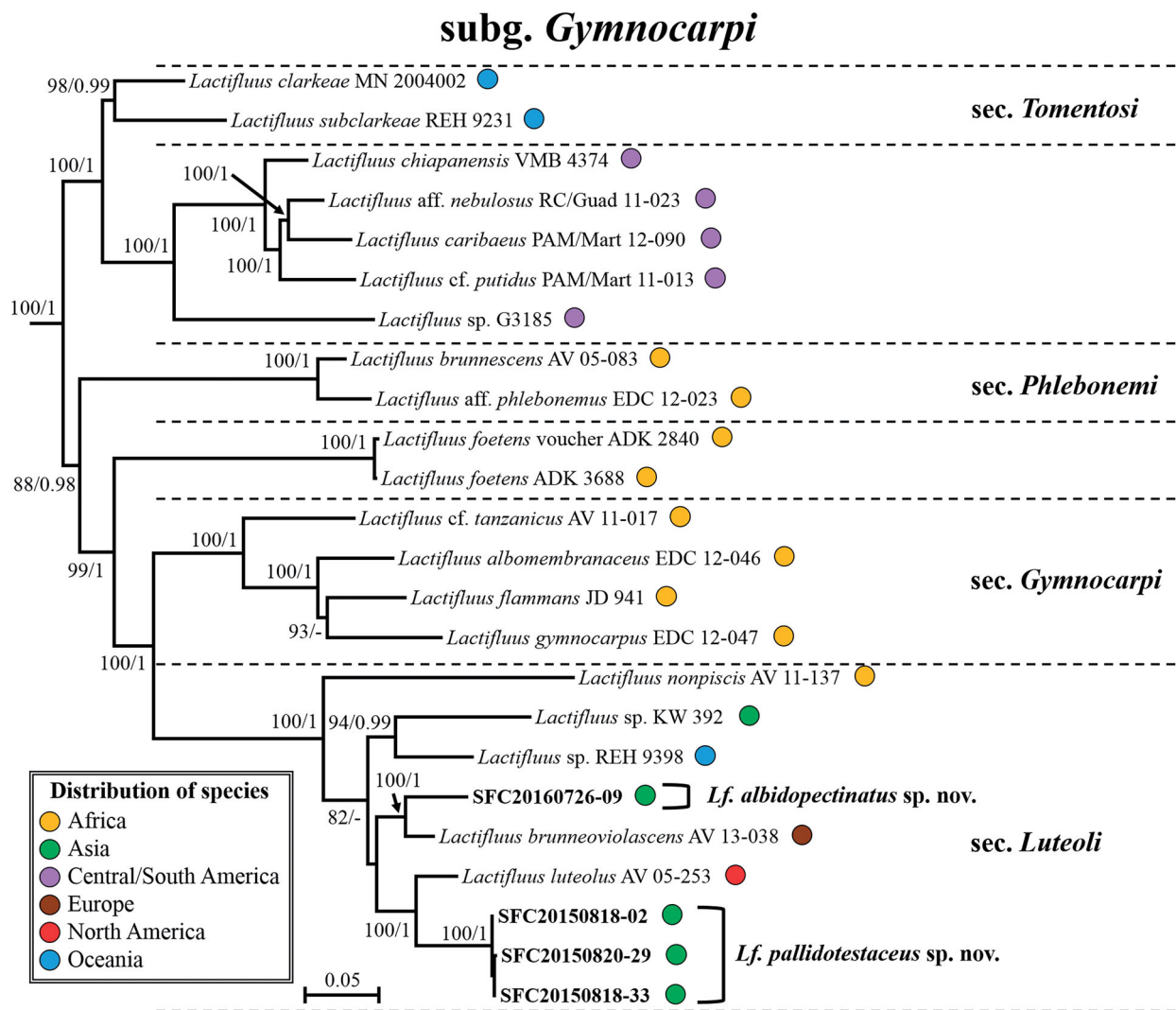
**Figure 2.** Maximum likelihood (ML) phylogenetic tree of *Lactifluus* subg. *Lactariopsis* based on concatenated ITS, nrLSU, *rpb2* and *rpb1* sequence data. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated. The color of circles after the specimen voucher name indicates the continental origin of each species; Africa (orange), Asia (green), Australasia (blue), Europe (brown), Central/South America (violet), North America (red).

and South America. Korean species under this section are characterized by capitate elements in the pileipellis and/or marginal cells, brownish discoloring white latex, and absence of true cystidia.

*Lactifluus* subg. *Lactariopsis* has four well supported sections, some unnamed clades, and several isolated species (Figure 2). The three Korean species (*Lf. multiseparatus* sp. nov., *Lf. orientivellereus* sp. nov., and *Lf. pilosus*) belong to *Lf.* sect. *Albati*. Species in this section are characterized by a whitish and velutinous pileus, a lamprotrichoderm

as pileipellis structure, and the presence of pleuromacrocytidia.

*Lactifluus* subg. *Lactifluus* is the most diverse subgenus with four well-supported sections and two isolated clades in our analysis. This subgenus lacks African taxa. Three Korean species (*Lf. betulicola* sp. nov., *Lf. luminosus* sp. nov., and *Lf. stellatus* sp. nov.) belong to *Lf.* sect. *Gerardii*. These Korean species share characters like spore ornamentation forming a complete reticulum, a palisade as pileipellis structure, the absence of macrocytidia, distant



**Figure 3.** Maximum Likelihood (ML) phylogenetic tree of *Lactifluus* subg. *Gymnocarpi* based on concatenated ITS, nrLSU, *rpb2* and *rpb1* sequence data. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated. The color of circles after the specimen voucher name indicates the continental origin of each species; Africa (orange), Asia (green), Australasia (blue), Europe (brown), Central/South America (violet), North America (red).

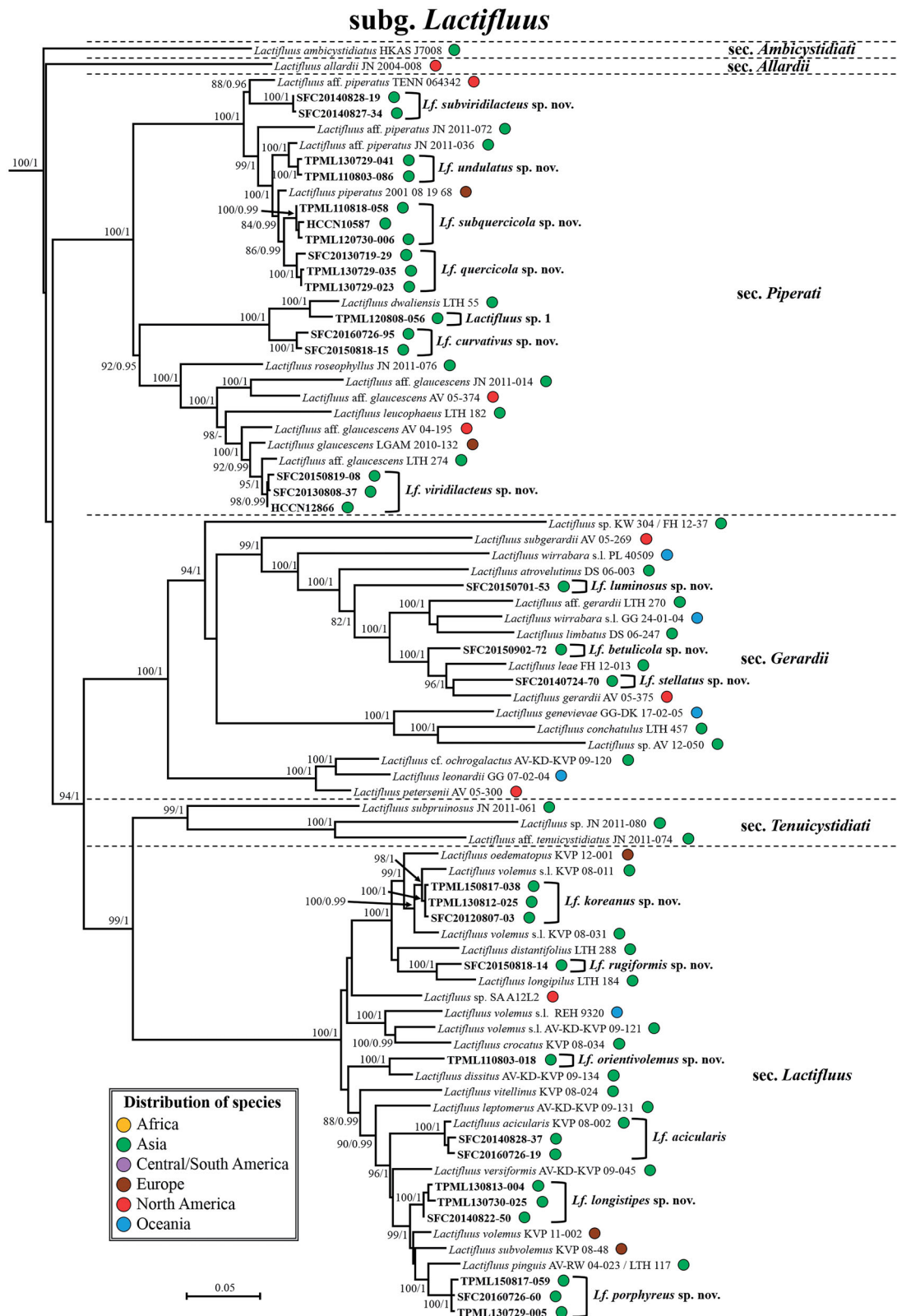
lamellae, and unchanging white latex. Six Korean species (*Lf. acicularis*, *Lf. koreanus* sp. nov., *Lf. longistipes* sp. nov., *Lf. orientivolemus* sp. nov., *Lf. porphyreus* sp. nov., and *Lf. rugiformis* sp. nov.) are members of *Lf.* sect. *Lactifluus*. This section contains species occurring throughout Europe, North America, and Asia that were previously referred to as *Lf. volemus*. Korean species in this section have an orange to orange brown pileus, a lampropalisade as pileipellis structure, pleurolamprocystidia, and brownish discoloring latex. The remaining seven Korean species (*Lf. curvativus* sp. nov., *Lf. quercicola* sp. nov., *Lf. subquercicola* sp. nov., *Lf. subviridilacteus* sp. nov., *Lf. undulatus* sp. nov., *Lf. viridilacteus* sp. nov., and *Lactifluus* sp. 1) belong to *Lf.* sect. *Piperati*. All Korean species in this section are characterized by firm and whitish basidiocarps, thin-walled macrocystidia, a hyphoepithelium as pileipellis structure comprising dermatocystidia in

the suprapellis, a low spore ornamentation forming an incomplete reticulum, and white latex.

*Lactifluus* subg. *Pseudogymnocarpi* contains four well supported sections and one unnamed clade (Figure 1). The three Korean species (*Lf. holophyllus*, *Lf. Luteolamellatus*, and *Lf. pseudohygrophoroides*) are members of *Lf.* sect. *Pseudogymnocarpi*. This section contains African, European, North American, and Asian species. These Korean species all have a yellowish orange to orange colored pileus, a lampropalisade as pileipellis structure, and lack of true cystidia.

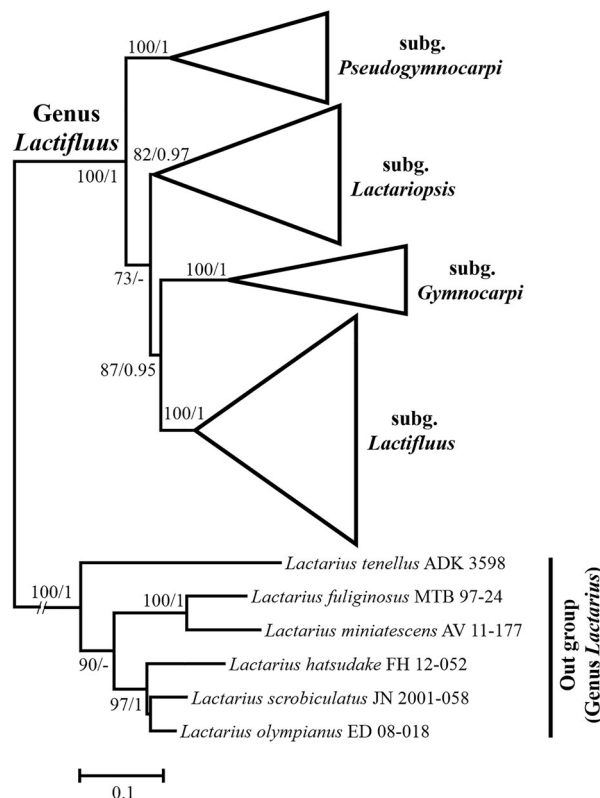
#### 4. Taxonomy

Taxonomic keys to species in South Korea and the full descriptions of the new species are provided below. Abbreviations in Figures 6–9 and 10–23 are as follows: **ba** basidia, **cc** cheilocystidia, **cl**



**Figure 4.** Maximum Likelihood (ML) phylogenetic tree of *Lactifluus* subg. *Lactifluus* based on concatenated ITS, nrLSU, *rpb2* and *rpb1* sequence data. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated. The color of circles after the specimen voucher name indicates the continental origin of each species; Africa (orange), Asia (green), Australasia (blue), Europe (brown), Central/South America (violet), North America (red).





**Figure 5.** Maximum Likelihood (ML) phylogenetic tree of Korean *Lactifluus* inferred using RAxML based on a concatenated dataset of ITS, nrLSU, *rpb2*, and *rpb1*. ML bootstrap support values/Bayesian posterior probability greater than 70%/0.95 are indicated.

cheilolamprocystidia, **cmc** cheilomacrocyctidia, **dc** dermatocystidia, **mc** marginal cells, **pc** pseudocystidia, **plc** pleurolamprocystidia, **pmc** pleuromacrocyctidia, **pp** pileipellis. All scale bars are 10 µm in length, horizontal scale bars are for pileipellis, and vertical scale bars are for other microscopic features.

#### 4.1. Key to the species of *Lactifluus* subgenus *Pseudogymnocarpi* section *Pseudogymnocarpi* in South Korea

- Lamellae adnate to slightly decurrent; pileipellis a lamprolalisade; lack of true cystidia
- 1. Lamellae yellowish; latex white, slowly changing brownish ..... *Lf. luteolamellatus*
- 1. Lamellae whitish to pale cream; latex white, unchanging ..... 2
- 2. Associated with *Abies*; pseudocystidia 3–6 µm broad ..... *Lf. holophyllus*
- 2. Associated with *Pinus* or *Quercus*; pseudocystidia 6–8 µm broad ..... *Lf. pseudohygrophoroides*

#### 4.2. Key to the species of *Lactifluus* subgenus *Gymnocarpi* section *Luteoli*

- Brownish discoloration of latex; no true cystidia; capitate marginal cells and/or pileipellis terminal elements

1. Pileus whitish; pileus margin radially folded to pectinate in age ..... *Lf. albidopectinatus*
1. Pileus grayish red to reddish brown; pileus margin entire ..... *Lf. pallidotestaceus*

#### 4.3. *Lactifluus* subgenus *Gymnocarpi* section *Luteoli*

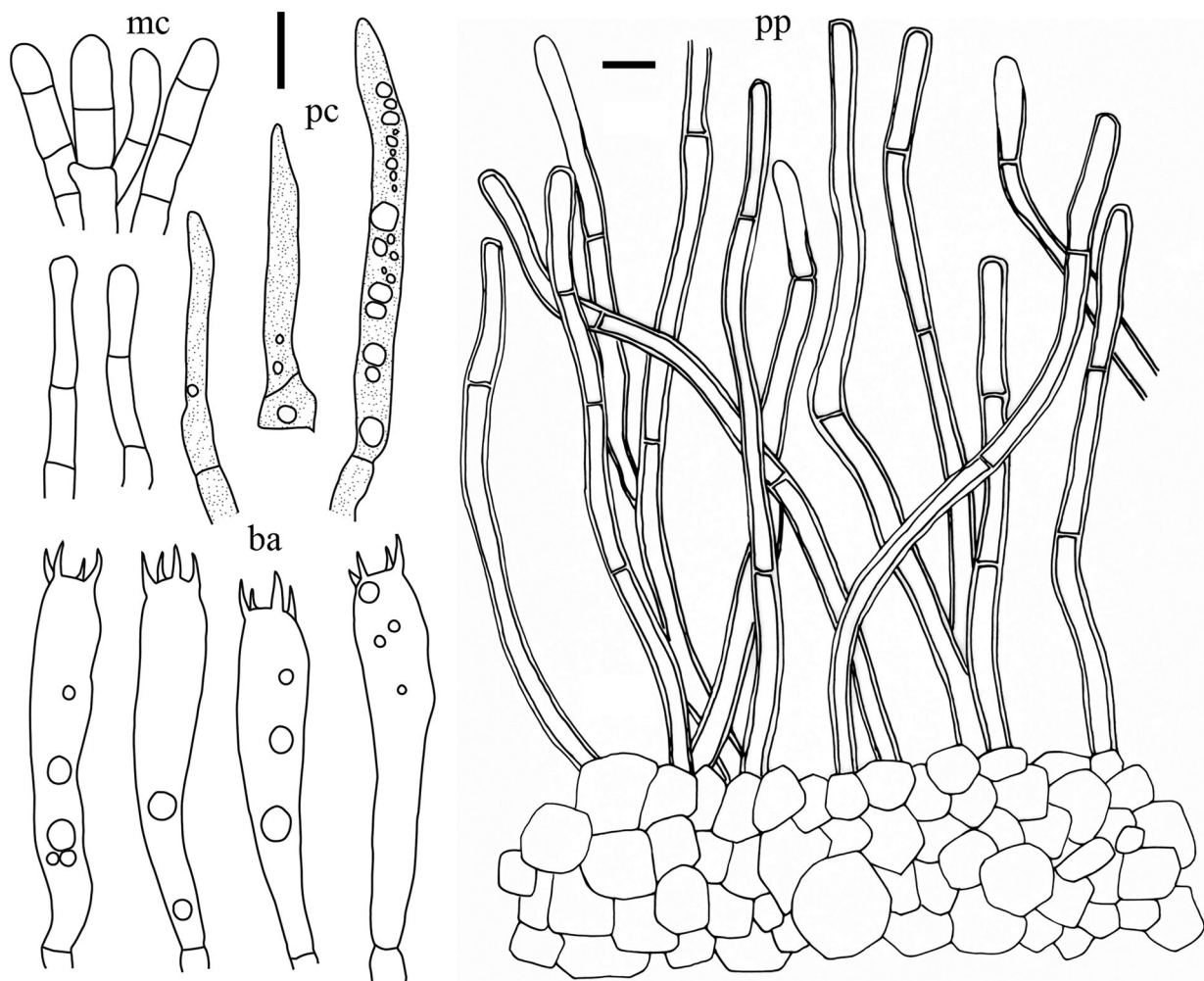
*Lactifluus albidopectinatus* H. Lee & Y. W. Lim **sp. nov.** (Figures 6, 24(g), 25(g))

Mycobank: MB 832062

**Etymology:** “albido” means a little soiled white color and “pectinatus” means comb-shaped. The name refers to the pileus color and pectinate margin.

**Holotypus:** SOUTH KOREA: Incheon-si, Ongjin-gun, Jangbong Island, alt. 70 m, N37°31'55" E126°21'11", 26 Jul 2016, SFC20160726-09 (**holotypus**, SFC)

**Pileus** 25–60 mm diam., convex to depressed with decurved margin; margin even and entire when young, radially folded to pectinate in age; surface dry, slightly tomentose, white (1A-) often tinged with faint ochraceous to light brown. **Lamellae** adnate, sometimes slightly decurrent, rather crowded, never forked, up to 3 mm broad, with 2–4 series of lamellulae between lamellae, whitish. **Stipe** 30–70 × 10–20 mm, cylindrical, sometimes tapering downwards, slightly velvety; surface whitish to



**Figure 6.** Microscopic features of *Lactifluus albidopectinatus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

concolorous with pileus; base with white tomentosum. *Latex* abundant, white changing slowly to brownish.

*Basidiospores* ( $n=20$ ), (8.2–)8.4–9.3–10.1  $\times$  7.6–8.5–9.3  $\mu$ m,  $Q=1.03$ –1.09–1.17, mostly subglobose, occasionally globose or broadly ellipsoid; ornamentation amyloid, composed of coarse ridges and spines connected by lower lines forming an almost completely reticulum, up to 0.4  $\mu$ m high, sharp and irregular, often branched; plage non-amyloid to centrally amyloid. *Basidia* 42.5–53.5  $\times$  8.5–9.5  $\mu$ m, 4-spored, subclavate, filled with guttulate and granular contents. *Pleuromacrocystidia* absent. *Lamellar edge* consisting of marginal cells; marginal cells 9–60.5  $\times$  2.5–5.5  $\mu$ m, often projecting with slightly inflated and rounded apex with brownish contents. *Pseudocystidia* numerous, 3–5  $\mu$ m diam., aseptate, distinctly emergent beyond the hymenium, cylindrical, never branched, with refringent contents; apex tapering. *Pileipellis* 180–280  $\mu$ m thick, a lamp-palisade; terminal elements 25–88  $\times$  3–5  $\mu$ m, parallel to ascending to upright, cylindrical, sometimes with slightly inflated apex, thin to thick-walled;

subpellis composed of isodiametric cells, 10–40  $\mu$ m in diam.

*Habitat*: Scattered to gregarious on soil in mixed forests dominated by *Quercus*.

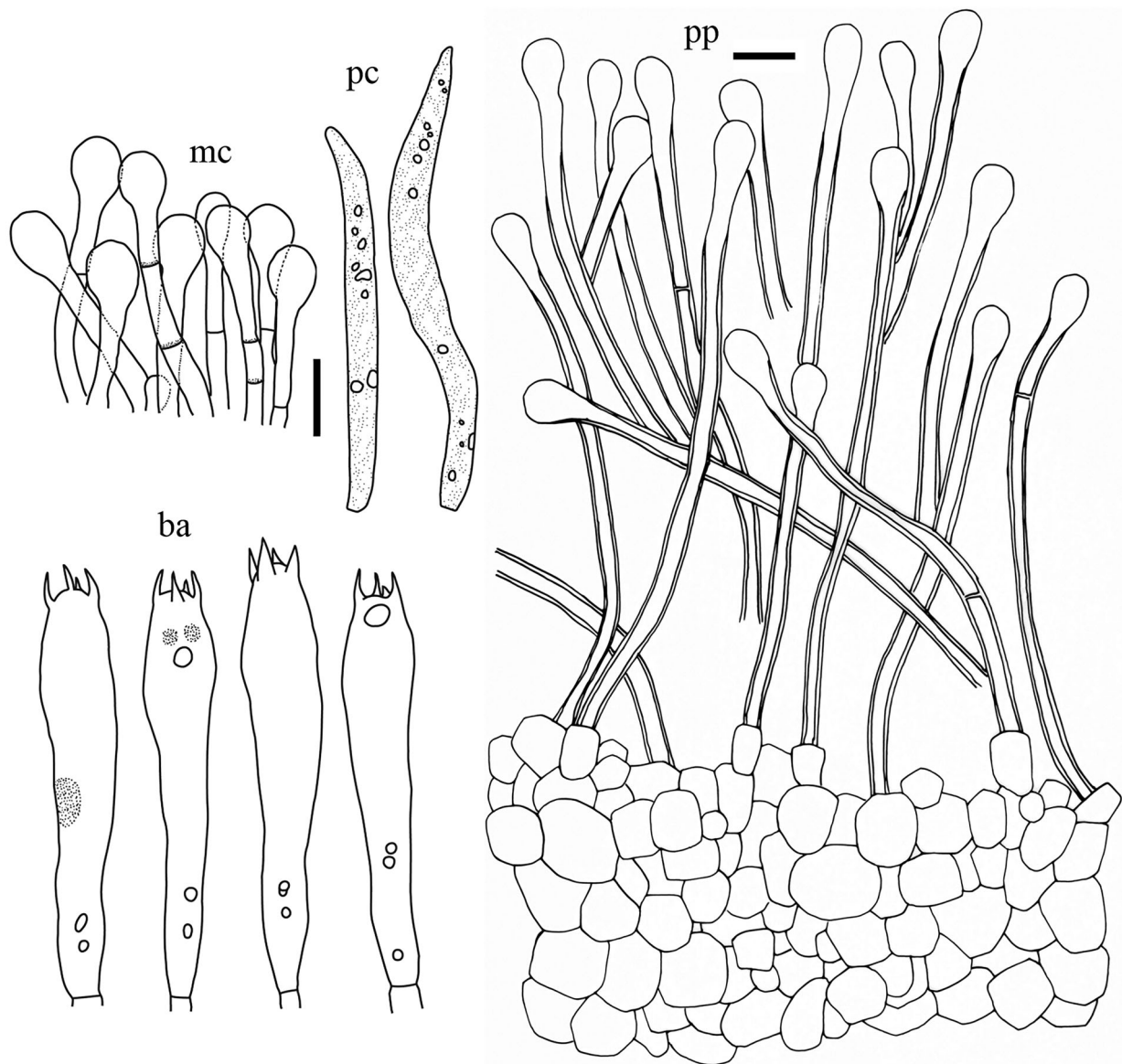
*Comments*: *Lactifluus albidopectinatus* has a whitish colored pileus, which is a rare character of species in sect. *Luteoli*. This species is phylogenetically closely related to *Lf. brunneoviolascens* (Figure 3), a species found in Europe, but differs in the size and shape of basidiospores. The ellipsoid basidiospores of *Lf. brunneoviolascens* are smaller (8–9  $\times$  5.5–6.5  $\mu$ m) than the subglobose spores of *Lf. albidopectinatus* [58] and the terminal elements of the pileipellis are more distinctly capitate.

***Lactifluus pallidotestaceus* H. Lee & Y. W. Lim sp. nov.** (Figures 7, 24(h), 25(h))

Mycobank: MB 832063

*Etymology*: “pallido” means pale and “testaceus” means brick-red color. The name refers to the color of the basidiomata.

*Holotypus*: SOUTH KOREA: Incheon-si, Ganghwa-gun, Mt. Mani, alt. 133 m, N37°36'30''



**Figure 7.** Microscopic features of *Lactifluus pallidotestaceus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

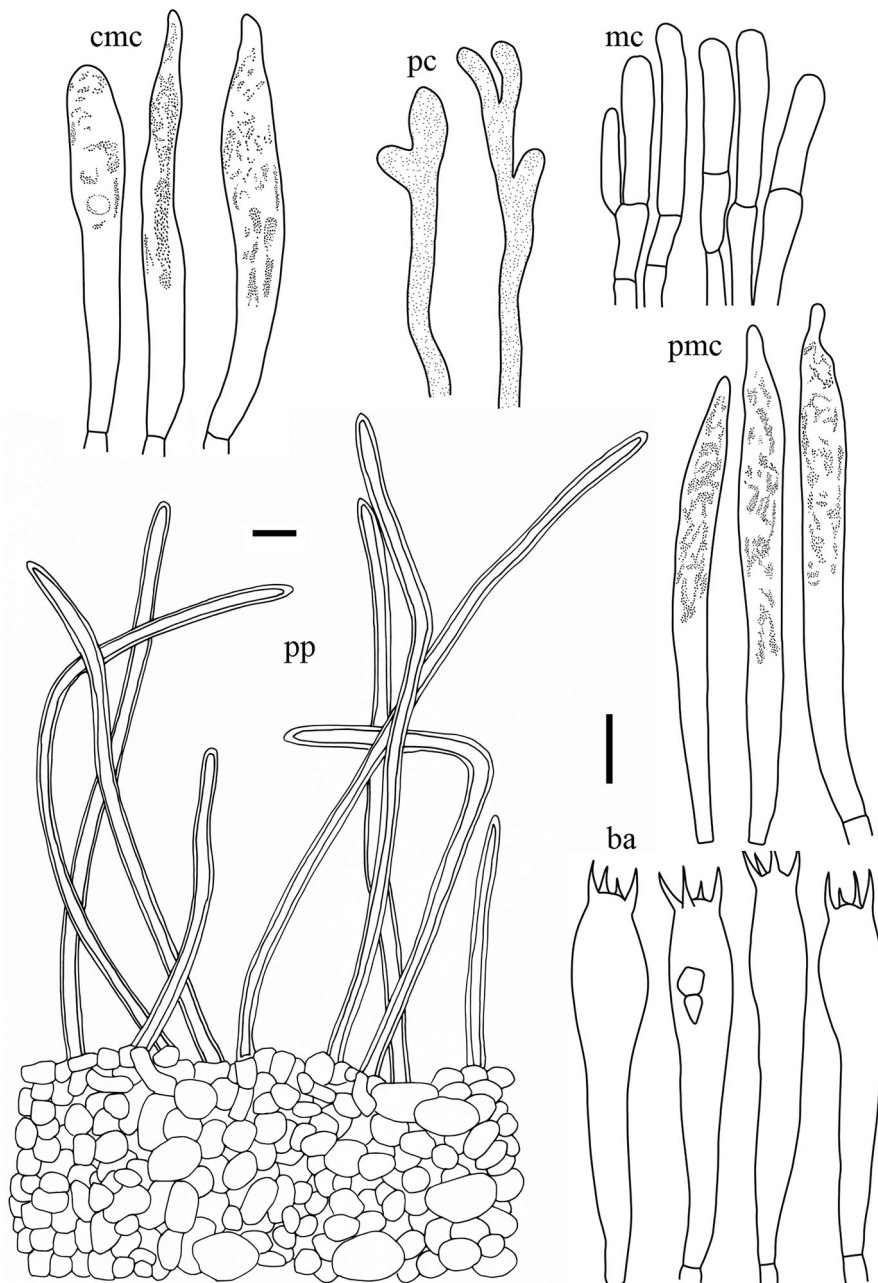
E126°25'51", 18 Aug 2015, SFC20150818-02 (**holotypus**, SFC)

*Pileus* 30–50 mm diam., convex to depressed with decurved margin; margin even and entire; surface dry, velutinous, grayish red (7B3) to reddish brown (8D6) with whitish to faint ochraceous tinges. *Lamellae* adnate to broadly adnate, rather crowded, up to 5 mm broad, with 2–4 series of lamellulae between lamellae, whitish to pale cream. *Stipe* 45–80  $\times$  8–15 mm, cylindrical, sometimes tapering upwards, sometimes irregularly compressed; surface slightly velvety, concolorous with pileus; base with dense white tomentosum. *Latex* abundant, white changing brownish slowly, staining lamellae brownish.

*Basidiospores* ( $n = 40$ ), (7.1–)7.2–7.7–7.8–8.5  $\times$  6.4–6.8–6.9–7.4  $\mu$ m,  $Q = 1.03$ –1.13–1.25, subglobose to broadly ellipsoid; ornamentation amyloid up to 0.5  $\mu$ m high, composed of lower short ridges and

warts; rarely branched, never forming a reticulum; plage centrally amyloid. *Basidia* 56.5–64  $\times$  9–10.5  $\mu$ m, 4-spored, subclavate, mostly filled with guttulate and granular contents. *Pleuromacrocytidia* absent. *Lamellar edge* consisting of marginal cells; marginal cells 10–40  $\times$  4–7.5  $\mu$ m, subcylindrical to subclavate, some projecting with strongly inflated, capitate apex with brownish contents. *Pseudocystidia* rare, 2.5–5  $\mu$ m diam., aseptate, distinctly emergent beyond the hymenium, cylindrical, never branched, with refringent contents; apex tapering, *Pileipellis* 210–300  $\mu$ m thick, a lampropalisade; terminal elements ascending to upright, cylindrical with inflated and rounded, capitate apex, thin- to thick-walled, 19.5–50  $\times$  3–6 (up to 8.5 at the apex)  $\mu$ m; subpellis composed of isodiametric cells, 5–18.5  $\mu$ m in diam.

*Habitat*: Scattered to gregarious on soil in mixed forests dominated by *Quercus*.



**Figure 8.** Microscopic features of *Lactifluus multiseparatus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

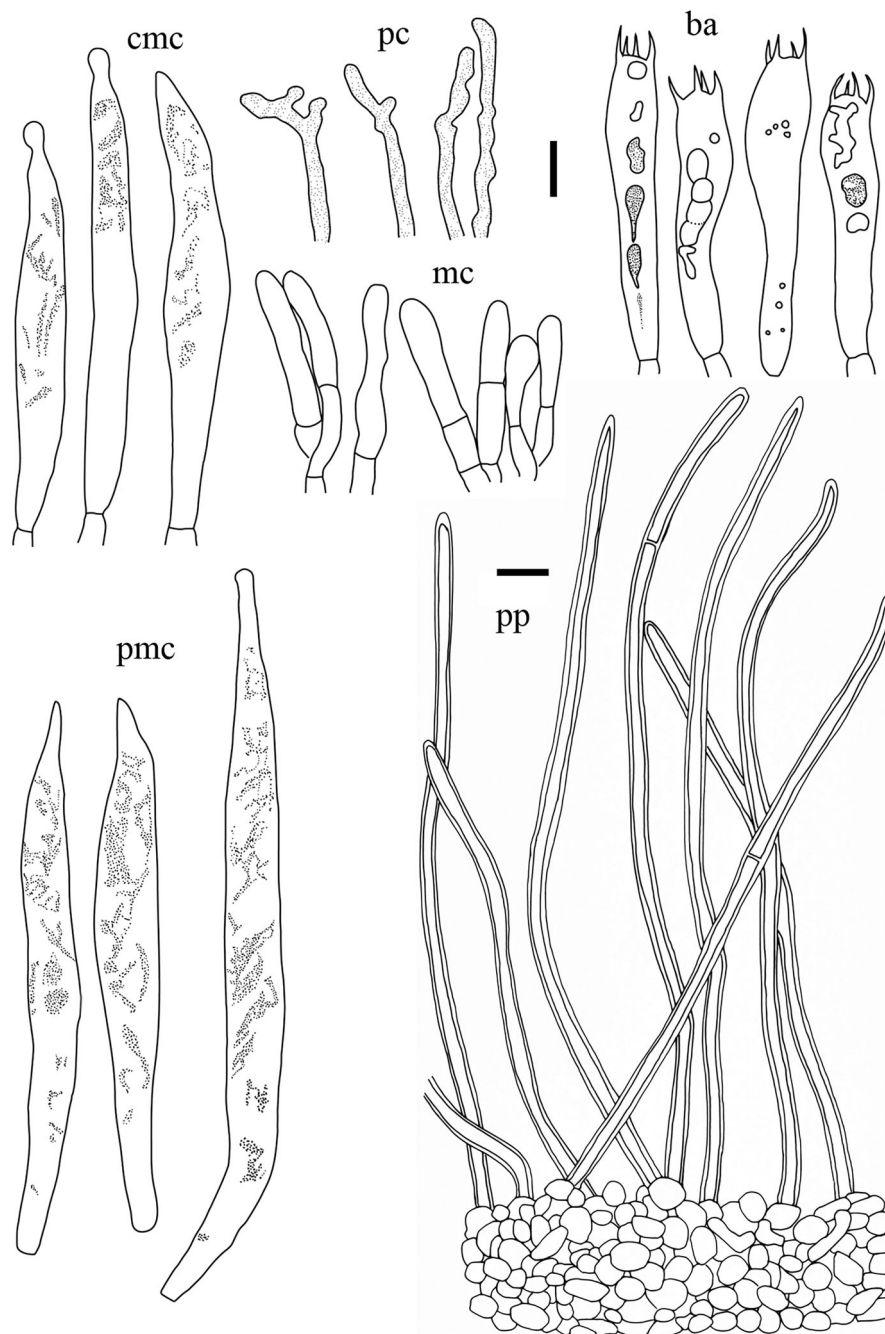
*Additional studied material:* SOUTH KOREA: Incheon-si, Ganghwa-gun, Mt. Mani, alt. 183 m, N37°36'32" E126°26'05", 18 Aug 2015, SFC20150818-33 (SFC); Gyeonggi-do, Gwacheon-si, Mt. Cheonggye, alt. 537 m, N37°25'15" E127°02'37", 20 Aug 2015, SFC2015082-29 (SFC).

*Comments:* In the field, *Lf. pallidotestaceus* is easily recognized by its medium-sized, apricot colored velutinous pileus, brown staining latex, and warty basidiospores. This species differs from the only other Korean species in the same section (*Lf. albidopectinatus*) by the apricot colored pileus and the distinctly capitate marginal cells. This species is phylogenetically closely related to *Lf. luteolus* from North America (Figure 3). *Lactifluus pallidotestaceus*

has subglobose to broadly ellipsoid basidiospores and lacks cheilocystidia, while *Lf. luteolus* has ellipsoid basidiospores and cheilocystidia in the lamellar edge [43]. *Lactifluus luteolus* also lacks the reddish colors in the pileus, which is off-white to buff.

#### 4.4 Key to the species of *Lactifluus* subgenus *Lactariopsis* section *Albati*

- Firm and white basidiocarps; trichoderm or lamp-rotrichoderm as pileipellis; presence of macrocystidia
- 1. Lamellae grayish cream, distant; latex white, changing pale yellow when dry..... *Lf. pilosus*



**Figure 9.** Microscopic features of *Lactifluus orientivellereus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

1. Lamellae white, crowded; latex white, unchanging.....2
2. Associated with *Betula*; pseudocystidia 4–8  $\mu$ m broad.....*Lf. multiseparatus*
2. Associated with *Abies*; pseudocystidia 2–4.5  $\mu$ m broad.....*Lf. orientivellereus*

#### 4.5. *Lactifluus* subgenus *Lactariopsis* section *Albati*

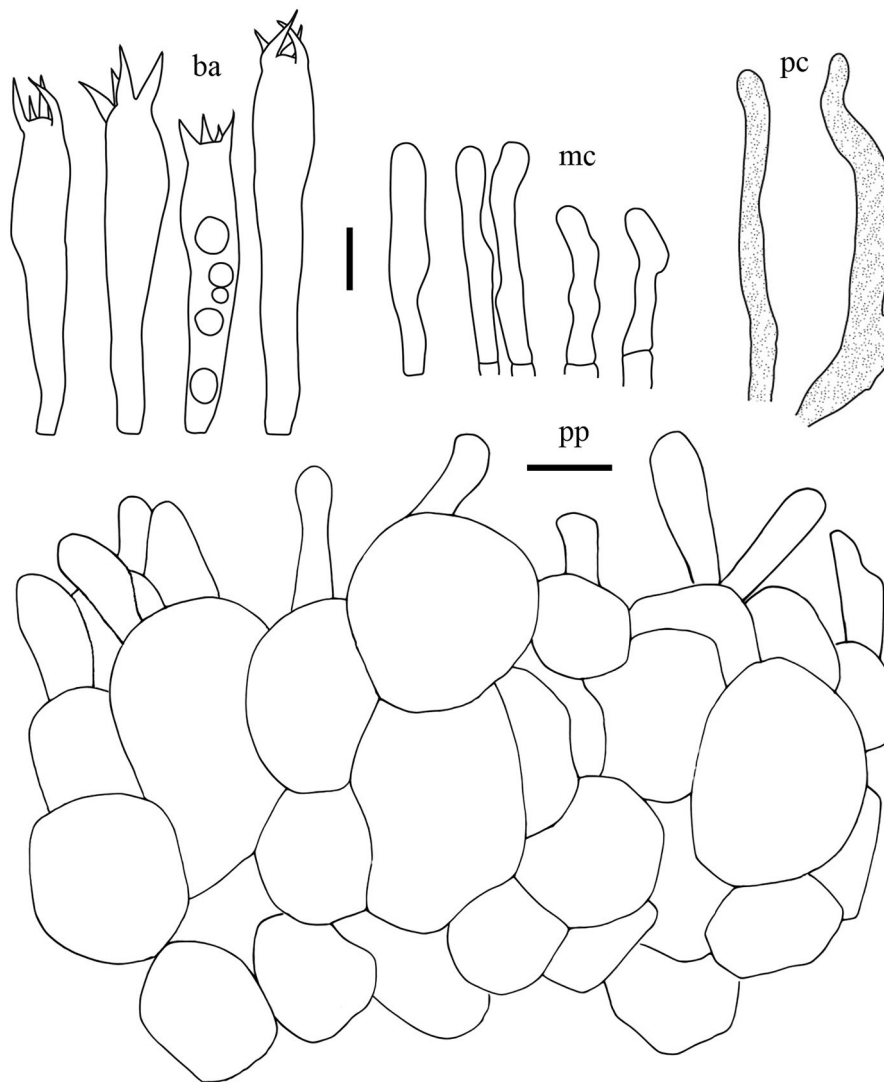
*Lactifluus multiseparatus* H. Lee & Y. W. Lim sp. nov. (Figures 8, 24(d), 25(d))

Mycobank: MB 832064

*Etymology*: “multi” means many and “separatus” means furcate. The name refers to the multi-furcate lamellae.

*Holotypus*: SOUTH KOREA: Gangwon-do, Inje-gun, Wondae-ri birch forest, alt. 549 m, N37°59'31" E128°12'45", 2 Sep 2015, SFC20150902-104 (**holotypus**, SFC)

*Pileus* 50–110 mm, convex when young, later slightly depressed in the center; margin inrolled and entire; surface dry, slightly velvety, white (1 A-) with pale cream to brownish spots in age. *Lamellae* decurrent, crowded, up to 2 mm broad, multi-furcate, with lamellulae of different lengths, whitish to pale cream. *Stipe* 40–80  $\times$  15–30 mm, cylindrical to



**Figure 10.** Microscopic features of *Lactifluus betulicola* sp. nov. All scale bars = 10  $\mu\text{m}$ . Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

slightly tapering downwards, often irregularly compressed, central, sometimes eccentric; surface dry, velvety, and concolorous with pileus. *Latex* transparent changing to white in a few second, changing pale yellow slowly, staining lamellae brownish.

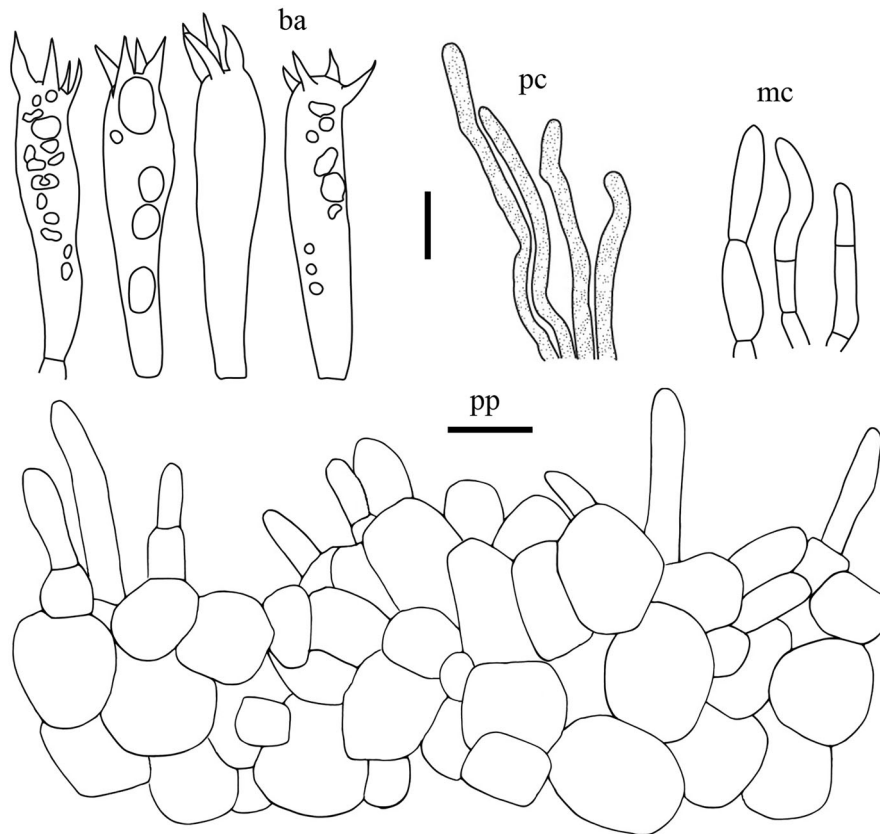
*Basidiospores* ( $n = 60$ ) (7.0–)7.1–8.0–8.1–9.1  $\times$  (5.6–)5.8–6.7–7.6  $\mu\text{m}$ ,  $Q = 1.04$ –1.19–1.30, subglobose to broadly ellipsoid; ornamentation amyloid, mostly up to 0.2  $\mu\text{m}$  high, consisting of ridges forming an incomplete reticulum; some ridges isolated; isolated warts common. *Basidia* 53.5–68.5  $\times$  8.5–12  $\mu\text{m}$ , 4-spored, subclavate, filled with granular contents. *Pleuromacrocystidia* 66–96.5  $\times$  6–10  $\mu\text{m}$ , subfusiform, abundant; apex obtuse, rarely moniliform. *Lamella edge* sterile with cheilomacrocystidia and marginal cells; cheilomacrocystidia 56.5–73.5  $\times$  6–10  $\mu\text{m}$ , narrowly clavate, abundant, with apex obtuse to subacute; marginal cells 12.5–30.5  $\times$  4–8  $\mu\text{m}$ , cylindrical to subclavate, 1–2 septate. *Pseudocystidia* rather abundant, 4–8  $\mu\text{m}$  diam., aseptate, indistinctly emergent beyond the hymenium,

cylindrical, often branched, with refringent contents; apex rounded or inflated. *Pileipellis* a lamprotrichoderm, 120–280  $\mu\text{m}$  thick; terminal elements 40–210  $\times$  4–7  $\mu\text{m}$ , parallel to erect, cylindrical, rarely 1–2 septate, thin to thick walled; subpellis composed of isodiametric cells, 4–22  $\mu\text{m}$  in diam.

*Habitat*: Scattered to gregarious on soil in mixed forests dominated by *Betula*.

*Additional studied material*: SOUTH KOREA: Gangwon-do, Inje-gun, Wondae-ri birch forest, alt. 493 m, N37°59'28" E128°13'26", 2 Sep 2015, SFC20150902-83 (SFC); Gyeongsangbuk-do, Sangjusi, Seongjubong Natural Recreation Forest, alt. 264 m, N36°31'59" E128°02'24", 9 Aug 2013, SFC20130809-37 (SFC).

*Comments*: *Lactifluus multiseparatus* is characterized by whitish basidiomata with transparent latex that changes yellowish when isolated and has brownish discoloration on lamellae. Another white Korean species (*Lf. orientivellereus*) is phylogenetically closely related to *Lf. multiseparatus* (Figure 2).



**Figure 11.** Microscopic features of *Lactifluus luminosus* sp. nov. All scale bars = 10  $\mu\text{m}$ . Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

However, the former species is distinguished from the latter species by unchanging white latex and less interconnection of spore ornamentation.

***Lactifluus orientivellereus* H. Lee & Y. W. Lim sp. nov.** (Figures 9, 24(e), 25(e))

Mycobank: MB 832065

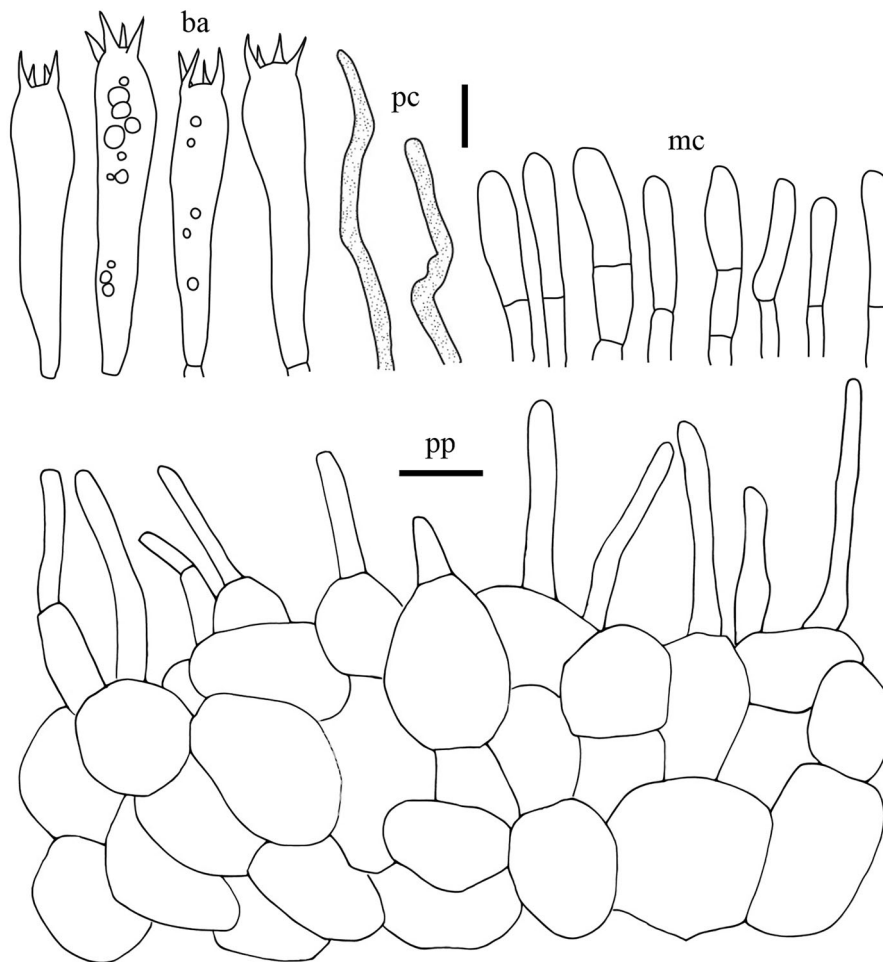
**Etymology:** “orienti” means eastern and ‘vellereus’ refers to the European *Lactifluus vellereus*. This name refers to the similar morphology with *Lf. vellereus* and its origin in East Asia (South Korea).

**Holotypus:** SOUTH KOREA: Gangwon-do, Hongcheon-gun, Mt. Baegam, alt. 937 m, N37°50'50" E128°10'44", 9 Sep 2015, TPML150909-054 (**holotypus**, TPML; **isotypus**, SFC)

**Pileus** 80–170 mm, convex when young, later infundibuliform and deeply depressed in the center; margin inrolled when young, expanding in age, sometimes wavy; surface dry, velvety, white (A1-) tinged with ochreous to yellowish brown. **Lamellae** slightly decurrent, very crowded, up to 3 mm broad, sometimes forked, with abundant lamellulae of different lengths, white turning grayish yellow when bruised. **Stipe** 10–45  $\times$  10–25 mm, cylindrical to slightly tapering downwards, central, sometimes eccentric; surface dry, velvety, and concolorous with pileus. **Latex** white, unchanging.

**Basidiospores** ( $n=40$ ) (6.8–)7.2–8.2–8.4–9.6  $\times$  (5.8–)6.0–6.4–6.5–7.3(–7.5)  $\mu\text{m}$ ,  $Q=1.08$ –1.26–1.48, subglobose to ellipsoid; ornamentation amyloid, mostly up to 0.2  $\mu\text{m}$  high, consisting of irregular or linear warts, often connected by an incomplete to almost complete reticulum; isolated warts sometimes present; plage non-amyloid. **Basidia** 46–61  $\times$  8–11  $\mu\text{m}$ , 4-spored, subcylindrical to subclavate, filled with guttulate and granular contents. **Pleuromacrocystidia** 76–110.5  $\times$  8–13  $\mu\text{m}$ , subfusiform to narrowly fusiform, abundant; apex obtuse, subacute to moniliform. **Lamella edge** sterile with cheilomacrocystidia and marginal cells; cheilomacrocystidia 67–87  $\times$  9–11.5  $\mu\text{m}$ , narrowly clavate, abundant, apex obtuse to moniliform; marginal cells 11–36  $\times$  3.5–8  $\mu\text{m}$ , cylindrical to subclavate, 1–3 septate. **Pseudocystidia** very abundant, 2–4.5  $\mu\text{m}$  diam., aseptate, distinctly emergent beyond the hymenium, cylindrical, often branched, with refringent contents; apex rounded. **Pileipellis** a lamprotrichoderm, 180–300  $\mu\text{m}$  thick; terminal hyphae 40–250  $\times$  3–5  $\mu\text{m}$ , mostly erect, cylindrical, 1–2 septate, thin to thick walled; subpellis composed of filamentous hyphae and isodiametric cells, 5–12  $\mu\text{m}$  in diam.

**Habitat:** Solitary to scattered on soil in *Abies holophylla* forest.



**Figure 12.** Microscopic features of *Lactifluus stellatus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

*Additional studied material:* SOUTH KOREA: Gangwon-do, Pyeongchang-gun, Mt. Odae, alt. 916 m, N37°46'42" E128°34'24", 12 Aug 2013, TPML130812-058 (TPML); Chungcheongnam-do, Geumsan-gun, Mt. Seodae, alt. 352 m, N36°13'45" E127°31'50", 26 Sep 2012, KA12-1562 (KA).

*Comments:* *Lactifluus orientivellereus* is phylogenetically closely related to *Lf. bertillonii*, *Lf. vellereus*, and *Lf. subvellereus* (Figure 2). These latter three species are not found in Asia and can be distinguished from *Lf. orientivellereus* by the more distant lamellae and smaller size of the pleurocystidia: *Lf. bertillonii* (50–75  $\times$  6–12  $\mu$ m), *Lf. vellereus* (50–85  $\times$  6–12  $\mu$ m), and *Lf. subvellereus* (45–60  $\times$  5–7.5  $\mu$ m) [43,44]. *Lactifluus orientivellereus* is also phylogenetically closely related to *Lf. pilosus* found in Asia (Thailand and Korea). However, *Lf. pilosus* interacts with *Castanopsis* or *Lithocarpus* [8], while *Lf. orientivellereus* grows under *Abies* trees. Additionally, *Lf. orientivellereus* has more crowded lamellae compared to the aforementioned four species.

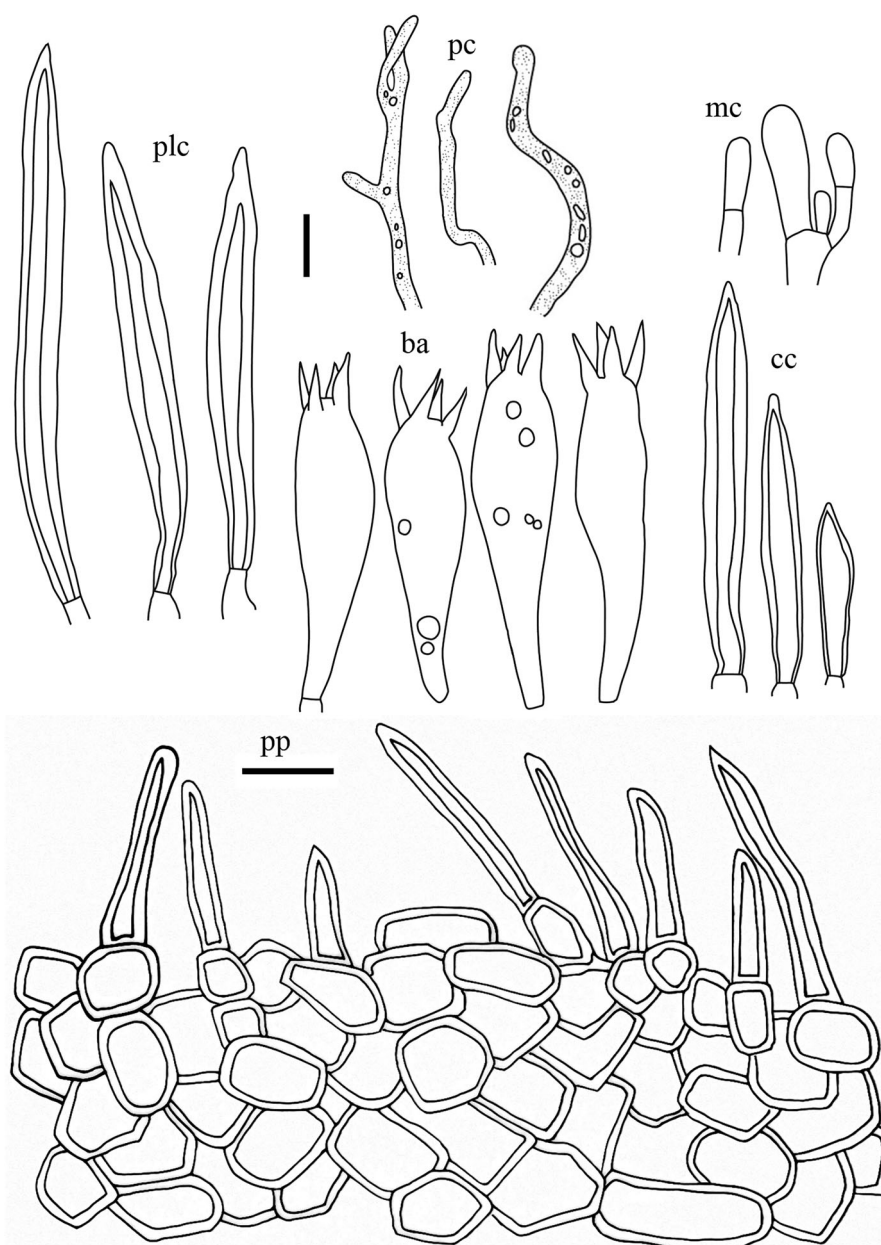
#### 4.6. Key to the species of *Lactifluus subgenus Lactifluus section Gerardii*

- Distant lamellae; white spore print; reticulate spore ornamentation; palisade structure in the pileipellis; no macrocystidia
- 1. Latex yellowish brown to light brown, staining lamellae reddish brown..... *Lf. ochrogalactus*
- 1. Latex white, unchanging..... 2
- 2. Pileus pale yellow to grayish orange; pileipellis 45–80  $\mu$ m thick ..... *Lf. luminosus*
- 2. Pileus brown to dark brown; pileipellis thicker than 70  $\mu$ m..... 3
- 3. Associated with *Betula*; pseudocystidia 4–8  $\mu$ m broad *Lf. betulicola*
- 3. Associated with *Pinus* or *Quercus*; pseudocystidia 2–4  $\mu$ m broad ..... *Lf. stellatus*

#### 4.7. *Lactifluus subgenus Lactifluus section Gerardii*

*Lactifluus betulicola* H. Lee & Y. W. Lim sp. nov. (Figures 10, 26(a), 27(a))





**Figure 13.** Microscopic features of *Lactifluus koreanus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

Mycobank: MB 832066

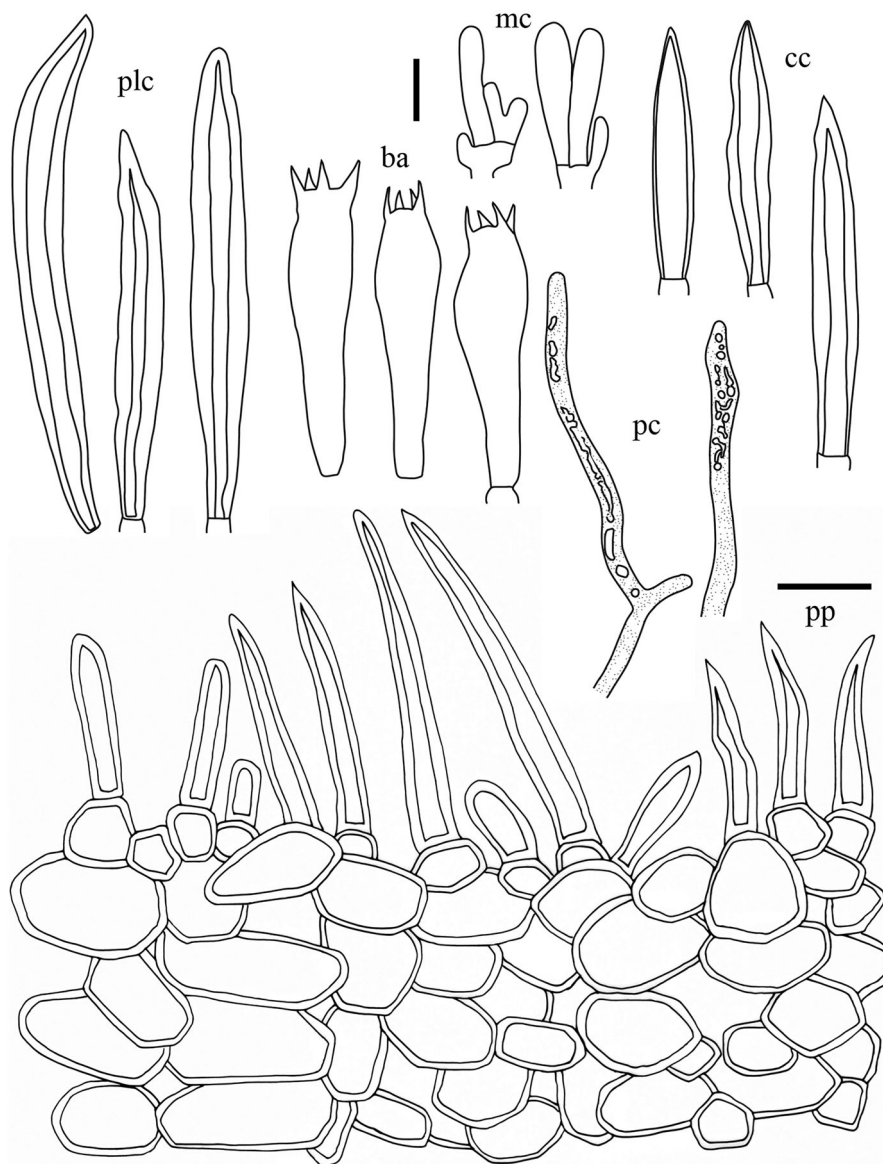
**Etymology:** “betuli” means birch tree and “cola” means dweller. The name refers to the presence of the species in *Betula* forest and the putative ectomycorrhizal association with *Betula*.

**Holotypus:** SOUTH KOREA: Gangwon-do, Inje-gun, Wondae-ri birch forest, alt. 571 m, N37°59'43" E128°11'59", 19 Jul 2013, SFC20150902-72 (**holotypus**, SFC)

**Pileus** 30–70 mm diam., planoconvex to concave, center depressed, sometimes papillate; margin irregularly wavy to slightly crenate; surface dry, velutinous, rugulose to rugose, grayish brown (6E5) to dark brown (7F6), sometimes with whitish spots. **Lamellae** deeply decurrent, distant, up to 7 mm broad, white to pale cream, pale brown in age, not

staining when bruised; decurrent teeth concolorous with stipe apex. **Stipe** 25–50  $\times$  5–10 mm, cylindrical, slightly tapering downwards; surface dry, velutinous, concolorous with pileus, sometimes with whitish spots. **Latex** abundant, watery white, unchanging.

**Basidiospores** ( $n=20$ ) 6.6–7.8–8.5(–8.9)  $\times$  (6.0–)6.1–6.9–7.8  $\mu$ m,  $Q=1.00$ –1.13–1.23, globose to broadly ellipsoid; ornamentation amyloid, mostly up to 0.7  $\mu$ m high, consisting of low and rounded ridges, completely reticulate; isolated warts rare; plage almost totally amyloid. **Basidia** 47–65.5  $\times$  8.5–11  $\mu$ m, subclavate to narrowly subclavate, 4-spored, filled with granular contents. **Macrocystidia** absent. **Lamellar edge** sterile, filled with marginal cells; marginal cells 10–40.5  $\times$  3.5–6  $\mu$ m, narrowly



**Figure 14.** Microscopic features of *Lactifluus longistipes* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

subcylindrical, sometimes slightly tortuous, with rounded apex. *Pseudocystidia* abundant, 4–8  $\mu$ m diam., cylindrical to irregular, sometimes branching. *Pileipellis* a palisade, 85–160  $\mu$ m thick; terminal elements 16.5–29  $\times$  3.5–7  $\mu$ m, subcylindrical, mostly erect, sometimes oblique; subpellis composed of globose to subglobose cells, 9–38  $\mu$ m in diam.

*Habitat:* Solitary to gregarious on soil in deciduous forests dominated by *Betula*.

*Comments:* *Lactifluus betulicola* is phylogenetically closely related to typical sect. *Gerardii* species, *Lf. gerardii* from North America and *Lf. leae* from Thailand (Figure 4). However, this species differs from *Lf. gerardii* by the size of basidiospores and the lack of macrocystidia. *Lactifluus gerardii* has macrocystidia and bigger basidiospores (8–10  $\times$  7.5–9  $\mu$ m) than *Lf. betulicola* [43]. In addition, *Lf. betulicola* is associated with *Betula* trees

while *Lf. leae* grows with *Dipterocarpus*, *Castanopsis*, and *Lithocarpus* [59]. The almost totally amyloid plage is a rather unique character, most often encountered in the pleurotoid species of this section.

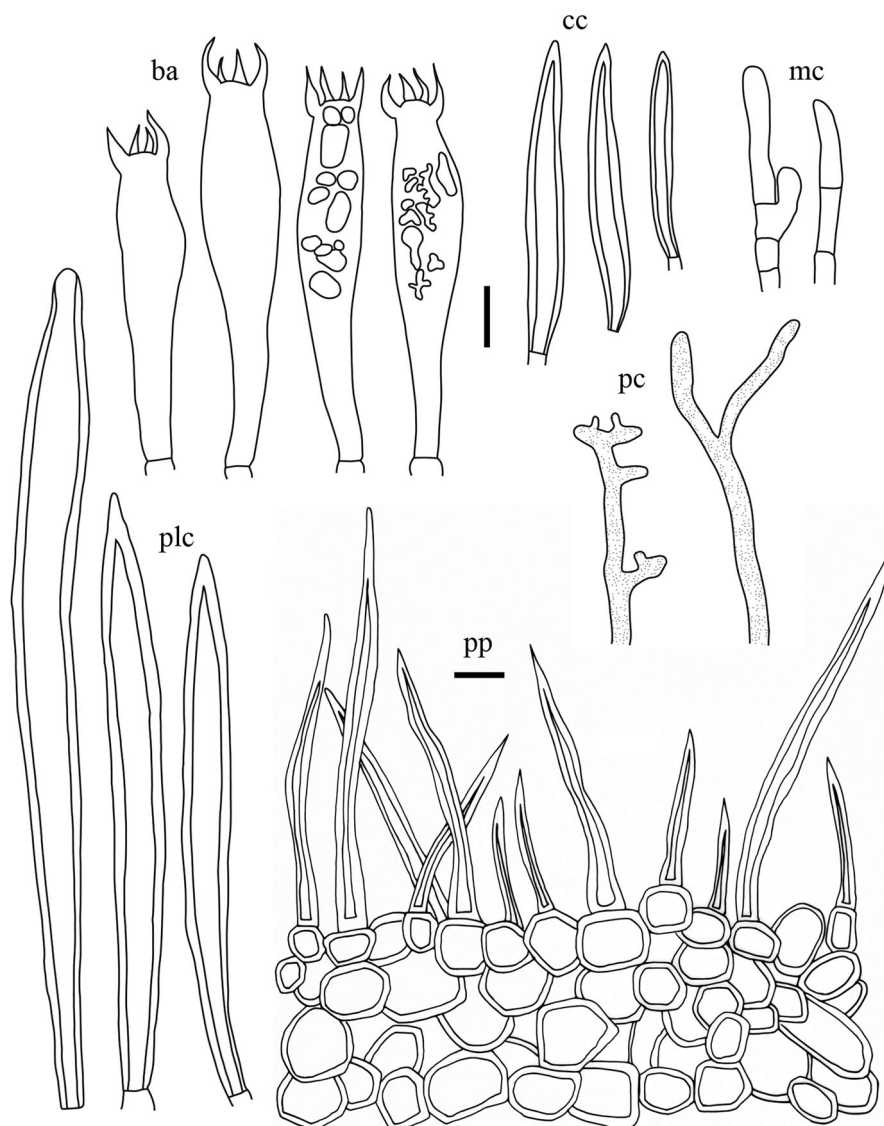
***Lactifluus luminosus*** H. Lee & Y. W. Lim sp. nov. (Figures 11, 26(b), 27(b))

Mycobank: MB 832067

*Etymology:* “luminosus” means bright. The name refers to the bright and yellowish colored pileus.

*Holotypus:* SOUTH KOREA: Jeju-do, Jeju-si, Dongbaek-dongsan wetland, alt. 150 m, N33°30'58" E126°43'23", 1 Jul 2015, SFC20150701-53 (**holotypus**, SFC)

*Pileus* 10–45 mm diam., convex with depressed center to irregularly concave; center with small papilla; surface slightly rugulose when young, becoming strongly radially wrinkled in age; margin



**Figure 15.** Microscopic features of *Lactifluus orientivolemus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

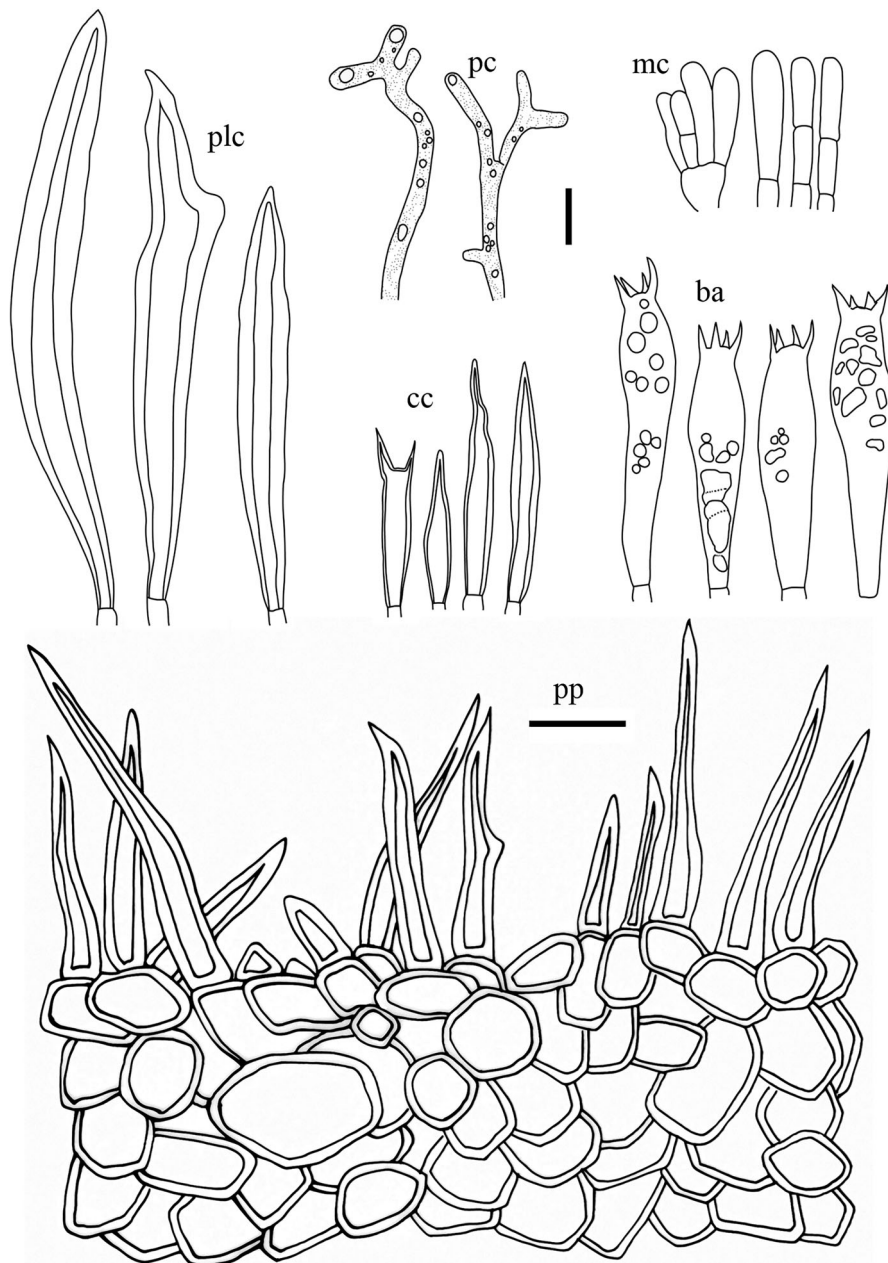
irregularly wavy to slightly crenate; surface, dry, velutinous, pale yellow (4A3) to grayish orange (5B4) with darker center, becoming paler toward margin with whitish spots. *Lamellae* deeply decurrent, distant, up to 8 mm broad, whitish to pale cream colored. *Stipe* 40–95  $\times$  6–20 mm, cylindrical, often tapering downwards, sometimes slightly curved, sometimes subfasciculate to fasciculate with 2 basidiomata with connected stipes; surface dry, faintly longitudinally wrinkled, minutely velutinous, concolorous with pileus or paler, sometimes with whitish spots. *Latex* moderately abundant, white or watery white, unchanging.

*Basidiospores* ( $n = 20$ ) 7.1–8.1–9.1(–9.2)  $\times$  (6.1–)6.5–7.5–8.6  $\mu$ m,  $Q = 1.01$ –1.08–1.15, globose to subglobose; ornamentation amyloid, consisting of low and straight ridges, mostly up to 0.5  $\mu$ m high, completely reticulate, isolated warts very rare; plage totally amyloid. *Basidia* 45–60  $\times$  8–10.5  $\mu$ m,

subclavate, 4-spored, filled with granular contents. *Macrocytidia* absent. *Lamellar edge* sterile, composed of marginal cells; marginal cells 17–25  $\times$  3.5–5  $\mu$ m, subcylindrical to narrowly subcylindrical, tapering toward apex. *Pseudocystidia* numerous, 1.5–3.5  $\mu$ m diameter, cylindrical, sometimes branching; apex obtuse, sometimes irregular. *Pileipellis* a palisade, 45–80  $\mu$ m thick; terminal elements 10–28  $\times$  4.5–6.5  $\mu$ m, subcylindrical, sometimes septate, mostly erect, sometimes oblique; subpellis composed of globose to subglobose cells, 7–22.5 in diam.

*Habitat*: Solitary to gregarious on soil in evergreen *Quercus* forests.

*Comments*: *Lactifluus luminosus* can be easily recognized by its strikingly bright yellow pileus. This species has the lightest basidiomata among the three Korean species (*Lf. luminosus*, *Lf. betulicola*, and *Lf. stellatus*) in the *Lf.* sect. *Gerardii*. In addition, the



**Figure 16.** Microscopic features of *Lactifluus porphyreus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

relatively low ornamentation of basidiospores and short marginal cells are useful characters to identify this species.

***Lactifluus stellatus*** H. Lee & Y. W. Lim **sp. nov.**  
(Figures 12, 26(c), 27(c))

Mycobank: MB 832068

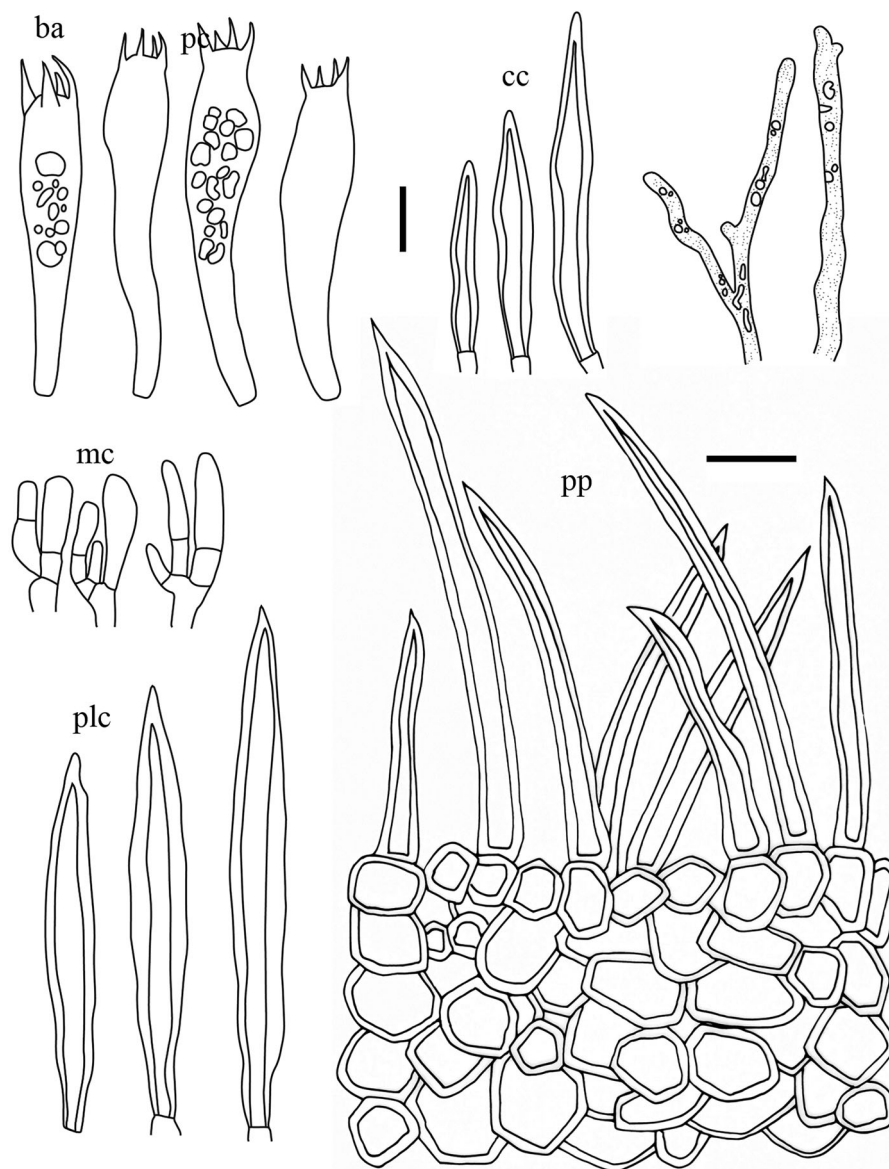
**Etymology:** “stellatus” means star or star shaped. The name refers to numerous, tiny white spots on pileus, which is reminiscent of star clusters.

**Holotypus:** SOUTH KOREA: Jeollabuk-do, Jangsu-gun, Bangwhadong Natural Recreation Forest, alt. 441 m, N35°34'59" E127°31'53", 24 Jul 2014, SFC20140724-70 (**holotypus**, SFC)

**Pileus** 10–40 mm diam., convex to concave when young, irregularly infundibuliform in age, center

depressed, rugulose with radical wrinkles; margin irregularly wavy, often crenate; surface dry, velutinous, light brown (6D4) to dark brown (9F5) with numerous white spots, scattered like a star cluster. **Lamellae** deeply decurrent, distant, up to 12 mm broad, white to pale cream, staining orange brown when bruised; decurrent teeth concolorous with stipe apex. **Stipe** 25–40  $\times$  5–10 mm, subcylindrical, tapering downwards; surface dry, velutinous, concolorous with pileus. **Latex** abundant, watery white, unchanging.

**Basidiospores** ( $n=20$ ) 5.8–7.1–8.4(–8.5)  $\times$  5.4–6.5–7.7(–7.8)  $\mu$ m,  $Q=1.02$ – $1.09$ – $1.15$ , globose to subglobose; ornamentation amyloid, up to 0.3  $\mu$ m high, consisting of low and rounded ridges, completely reticulate; isolated warts rare; plage centrally



**Figure 17.** Microscopic features of *Lactifluus rugiformis* sp. nov. All scale bars = 10  $\mu\text{m}$ . Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

amyloid. *Basidia* 52.5–65.5  $\times$  7.5–11  $\mu\text{m}$ , subclavate, 2- or 4-spored, filled with granular contents. *Macrocystidia* absent. *Lamellar edge* sterile, composed of marginal cells; marginal cells 7.5–31  $\times$  4.5–8  $\mu\text{m}$ , subcylindrical to subclavate, 1–2 septate. *Pseudocystidia* rare, 2–4  $\mu\text{m}$  diam., cylindrical, sometimes branching; apex obtuse. *Pileipellis* a palisade, 70–110  $\mu\text{m}$  thick; terminal elements 8.5–25  $\times$  2–4.5  $\mu\text{m}$ , subcylindrical, sometimes septate, mostly erect, sometimes oblique; subpellis composed of globose to subglobose cells, 5.5–23  $\mu\text{m}$  in diam.

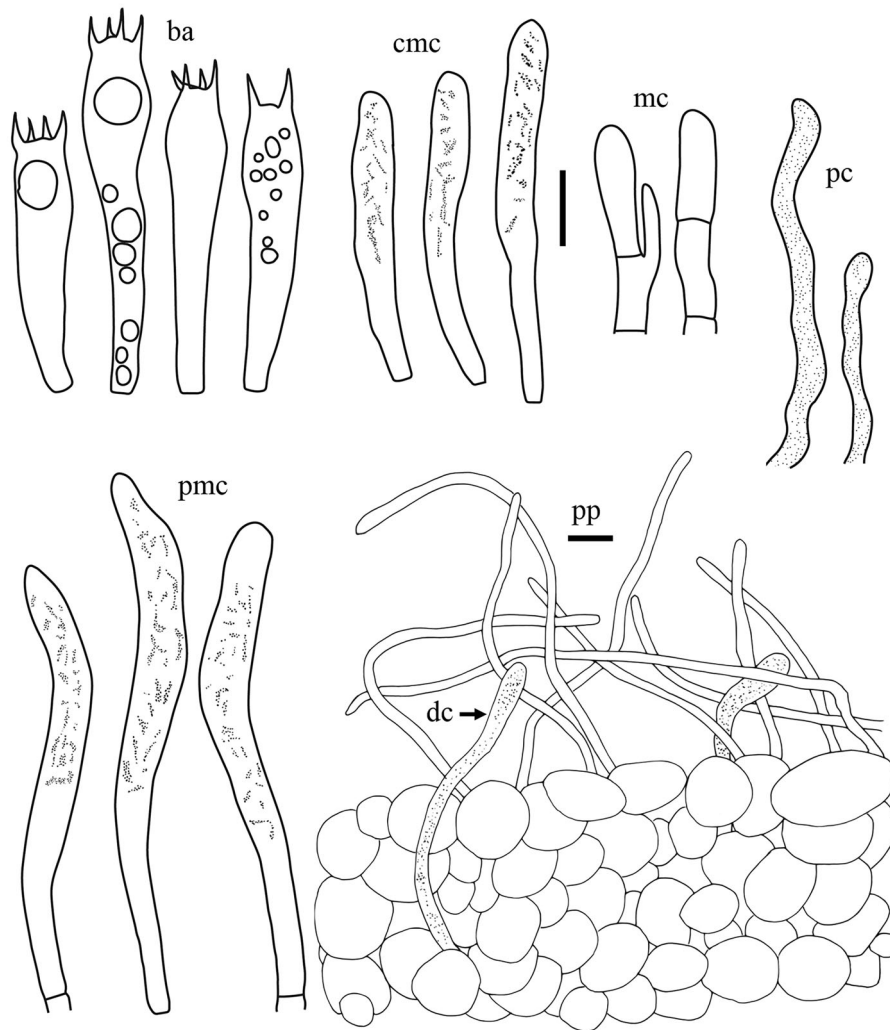
*Habitat:* Solitary to gregarious on soil in mixed forests dominated by *Quercus* and *Pinus*.

*Comments:* *Lactifluus stellatus* is a unique species in the sect. *Gerardii*, and this species can be characterized by the uneven dark brown color of pileus with striking whitish spots, the strong rugulose

surface, and the low ornamentation of reticulate basidiospores. This species is phylogenetically closely related to *Lf. gerardii*, *Lf. leae*, and *Lf. betulicola*, but can be distinguished from the aforementioned three species by the height of spore ornamentation. The basidiospore ornamentation *Lf. gerardii* (0.5–0.8  $\mu\text{m}$ ), *Lf. leae* (0.5–1.0  $\mu\text{m}$ ) and *Lf. betulicola* (up to 0.7  $\mu\text{m}$ ) is distinctly higher than that of *Lf. betulicola* (maximum 0.3  $\mu\text{m}$ ) [41].

#### 4.8. Key to the species of *Lactifluus* subgenus *Lactifluus* section *Lactifluus*

- Grayish-yellow, orange to orange-brown or reddish-brown pileus; white latex discoloring brownish on lamellae; reticulate spore ornamentation; lampropalisade type pileipellis; presence of lamprocystidia



**Figure 18.** Microscopic features of *Lactifluus curvativus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

1. Pileus light yellow to light brown, spore ornamentation up to 2.0  $\mu$ m .....*Lf. pinguis*
1. Pileus darker colored, spore ornamentation never up to 2.0  $\mu$ m ..... 2
2. Pileipellis terminal elements up to 100  $\mu$ m or more ..... 3
2. Pileipellis terminal hyphae never up to 100  $\mu$ m... 4
3. Spore ornamentation up to 1.4  $\mu$ m, pileus brown to orange brown.....*Lf. acicularis*
3. Spore ornamentation up to 0.9  $\mu$ m, pileus light orange..... *Lf. orientivolemus*
4. Subpellis thickness up to 140  $\mu$ m. *Lf. koreanus*
4. Subpellis thickness never up to 140  $\mu$ m..... 5
5. Associated with *Pinus* trees.....*Lf. porphyreus*
5. Associated with *Quercus* trees..... 6
6. Ratio of stipe length/pileus diameter > 1.5, spore ornamentation up to 0.6  $\mu$ m ..... *Lf. longistipes*
6. Ratio of stipe length/pileus diameter < 0.7, spore ornamentation up to 1.7  $\mu$ m ..... *Lf. rugiformis*

#### 4.9. *Lactifluus* subgenus *Lactifluus* section *Lactifluus*

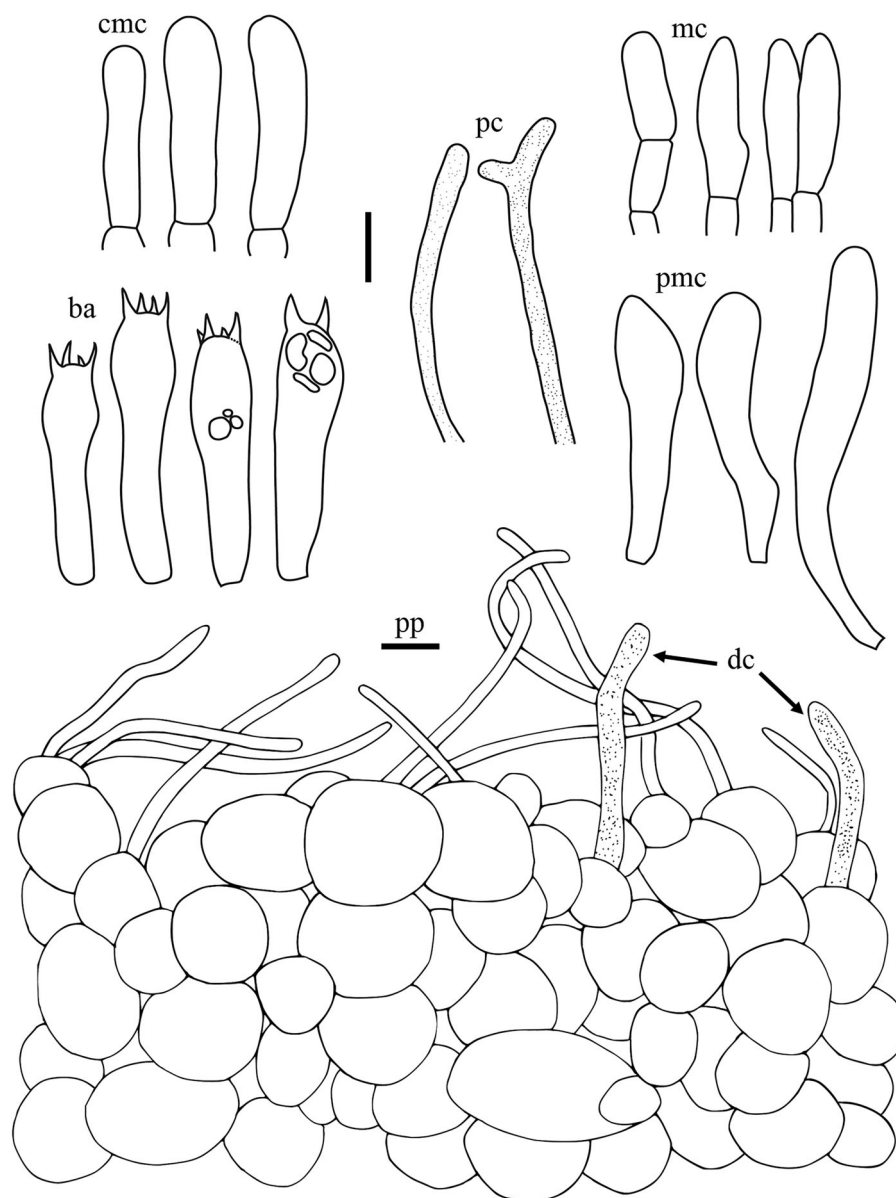
***Lactifluus koreanus*** H. Lee & Y. W. Lim sp. nov. (Figures 13, 26(e), 27(e))

Mycobank: MB 832069

*Etymology*: “koreanus” means Korean. The name refers to the distribution of this species. This species is very common in Korea, including Jeju Island.

*Holotypus*: SOUTH KOREA: Jeju-do, Seogwiposi, Hannam Experimental Forest, alt. 451 m, N33°22'29" E126°39'54", 7 Aug 2015, SFC20120807-03 (**holotypus**, SFC)

*Pileus* 35–85 mm diam., convex when young, becoming applanate with slightly depressed center to infundibuliform when mature, rugulose when young; margin entire, sometimes wavy; surface dry, slightly velutinous, pruinose when young, light orange (6A4) to grayish red (7B6), getting paler toward margin, sometimes with orange brown

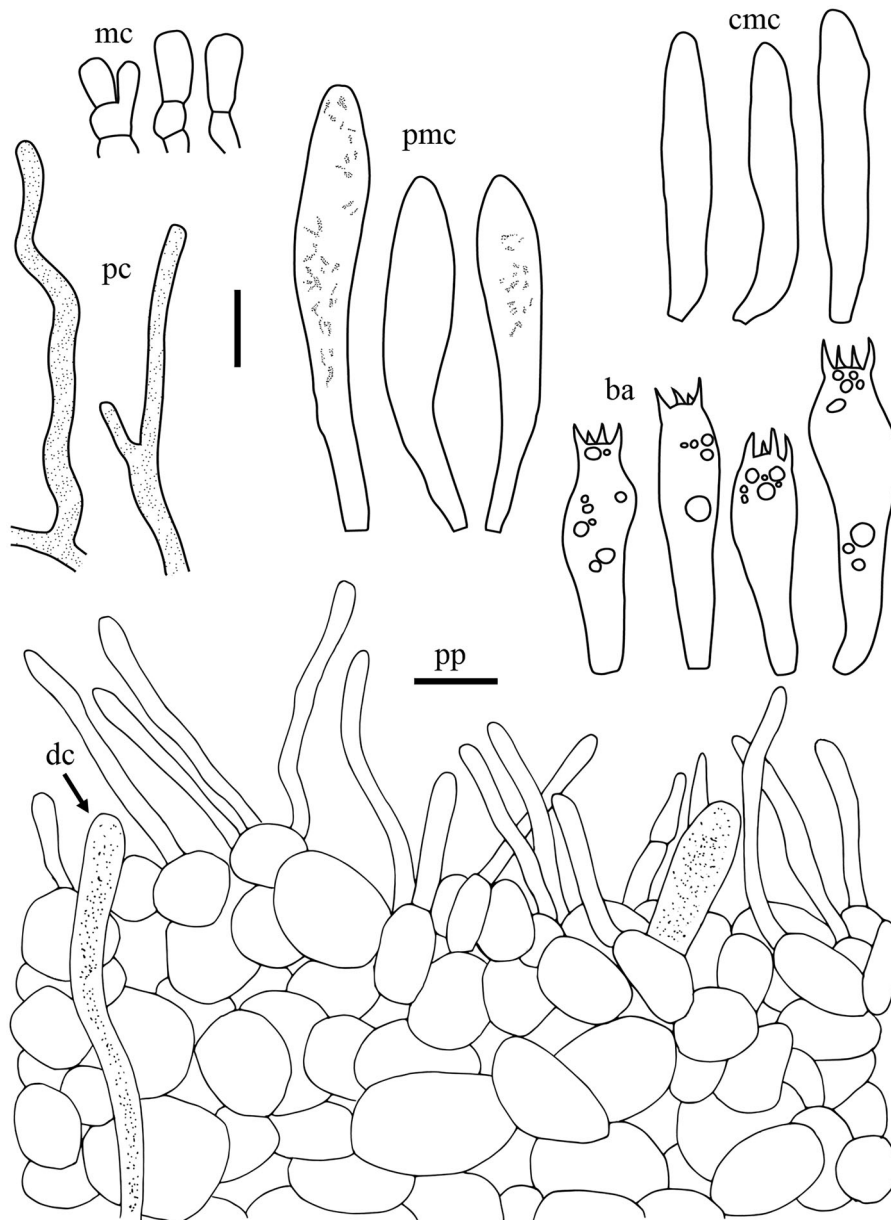


**Figure 19.** Microscopic features of *Lactifluus quercicola* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

tinges. *Lamellae* subdecurrent to decurrent, crowded, up to 6 mm broad, sometimes forked near the stipe, with numerous lamellulae of different length, cream colored, discoloring grayish brown to dark brown when bruised. *Stipe* 45–75  $\times$  5–15 mm, cylindrical to slightly tapering upwards, centrally attached, surface dry, sometimes velutinous, pruinose when young, concolorous to pileus or darker tinged often with brownish. *Latex* abundant, sticky, white, slowly changing to brown.

*Basidiospores* ( $n=40$ ) 7.1–7.8–8.0–8.6  $\times$  6.6–7.3–7.4–8.2(–8.3)  $\mu$ m,  $Q=1.02$ –1.06–1.11, globose to subglobose; ornamentation amyloid, up to 1.3  $\mu$ m high, forming a complete reticulum, with rather irregular edges; isolated warts rare; plage totally amyloid. *Basidia* 46.5–61  $\times$  10–13  $\mu$ m,

subclavate to clavate, 4-spored, filled with guttulate and granular contents. *Pleurolamprocystidia* abundant, 50–95  $\times$  6–10  $\mu$ m, cell wall 1.5–3.5  $\mu$ m thick, subfusiform to subcylindrical, with acute apex, emergent, arising from the subhymenium. *Lamellar edge* composed of basidia, cheilocystidia, and marginal cells; cheilocystidia 21.5–64.5  $\times$  3.5–7.5  $\mu$ m, mostly thick-walled, fusiform to subfusiform, with subacute to acute apex; marginal cells 7–26  $\times$  2.5–7  $\mu$ m, subcylindrical to clavate, 1–2 septate. *Pseudocystidia* rather abundant, 2–5.5  $\mu$ m diam., emergent, subcylindrical, often branched; apex obtuse. *Pileipellis* a lampropalisade; terminal elements 14–42.5  $\times$  2–4  $\mu$ m, cell wall 0.5–1.0  $\mu$ m thick, subcylindrical to subfusiform with tapering apices, acuminate or obtuse apex; subpellis 85–140  $\mu$ m



**Figure 20.** Microscopic features of *Lactifluus subquercicola* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

thick, composed of rounded to elongated, thick-walled cells, 6–20  $\mu$ m in diam.

*Habitat:* Scattered on soil in *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material:* SOUTH KOREA: Gangwon-do, Pyeongchang-gun, Mt. Odae, alt. 874 m, N37°46'51" E128°34'41", 12 Aug 2013, TPML130812-025 (TPML); Gangwon-do, Chuncheon-si, Mt. Daeryong, alt. 748 m, N37°50'47" E127°48'59", 17 Aug 2015, TPML150817-038 (TPML).

*Comments:* *Lactifluus koreanus* shares common characters of the *Lf. volemus* group such as an orange-colored pileus, brownish discoloring white latex, and pleurolamprocystidia. *Lactifluus koreanus* has the thickest subpellis (85–140  $\mu$ m) among the Korean species in *Lf.* sect. *Lactifluus*. This species is

phylogenetically closely related to *Lf. oedematopus* distributed in Europe (Figure 4). However, *Lf. oedematopus* has larger basidiospores (7.5–8.4–8.9–10.1  $\times$  6.7–7.5–8.2–9.2  $\mu$ m) and broader terminal elements (3–10  $\mu$ m) than those of *Lf. koreanus* [26].

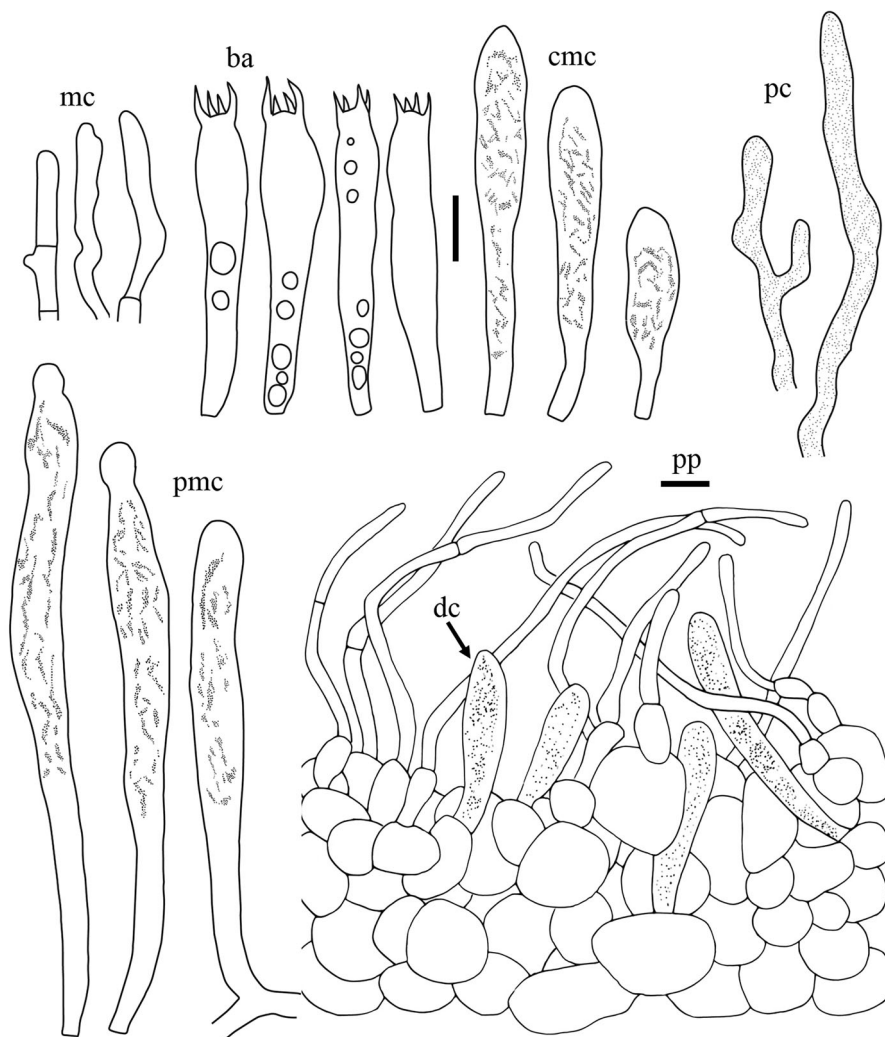
***Lactifluus longistipes* H. Lee & Y. W. Lim sp. nov.** (Figures 14, 26(f), 27(f))

Mycobank: MB 832070

*Etymology:* “longi” means long and “stipes” means stipe. The name refers to the long stipe relative to the pileus diameter.

*Holotypus:* SOUTH KOREA: Gangwon-do, Inje-gun, Mt. Jeombong, alt. 813 m, N38°02'35" E128°27'40", 22 Aug 2014, SFC20140822-50 (**holotypus**, SFC)





**Figure 21.** Microscopic features of *Lactifluus subviridilacteus* sp. nov. All scale bars = 10  $\mu\text{m}$ . Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

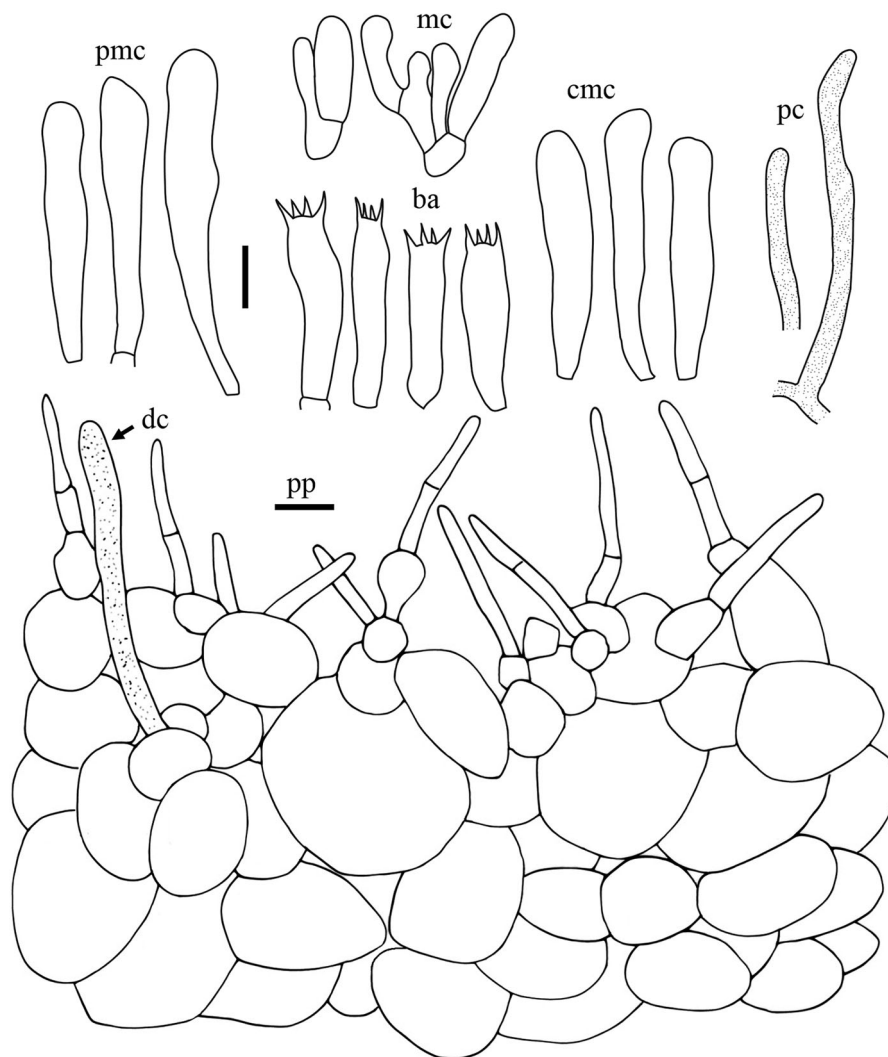
*Pileus* 35–70 mm diameter, convex when young, becoming applanate with depressed center when mature; margin entire; surface dry, velvety, smooth, dark brown to reddish brown (7E8–8E7) in the center, gradually getting paler toward margin; margin tinged with grayish brown. *Lamellae* subdecurrent to decurrent, crowded, up to 4 mm broad, with 3–4 series of lamellulae between lamellae, pale cream, discoloring ocher when bruised. *Stipe* 70–105  $\times$  8–15 mm, cylindrical to slightly tapering upwards, centrally attached, surface dry, velvety, longitudinally rugose, yellowish orange, paler upward. *Latex* copious, sticky, white changing light brown.

*Basidiospores* ( $n=40$ ) 7.1–8.3–8.6–9.6  $\times$  6.9–7.9–8.0–9.1  $\mu\text{m}$ ,  $Q=1.00$ –1.05–1.06–1.10, globose to subglobose; ornamentation amyloid up to 0.6  $\mu\text{m}$  high, almost completely reticulate, warts rare; plage distally to almost totally amyloid. *Basidia* 45–52  $\times$  9–12.5  $\mu\text{m}$ , clavate to subclavate, 4-spored, filled with granular contents. *Pleurolamprocystidia* 52–90.5  $\times$  6.5–10  $\mu\text{m}$ , thick-walled with cell wall

1.5–3.5  $\mu\text{m}$  thick, fusiform to subfusiform, acuminate, sometimes obtuse, emergent, arising from the subhymenium, abundant. *Lamellae-edge* composed of basidia, cheilocystidia, and marginal cells; cheilocystidia 40–59.5  $\times$  5.5–8.5  $\mu\text{m}$ , mostly thick-walled (1–2.5  $\mu\text{m}$ ), fusiform to subfusiform, acuminate; marginal cells 10–35.5  $\times$  3–9  $\mu\text{m}$ , subcylindrical to clavate, often septate. *Pseudocystidia* abundant, 2.5–4.5  $\mu\text{m}$  diameter, emergent, cylindrical; apex obtuse. *Pileipellis* a lampropalisade; terminal elements 8.5–58.5  $\times$  2–4  $\mu\text{m}$ , cell wall 0.5–1.5  $\mu\text{m}$  thick, subcylindrical to subfusiform, slightly acuminate or obtuse apex; subpellis 65–110  $\mu\text{m}$  thick, composed of rounded to elongated cells, thick-walled, 5.5–30  $\mu\text{m}$  in diam.

*Habitat*: Solitary or scattered on soil in *Quercus* forests.

*Additional studied material*: SOUTH KOREA: Gyeongsangbuk-do, Uljin-gun, Mt. Tonggo, alt. 506 m, N36°55'17" E129°11'34", 30 Jul 2013, TPML130730-025 (TPML); Gangwon-do, Hongcheon-gun, Mt. Odae, alt. 876 m, N37°40'48"



**Figure 22.** Microscopic features of *Lactifluus undulatus* sp. nov. All scale bars = 10  $\mu$ m. Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

E128°27'09", 13 Aug 2013, TPML130813-004 (TPML).

*Comments:* *Lactifluus longistipes* can be easily distinguished from other species in sect. *Lactifluus* by its long stipe relative to the pileus size. The ratio of stipe length/pileus diameter is between 1.5 and 2. This species also has the lowest spore ornamentation among the Korean species of *Lf.* sect. *Lactifluus*. This species is phylogenetically closely related to *Lf. volemus* and *Lf. subvolemus* from Europe (Figure 4). However, these two European species have larger basidiospores and higher spore ornamentation (up to about 1.5  $\mu$ m in *Lf. volemus* and up to about 1.6  $\mu$ m in *Lf. subvolemus*) than those of *Lf. longistipes* (up to 0.6  $\mu$ m) [26].

***Lactifluus orientivolemus* H. Lee & Y. W. Lim sp. nov.** (Figures 15, 26(g), 27(g))

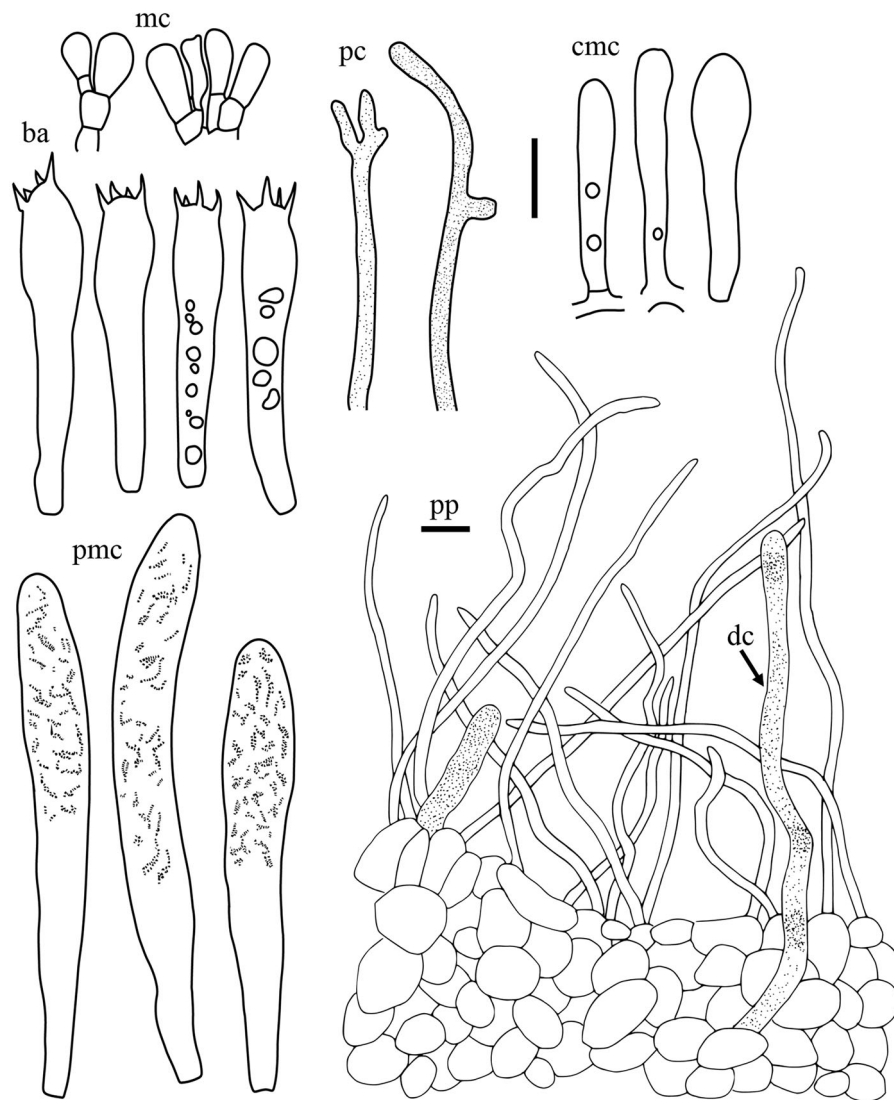
Mycobank: MB 832071

*Etymology:* "orienti" means eastern and "volemus" refers to the European *Lactifluus volemus*. This

name refers to the similar morphology with *Lf. volemus* and its origin in East Asia (South Korea).

*Holotypus:* SOUTH KOREA: Gyeongsangbuk-do, Sangju-si, Mt. Songni, alt. 419 m, N36°28'58" E127°56'23", 3 Aug 2011, TPML110803-018 (**holotypus**, TPML; **isotypus**, SFC)

*Pileus* 40–80 mm diam., convex with slightly depressed center when young, becoming applanate to deeply depressed, smooth to slightly rugulose when young; margin entire, sometimes wavy in age; surface dry, pruinose, velutinous, very pale yellowish white or straw-colored, locally slightly browning, light orange (5A5) in center and light yellow (4A5) on margin. *Lamellae* decurrent with small tooth, crowded, up to 5 mm broad, with numerous lamellulae of different length, whitish to cream, discoloring pale brown when bruised. *Stipe* 40–80  $\times$  10–18 mm, cylindrical to slightly tapering downwards, centrally attached, surface dry, yellowish brown tinged with orange brown. *Latex* abundant, sticky, white changing light brown.



**Figure 23.** Microscopic features of *Lactifluus viridilacteus* sp. nov. All scale bars = 10  $\mu\text{m}$ . Horizontal scale bars are for pileipellis and vertical scale bars are for other microscopic features.

*Basidiospores* ( $n = 20$ )  $8.2\text{--}9.1\text{--}9.9 \times 7.6\text{--}8.6\text{--}9.5 \mu\text{m}$ ,  $Q = 1.03\text{--}1.06\text{--}1.09$ , globose to subglobose; ornamentation amyloid, up to  $0.9 \mu\text{m}$  high, composed of ridges forming a complete reticulum; isolated warts rare; plage distally to totally amyloid. *Basidia*  $56.5\text{--}69 \times 9.5\text{--}13 \mu\text{m}$ , clavate to subclavate, 4-spored, filled with granular contents. *Pleurolamprocystidia*  $65\text{--}149.5 \times 6.5\text{--}11 \mu\text{m}$ , cell wall  $2.5\text{--}4 \mu\text{m}$  thick, subfusiform to subclavate, acuminate, strongly emergent, arising from the subhymenium, abundant. *Lamellar edge* composed of basidia, cheilocystidia, and marginal cells; cheilocystidia  $30.5\text{--}71.5 \times 3\text{--}6.5 \mu\text{m}$ , mostly thick-walled ( $0.5\text{--}3 \mu\text{m}$ ), subfusiform, acuminate; marginal cells  $10\text{--}32 \times 3.5\text{--}6 \mu\text{m}$ , subcylindrical to cylindrical, 1–3 septate. *Pseudocystidia*  $2.5\text{--}4.5 \mu\text{m}$  diameter, slightly emergent, cylindrical to conspicuously tortuous, often irregularly branched; apex obtuse. *Pileipellis* a lamprolalisade; terminal elements  $26.5\text{--}138 \times$

$2\text{--}5.5 \mu\text{m}$ , cell wall  $0.5\text{--}2 \mu\text{m}$  thick, subcylindrical to subfusiform, with acuminate apex; subpellis  $55\text{--}110 \mu\text{m}$  thick, composed of rounded to elongated cells, thick-walled,  $4.5\text{--}24 \mu\text{m}$  in diam.

*Habitat*: Scattered on soil in the mixed forests dominated by *Quercus* and *Pinus*.

*Comments*: *Lactifluus orientivolemus* has the longest terminal elements in the pileipellis among the Korean species of this section. This species is macromorphologically very similar to *Lf. rugiformis*, which can cause misidentifications in the field. However, microscopic characters of *Lf. orientivolemus*, such as larger pleurolamprocystidia and basidia, distinguish the species from *Lf. rugiformis*. *Lactifluus orietivolemus* is phylogenetically closely related to *Lf. dissitus* from India (Figure 4). However, *Lf. dissitus* has shorter terminal elements in the pileipellis ( $11\text{--}55 \times 3\text{--}6 \mu\text{m}$ ) [25] than *Lf. orientivolemus* ( $26.5\text{--}138 \times 2\text{--}5.5 \mu\text{m}$ ).



**Figure 24.** Fruiting bodies of Korean species of *Lactifluus* subg. *Pseudogymnocarpi* (a~c), subg. *Lactariopsis* (d~f), subg. *Gymnocarpi* (g~h). (a) *Lf. holophyllus*, (b) *Lf. luteolamellatus*, (c) *Lf. pseudohygrophoroides*, (d) *Lf. multiseparatus*, (e) *Lf. orientivellereus*, (f) *Lf. pilosus*, (g) *Lf. albidopectinatus*, (h) *Lf. pallidotestaceus*. The color of the two circles at the top-right of each species picture indicates the color (left circle) and color change (right circle) of the latex. Scale bar = 1 cm.

***Lactifluus porphyreus*** H. Lee & Y. W. Lim **sp. nov.**  
(Figures 16, 26(h), 27(h))

Mycobank: MB 832072

**Etymology:** “porphyreus” means red brown. The name refers to the reddish-brown colored pileus of this species.

**Holotypus:** SOUTH KOREA: Incheon-si, Ongjin-gun, Jangbong-do Island, alt. 71 m, N37°32'09", E126°20'53", 26 Jul 2016, SFC20160726-60 (**holotypus**, SFC)

**Pileus** 45–100 mm diam., convex when immature, becoming applanate, subumbilicate or infundibuliform when mature; margin regular or slightly wavy; surface dry, velutinous, pruinose when young, rugose at margin, ochraceous orange (5A8–6B7) with reddish brown (5A6) tinges, getting paler toward the margin. **Lamellae** subdecurrent to decurrent, rather crowded, up to 5 mm, with numerous lamellulae of different length, whitish cream, discoloring brown when bruised. **Stipe** 45–90 × 8–20 mm, cylindrical to slightly tapering downwards, centrally attached, surface dry, slightly velutinous, concolorous with pileus, often getting paler toward the top. **Latex** abundant, sticky, white turning light brown slowly.

**Basidiospores** ( $n = 40$ ) 7.8–8.6–8.7–9.3(–9.5) × 7.0–7.9–8.1–8.9(–9.0)  $\mu\text{m}$ ,  $Q = 1.02\text{--}1.07\text{--}1.08\text{--}1.14$ , globose to subglobose, sometimes broadly ellipsoid; ornamentation amyloid; forming complete reticulum, ridges up to 0.8  $\mu\text{m}$  high, isolated warts very scarce; plage distally to almost complete amyloid. **Basidia** 47–61.5 × 11–12.5  $\mu\text{m}$ , clavate to subclavate, 4-spored, filled with guttulate and granular contents. **Pleurolamprocystidia** 60–115.5 × 7.5–13  $\mu\text{m}$ , cell wall 2.5–6  $\mu\text{m}$  thick, fusiform, subfusiform to subclavate, acuminate or with obtuse apex, sometimes with

wavy outline, distinctly emergent, arising from the subhymenium, very abundant. **Lamellae-edge** composed of basidia, cheilocystidia, and marginal cells; cheilocystidia 24–61 × 3.5–9.5  $\mu\text{m}$ , mostly thick-walled, fusiform to subfusiform, acuminate or furcate apex, sometimes with wavy outline; marginal cells 8.5–30.5 × 2–8  $\mu\text{m}$ , subcylindrical to cylindrical, 1–2 septate. **Pseudocystidia** numerous, 2.5–4.5  $\mu\text{m}$  diameter, slightly to distinctly emergent, cylindrical to tortuous; apex obtuse. **Pileipellis** a lamprosporangium; terminal elements 10.5–63.5 × 3–5  $\mu\text{m}$ , cell wall 0.5–1.5  $\mu\text{m}$  thick, subcylindrical to subfusiform, with acuminate apex; subpellis 60–100  $\mu\text{m}$  thick, composed of rounded to elongated cells, with thick-wall, 5–22  $\mu\text{m}$  in diam.

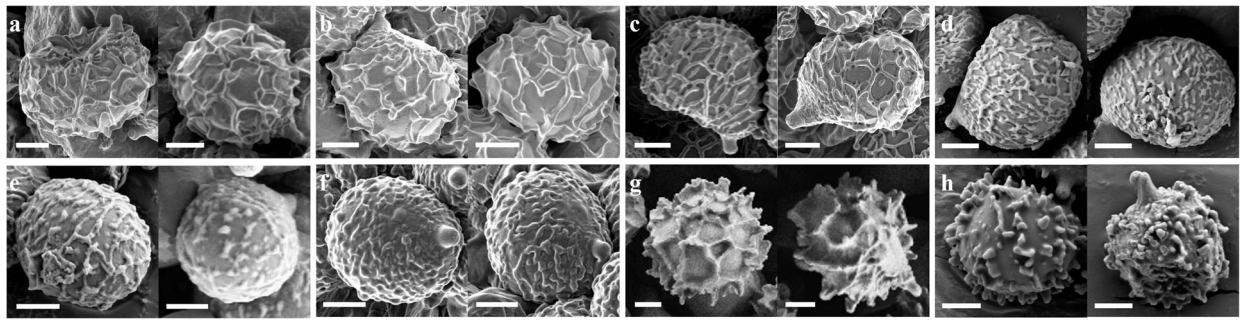
**Habitat:** Scattered on soil in *Pinus* forest or mixed forests dominated by *Quercus* and *Pinus*.

**Additional studied material:** SOUTH KOREA: Gyeongsangbuk-do, Yeongcheon-si, Mt. Bohyeon, alt. 659 m, N37°50'34" E128°59'01", 29 Jul 2013, TPML130729-005 (TPML); Gangwon-do, Chuncheon-si, Mt. Daeryong, alt. 591 m, N37°50'34" E127°48'35", 17 Aug 2015, TPML150817-059 (TPML).

**Comments:** *Lactifluus porphyreus* can be easily distinguished from other Korean *Lactifluus* species belonging this section by its pale yellowish-orange pileus and ectomycorrhizal host (*Pinus*). This species is phylogenetically closely related to *Lf. volemus* and *Lf. subvolemus* (Figure 4). However, the latter two species have larger basidiospores and higher spore ornamentations than *Lf. porphyreus* [26].

***Lactifluus rugiformis*** H. Lee & Y. W. Lim **sp. nov.**  
(Figures 17, 26(i), 27(i))

Mycobank: MB 832073



**Figure 25.** Scanning electron micrographs of basidiospores from Korean species of *Lactifluus* subg. *Pseudogymnocarpi* (a~c), subg. *Lactariopsis* (d~f), subg. *Gymnocarpi* (g~h). (a) *Lf. holophyllus*, (b) *Lf. luteolamellatus*, (c) *Lf. pseudohygrophoroides*, (d) *Lf. multiseparatus*, (e) *Lf. orientivellereus*, (f) *Lf. pilosus*, (g) *Lf. albidopectinatus*, (h) *Lf. pallidotestaceus*. Scale bar = 2  $\mu$ m.

**Etymology:** “rugiformis” means rugulose or wrinkled. The name refers to the distinctly rugulose pileus margin of this species.

**Holotypus:** SOUTH KOREA: Incheon-si, Ganghwa-gun, Mt. Mani, alt. 149 m, N37°36'29" E126°26'02", 18 Aug 2015, SFC20150818-14 (**holotypus**, SFC)

**Pileus** 50–110 mm diameter, convex with depressed center; margin decurved, smooth in age, distinctly rugulose toward the margin; surface dry, velutinous, rusty orange (6C8–7C8) tinged with a more brownish, darker in the center, slightly paler toward the margin. **Lamellae** decurrent, rather crowded, up to 3 mm broad, rarely furcate, with numerous lamellulae of different length, cream to pale orange. **Stipe** 30–70  $\times$  15–20 mm, subcylindrical, tapering downwards, centrally attached, surface dry, velutinous, longitudinally rugulose, concolorous with pileus. **Latex** abundant, sticky, white turning dark brown.

**Basidiospores** ( $n = 20$ ) 7.1–8.4–9.6  $\times$  6.7–7.9–9.2  $\mu$ m,  $Q = 1.01$ – $1.05$ – $1.09$ , globose to subglobose; ornamentation amyloid, composed of ridges up to 1.7  $\mu$ m high, forming complete reticulum, occasionally isolated warts present; plage totally amyloid. **Basidia** 49.5–60  $\times$  9–12.5  $\mu$ m, clavate to subclavate, 4-spored, filled with granular contents. **Pleurolamprocystidia** 41–108  $\times$  4–9.5  $\mu$ m, cell wall 1.5–3  $\mu$ m, subcylindrical to subfusiform, with acuminate apex, slightly to distinctly emergent, arising from the subhymenium, very abundant. **Lamellae edge** composed of basidia, cheilocystidia, and marginal cells; cheilocystidia 17–70.5  $\times$  2.5–7  $\mu$ m, mostly thick-walled (0.5–2  $\mu$ m), subcylindrical to subfusiform, with acuminate apex; marginal cells 9.5–36.5  $\times$  3–13  $\mu$ m, subcylindrical to subclavate, 1–3 septate. **Pseudocystidia** 2–4  $\mu$ m in diameter, slightly emergent, cylindrical to slightly tortuose. **Pileipellis** a lampropalisade; terminal elements 20–68  $\times$  2.5–4  $\mu$ m, cell wall 0.5–1.5  $\mu$ m thick, erect,

subcylindrical to cylindrical with obtuse apex; subpellis 65–100  $\mu$ m thick, composed of rounded to elongated cells, thick-walled, 5–22  $\mu$ m in diameter.

**Habitat:** Scattered on soil in mixed forests dominated by *Quercus*.

**Comments:** *Lactifluus rugiformis* can be recognized by the overall reddish-brown colors, the strongly rugulose pileus margin, the cream to pale orange lamellae, and the short stipe relative to the pileus size. The ratio of stipe length/pileus diameter is between 0.4 and 0.7. This species is phylogenetically closely related to *Lf. longipilus* from Thailand (Figure 4). However, the terminal elements in the pileipellis (10–130  $\mu$ m) of *Lf. longipilus* are longer than *Lf. rugiformis* [10].

#### 4.10. Key to the species of *Lactifluus* subgenus *Lactifluus* section *Piperati*

- Firm and whitish pileus; hyphoepithelium type pileipellis; presence of dermatocystidia in the pileipellis
- 1. Latex white, turning grayish green..... 2
- 1. Latex white, unchanging..... 3
- 2. Lamellae rather distant, suprapellis 30–70  $\mu$ m thick..... *Lf. subviridilacteus*
- 2. Lamellae very crowded, suprapellis 50–130  $\mu$ m thick..... *Lf. viridilacteus*
- 3. Associated with *Pinus* trees..... *Lf. undulatus*
- 3. Associated with *Quercus* trees..... 4
- 4. Lamellar edge sterile, marginal cells up to about 15  $\mu$ m long..... *Lf. subquercicola*
- 4. Lamellar edge with basidia, marginal cells up to about 25  $\mu$ m long..... 5
- 5. Spore ornamentation dense, almost completely reticulate..... *Lf. curvativus*
- 5. Spore ornamentation loose, incompletely reticulate..... *Lf. quercicola*



**Figure 26.** Fruiting bodies of Korean species of *Lactifluus* subg. *Lactifluus* sect. *Gerardii* (a~c), sect. *Lactifluus* (d~i), sect. *Piperati* (j~o). (a) *Lf. betulicola*, (b) *Lf. luminosus*, (c) *Lf. stellatus*, (d) *Lf. acicularis*, (e) *Lf. koreanus*, (f) *Lf. longistipes*, (g) *Lf. orientivolemus*, (h) *Lf. porphyreus*, (i) *Lf. rugiformis*, (j) *Lf. curvativus*, (k) *Lf. quercicola*, (l) *Lf. subquercicola*, (m) *Lf. subviridilacteus*, (n) *Lf. undulatus*, (o) *Lf. viridilacteus*. The color of the two circles at the top-right of each species picture indicates the color (left circle) and color change (right circle) of the latex. Scale bar = 1 cm.

#### 4.11. *Lactifluus* subgenus *Lactifluus* section *Piperati*

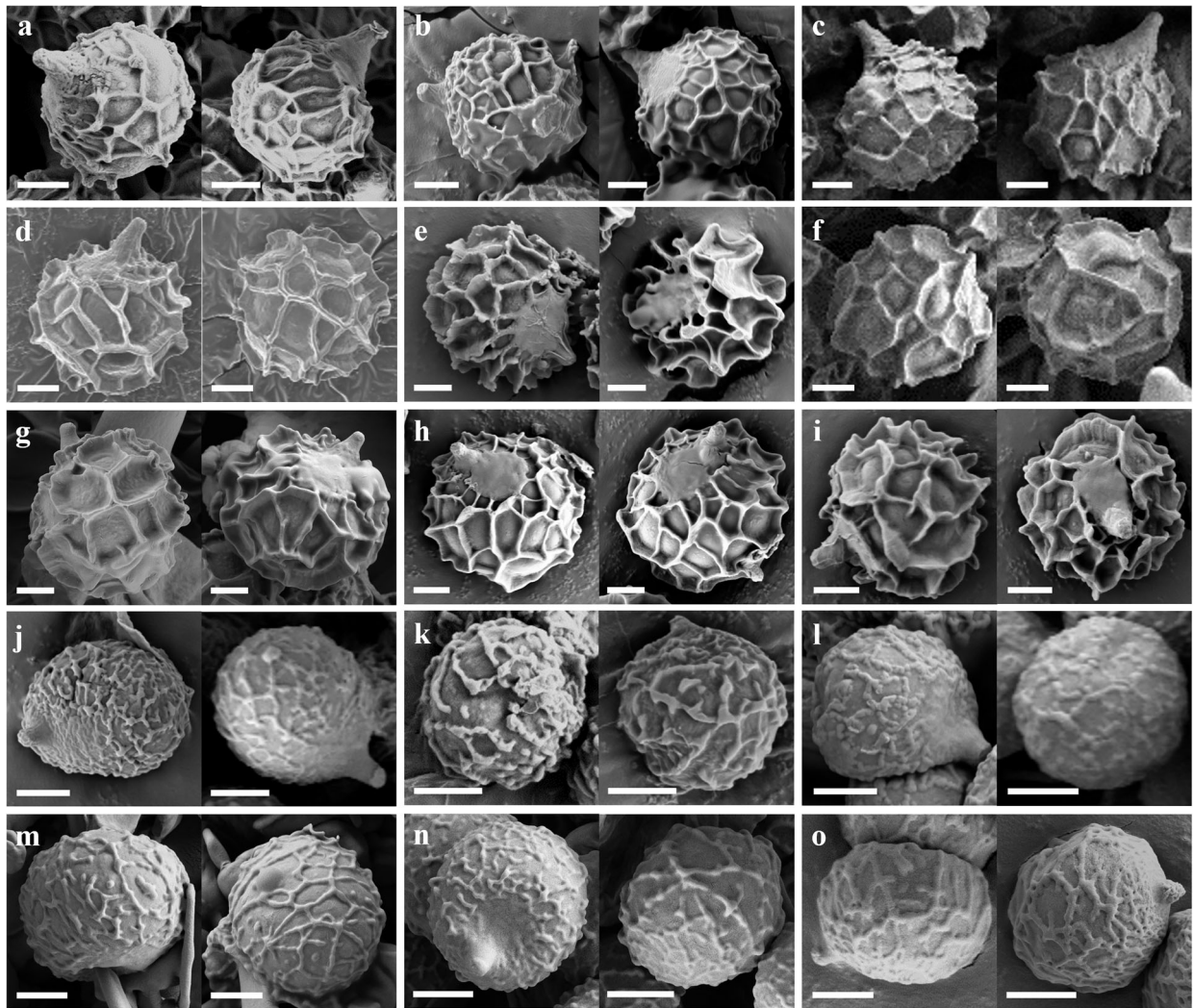
*Lactifluus curvativus* H. Lee & Y. W. Lim sp. nov.

(Figures 18, 26(j), 27(j))

Mycobank: MB 832074

*Etymology*: “curvativus” means slightly curved margin. This name refers to the in-rolled to decurved margin of this species.

*Holotypus*: SOUTH KOREA: Gyeonggi-do, Goyang-si, Seo-o-reung royal tombs, alt. 62 m,



**Figure 27.** Scanning electron micrographs of basidiospores from Korean species of *Lactifluus* subg. *Lactifluus* sect. *Gerardii* (a~c), sect. *Lactifluus* (d~i), sect. *Piperati* (j~o). (a) *Lf. betulicola*, (b) *Lf. luminosus*, (c) *Lf. stellatus*, (d) *Lf. acicularis*, (e) *Lf. koreanus*, (f) *Lf. longistipes*, (g) *Lf. orientivolemus*, (h) *Lf. porphyreus*, (i) *Lf. rugiformis*, (j) *Lf. curvativus*, (k) *Lf. quercicola*, (l) *Lf. subquercicola*, (m) *Lf. subviridilacteus*, (n) *Lf. undulatus*, (o) *Lf. viridilacteus*. The color of the two circles at the top-right of each species picture indicates the color (left circle) and color change (right circle) of the latex. Scale bar = 2  $\mu$ m.

N37°37'48" E126°53'54", 26 Jul 2016, SFC20160726-95 (**holotypus**, SFC)

*Pileus* 50–120 mm, at first convex with depressed center; margin entire, decurved, often crenate in age; surface smooth, dry, concentrically rugulose toward margin, whitish with ochraceous spots. *Lamellae* subdecurrent, very crowded, up to 1.5 mm broad, often furcate, white to pale cream, sometimes tinged with a pale ochraceous. *Stipe* 45–85  $\times$  20–35 mm, subcylindrical, often tapering downwards, smooth, often irregularly compressed, dry, white tinged sometimes with ochraceous tinges. *Latex* rather abundant, white, unchanging.

*Basidiospores* ( $n=40$ ) (5.4–)5.5–6.1–6.3–6.8  $\times$  5.1–5.4–5.5–5.9  $\mu$ m,  $Q=1.03$ –1.11–1.21, globose to broadly ellipsoid; ornamentation amyloid, up to 0.2  $\mu$ m high, consisting of irregularly rounded to elongate warts connected by lower lines, forming

almost complete reticulum; plage inamyloid. *Basidia* 36–49.5  $\times$  7.5–9  $\mu$ m, subcylindrical to subclavate, 4-spored, mostly filled with guttulate and granular contents. *Pleuromacrocystidia* abundant, 38–74  $\times$  4.5–6.5  $\mu$ m, subcylindrical to narrowly subfusiform, with obtuse apex. *Lamellae edge* composed of basidia, cheilomacrocystidia, and marginal cells; cheilomacrocystidia 18.5–43  $\times$  4–7  $\mu$ m, subcylindrical, with obtuse apex; marginal cells 12–28  $\times$  3–6  $\mu$ m, cylindrical to subclavate, 1–2 septate. *Pseudocystidia* 2–4  $\mu$ m in diam., slightly emergent, cylindrical to subcylindrical. *Pileipellis* a hyphoepithelium; suprapellis 30–50  $\mu$ m thick, consisting of thin, hyaline hyphae, 2–4  $\mu$ m broad; subpellis almost completely cellular, consisting of globose to subglobose cells, 5–22  $\mu$ m in diam.; dermatocystidia rather abundant in suprapellis, 3.5–6.5  $\mu$ m broad, subcylindrical to cylindrical.

*Habitat:* Solitary or scattered on soil in the *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material:* SOUTH KOREA: Gyeonggi-do, Incheon-si, Mt. Mani, alt. 326 m, N37°36'42" E126°26'05", 18 Aug 2015, SFC20150818-15 (SFC).

*Comments:* *Lactifluus curvativus* is characterized by white unchanging latex, very crowded lamellae, and small basidiospores. *Lactifluus dwaliensis* distributed in India is phylogenetically closely related to *Lf. curvativus* (Figure 4), however, *Lf. dwaliensis* can be distinguished from *Lf. curvativus* by its big basidiospores (8–10.5 × 7.2–8.6 µm) and sterile lamellar edge [60].

***Lactifluus quercicola*** H. Lee & Y. W. Lim **sp. nov.** (Figures 19, 26(k), 27(k))

Mycobank: MB 832075

*Etymology:* “querci” means oak tree and “cola” means dweller. The name refers to the presence of the species in *Quercus* forests and the putative ectomycorrhizal association with *Quercus*.

*Holotypus:* SOUTH KOREA: Gyeongsangbuk-do, Sangju-si, Mt. Noeum, alt. 163 m, N36°25'35" E128°06'24", 19 Jul 2013, SFC20130719-29 (**holotypus**, SFC)

*Pileus* 40–150 mm, at first convex with depressed center; margin decurved, crenulate or wavy in age; surface smooth, dry, matt or slightly shiny, whitish, sometimes with buff-colored spots. *Lamellae* adnate to decurrent, very crowded, up to 1.5 mm broad, often furcate, never multi-furcate, whitish to pale cream tinged with buff. *Stipe* 40–95 × 12–30 mm, cylindrical, often tapering downwards, smooth or uneven, dry, white tinged with pale cream, becoming buff or brownish from base. *Latex* rather abundant, white, unchanging.

*Basidiospores* ( $n = 60$ ) 4.7–5.5–5.7–6.4 × (4.1–)4.2–4.9–5.0–5.6 µm,  $Q = 1.04–1.11–1.12–1.22$ , globose to broadly ellipsoid; ornamentation amyloid, up to 0.3 µm high, consisting of irregularly rounded to elongate warts connected by low ridges, forming an incomplete reticulum; plage centrally amyloid. *Basidia* 29.5–42 × 5–9 µm, subcylindrical to subclavate, 2- or 4-spored, filled with granular contents. *Pleuromacrocystidia* abundant, 35.5–60 × 6.5–8 µm, subcylindrical to subclavate, sometimes with wavy out-line, with obtuse apex. *Lamellae edge* composed of basidia, cheilomacrocystidia, and marginal cells; cheilomacrocystidia 24–40 × 4.5–7 µm, subcylindrical to cylindrical, with obtuse apex; marginal cells 13–35.5 × 4–5.5 µm, cylindrical to subcylindrical, 1–2 septate. *Pseudocystidia* rare, 2–4 µm in diam., not emergent, cylindrical to slightly tortuose. *Pileipellis* a hyphoepithelium; suprapellis 20–50 µm thick, consisting of thin, hyaline hyphae, 2–3.5 µm

broad; subpellis almost completely cellular, consisting of globose to subglobose cells, 6–34 µm in diam.; dermatocystidia rather abundant in suprapellis, 4.5–7 µm broad, subcylindrical to cylindrical.

*Habitat:* Solitary or gregarious on soil in *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material:* SOUTH KOREA: Gyeongsangbuk-do, Yeongcheon-si, Mt. Bohyeon, alt. 695 m, N36°10'30" E128°59'07", 29 Jul 2013, TPML130729-023 (TPML); *ibid.*, Yeongdeok-gun, Mt. Chilbo, alt. 360 m, N36°37'26" E128°21'60", 29 Jul 2013, TPML130729-035 (TPML).

*Comments:* *Lactifluus quercicola* is phylogenetically closely related to *Lf. piperatus* (Figure 4). *Lactifluus piperatus* is distinguished from *Lf. quercicola* by bigger basidiospore size (7.0–10.4 × 5.2–7.5) [42]. Smaller marginal cells of *Lf. quercicola* (13–35.5 × 4–5.5 µm) and its association with *Quercus* are distinguishing features separating it from morphologically similar species, *Lf. subquercicola* (5–15 × 2–5.5 µm) and *Lf. undulatus* (association with Pine), respectively.

***Lactifluus subquercicola*** H. Lee & Y. W. Lim **sp. nov.** (Figures 20, 26(l), 27(l))

Mycobank: MB 832076

*Etymology:* ‘sub’ means almost and ‘quercicola’ refers *Lactifluus quercicola*. This name refers to the fact that this species is reminiscent of *Lf. quercicola*.

*Holotypus:* SOUTH KOREA: Gangwon-do, Inje-gun, Mt. Jeombong, alt. 864 m, N38°04'06" E128°23'43", 30 Jul 2012, TPML120730-006 (**holotypus**, TPML; **isotypus**, SFC)

*Pileus* 50–120 mm, planoconvex with decurved margin when young, with age expanding and becoming depressed in the center; margin crenulate and wavy in age; surface smooth, dry, slightly shiny, whitish, sometimes with brownish-orange-colored spots. *Lamellae* adnate to decurrent, very crowded, up to 2 mm broad, furcate, often multi-furcate, sometimes furcate toward stipe, pale cream to cream tinged with pale orange. *Stipe* 50–110 × 10–30 mm, cylindrical, often tapering downwards, smooth, dry, white tinged with whitish chrome or pale cream, becoming buff or brownish from base. *Latex* rather abundant, white, unchanging.

*Basidiospores* ( $n = 60$ ) 5.2–6.2–6.3–7.2(–7.4) × 4.5–5.2–5.4–6.3(–6.4) µm,  $Q = 1.03–1.11–1.14–1.22$ , globose to broadly ellipsoid; ornamentation amyloid, up to 0.2 µm high, consisting of irregularly rounded warts connected by fine lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 33–45 × 6.5–10.5 µm, subcylindrical to subclavate, 4-spored, filled with granular contents. *Pleuromacrocystidia* abundant, 49.5–63.5 × 7–9.5 µm, subclavate to subfusiform, with obtuse apex. *Lamellae edge* sterile, composed of



cheilomacrocytidia and marginal cells; cheilomacrocytidia  $17.5\text{--}45 \times 5.5\text{--}7.5 \mu\text{m}$ , subcylindrical, with obtuse apex; marginal cells  $5\text{--}15 \times 2\text{--}5.5 \mu\text{m}$ , subclavate to clavate, 1–2 septate. *Pseudocystidia* rare  $1.5\text{--}3.5 \mu\text{m}$  in diam., not emergent, cylindrical to slightly tortuous, often branching. *Pileipellis* a hyphoepithelium; suprapellis  $20\text{--}60 \mu\text{m}$  thick, consisting of thin, hyaline hyphae,  $1.5\text{--}3.5 \mu\text{m}$  broad; subpellis cellular, consisting of globose to subglobose cells,  $5\text{--}27 \mu\text{m}$  in diameter; dermatocystidia rather abundant in suprapellis,  $4\text{--}6.5 \mu\text{m}$  broad, subcylindrical to cylindrical.

*Habitat*: Solitary or gregarious on soil in *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material*: SOUTH KOREA: Gangwon-do, Goseong-gun, Mt. Hyangrobong, alt. 516 m, N38°18'51" E128°20'35", 18 Aug 2011, TPML110818-058 (TPML); Jeollabuk-do, Muju-gun, Mt. Deogyu, alt. 932 m, N35°51'31" E127°46'06", 17 Aug 2002, HCCN10587 (HCCN).

*Comments*: *Lactifluus subquercicola* has similar macromorphology with *Lf. quercicola* and *Lf. undulatus*, and species may be easily misidentified in the field. However, *Lf. subquercicola* can be distinguished from these two species based on the sterile lamellar edge.

***Lactifluus subviridilacteus* H. Lee & Y. W. Lim sp. nov.** (Figures 21, 26(m), 27(m))

Mycobank: MB 832077

*Etymology*: ‘sub’ means almost and ‘viridilacteus’ refers *Lactifluus viridilacteus*. This name refers to the fact that this species is reminiscent of *Lf. viridilacteus*.

*Holotypus*: SOUTH KOREA: Chungcheongnam-do, Buyeo-gun, Muryangsa Temple, alt. 163 m, N36°19'02" E126°41'36", 28 Aug 2014, SFC20140828-19 (**holotypus**, SFC)

*Pileus*  $50\text{--}120 \text{ mm}$ , convex to applanate with a depressed center; margin decurved; surface smooth, dry, slightly velutinous, rugulose, white to pale cream often with irregular brownish spots. *Lamellae* slightly decurrent, up to 8 mm broad, rather distant, never furcate, with 1–2 series of lamellulae between lamellae, whitish to pale cream tinged often with yellowish brown. *Stipe*  $40\text{--}85 \times 10\text{--}30 \text{ mm}$ , cylindrical; surface smooth, dry, white to pale cream tinged often with yellowish brown. *Latex* rather abundant, white, turning grayish green.

*Basidiospores* ( $n = 40$ )  $6.5\text{--}7.5\text{--}7.6\text{--}8.6(-8.7) \times 5.5\text{--}6.4\text{--}6.6\text{--}7.4(-7.7) \mu\text{m}$ ,  $Q = 1.11\text{--}1.17\text{--}1.24$ , subglobose to broadly ellipsoid; ornamentation amyloid, up to  $0.2 \mu\text{m}$  high, composed of irregular isolated warts, connected by low lines, forming an incomplete reticulum; plage mostly inamyloid, sometimes slightly amyloid at the center. *Basidia*  $49.5\text{--}56 \times 7.5\text{--}11 \mu\text{m}$ , subcylindrical to subclavate,

4-spored, filled with granular contents. *Pleuromacrocytidia* abundant  $71.5\text{--}113.5 \times 7.5\text{--}10.5 \mu\text{m}$ , subcylindrical to subfusiform, with obtuse or inflated and rounded apex, originating deep in the trama, mostly emergent. *Lamella edge* composed of basidia, strongly emergent cheilomacrocytidia, and marginal cells; cheilomacrocytidia  $39.5\text{--}80.5 \times 6\text{--}10 \mu\text{m}$ , subclavate, with obtuse apex; marginal cells  $15\text{--}32 \times 3\text{--}6 \mu\text{m}$ , cylindrical to subclavate, 1–2 septate. *Pseudocystidia*  $3.5\text{--}5 \mu\text{m}$  in diam., slightly emergent, cylindrical to slightly irregular. *Pileipellis* a hyphoepithelium; suprapellis  $30\text{--}70 \mu\text{m}$  thick, consisting of thin, hyaline hyphae,  $2.5\text{--}4 \mu\text{m}$  broad; subpellis cellular, consisting of globose to subglobose cells,  $9\text{--}28 \mu\text{m}$  in diameter; dermatocystidia very abundant in suprapellis,  $6\text{--}10 \mu\text{m}$  broad, subcylindrical to subclavate.

*Habitat*: Solitary or gregarious on soil in *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material*: SOUTH KOREA: Jeollabuk-do, Jangsu-gun, Bangwhadong Natural Recreation Forest, alt. 477 m, N35°35'48" E127°31'45", 27 Aug 2014, SFC20140827-34 (SFC).

*Comments*: *Lactifluus subviridilacteus* is characterized by white latex with grayish-green discoloration, rather distant and non-furcate lamellae. This species is sometimes confused with *Lf. subpiperatus* by relatively distant lamellae. However, they can be distinguished from each other as the former has pleuromacrocytidia, while the latter does not [43]. In addition, *Lf. subpiperatus* has smaller basidia ( $34\text{--}40 \times 7\text{--}8 \mu\text{m}$ ) and cheilomacrocytidia ( $20\text{--}34 \times 3\text{--}6 \mu\text{m}$ ) than *Lf. subviridilacteus* [43].

***Lactifluus undulatus* H. Lee & Y. W. Lim sp. nov.** (Figures 22, 26(n), 27(n))

Mycobank: MB 832078

*Etymology*: ‘undulatus’ means wavy. This name refers to the wavy margin of this species.

*Holotypus*: SOUTH KOREA: Gyeongsangbuk-do, Yeongdeok-gun, Mt. Chilbo, alt. 435 m, N36°37'27" E129°21'50", 29 Jul 2013, TPML130729-041 (**holotypus**, TPML; **isotypus**, SFC)

*Pileus*  $60\text{--}130 \text{ mm}$ , convex with slightly depressed center and decurved margin when young, later expanding and infundibuliform; margin uplifted and wavy in age; surface smooth, dry, slightly shiny, whitish, sometimes with ocher colored spots. *Lamellae* adnate, up to 3 mm broad, very crowded, often furcate, sometimes multi-furcate, whitish to pale cream tinged often with pale cream. *Stipe*  $45\text{--}90 \times 10\text{--}20 \text{ mm}$ , cylindrical, often tapering downwards; surface smooth, dry, white tinged often with pale cream, pale ocher at the base. *Latex* rather abundant, white, unchanging.

*Basidiospores* ( $n = 40$ )  $5.1\text{--}5.7\text{--}5.9\text{--}6.4 \times 4.6\text{--}5.2\text{--}5.3\text{--}5.9 \mu\text{m}$ ,  $Q = 1.03\text{--}1.09\text{--}1.11\text{--}1.15$ ,

globose to subglobose; ornamentation amyloid, up to 0.2 µm high, composed of irregular isolated warts, occasionally connected by low lines, forming an incomplete reticulum; plage inamyloid. *Basidia* 28.5–35.5 × 5.5–8 µm, subcylindrical, 4-spored, mostly filled with guttulate and granular contents. *Pleuromacrocyttidia* abundant, 41–77.5 × 6–8.5 µm, subcylindrical to subclavate, with obtuse or rounded apex, sometimes with irregularly wavy outline, originating deep in the trama. *Lamella edge* composed of basidia, cheilomacrocyttidia, and marginal cells; cheilomacrocyttidia 24–47 × 5.5–8 µm, subcylindrical to subclavate, with obtuse or rounded apex; marginal cells 8–21 × 3–6 µm, cylindrical to subcylindrical, 1–2 septate. *Pseudocystidia* rare, 2.5–3.5 µm in diameter, not emergent, cylindrical to subcylindrical. *Pileipellis* a hyphoepithelium; suprapellis 20–50 µm thick, consisting of thin, hyaline hyphae, 2–3.5 µm broad; subpellis almost completely cellular, consisting of globose to subglobose cells, 7.5–40 µm in diam.; dermatocystidia scarce in suprapellis, 3.5–6.5 µm broad, subcylindrical to cylindrical.

*Habitat:* Solitary or scattered on soil in *Pinus* forests.

*Additional studied material:* SOUTH KOREA: Chungcheongbuk-do, Boeun-gun, Mt. Songni, alt. 454 m, N36°32'37" E127°50'22", 3 Aug 2011, TPML110803-086 (TPML).

*Comments:* *Lactifluus undulatus* is macromorphologically very similar to *Lf. quercicola* and *Lf. subquercicola*. However, their habitats are different. While *Lf. quercicola* and *Lf. subquercicola* grow in oak forests, *Lf. undulatus* grows in pine forests.

***Lactifluus viridilacteus*** H. Lee & Y. W. Lim sp. nov. (Figures 23, 26(o), 27(o))

Mycobank: MB 832079

*Etymology:* “viridi” means greenish and “lacteus” means milky. The name refers to the grayish-green latex discoloration of this species.

*Holotypus:* SOUTH KOREA: Gyeonggi-do, Gwangju-si, Mt. Taewha, alt. 285 m, N37°18'29" E127°18'01", 19 Aug 2015, SFC20150819-08 (**holotypus**, SFC)

*Pileus* 50–150 mm, convex to applanate with a depressed center; surface smooth, dry, slightly velutinous, sometimes slightly rugose, white to pale cream with darker spots. *Lamellae* decurrent, very crowded, up to 2 mm broad, whitish, turning greenish by the latex and finally becoming brownish. *Stipe* 30–90 × 10–40 mm; surface smooth, dry, white to pale cream. *Latex* rather abundant, white becoming grayish green.

*Basidiospores* ( $n = 40$ ) 5.2–5.9–6.0–6.6 × 4.5–5.1–5.2–5.7(–5.8) µm,  $Q = 1.07–1.15–1.18–1.27$ , subglobose to broadly ellipsoid; ornamentation amyloid, up to 0.3 µm high, composed of irregular

isolated warts, aligned or connected by lower lines, but never forming a reticulum; plage inamyloid, sometimes with a slightly amyloid spot. *Basidia* 38–45 × 7.5–9 µm, subclavate, 4-spored, filled with guttulate and granular contents. *Pleuromacrocyttidia* abundant, 45–80 × 7.5–10.5 µm, subcylindrical to subclavate, originating deep in the trama, mostly strongly emergent; apex obtuse. *Lamella edge* composed of basidia, strongly emergent cheilomacrocyttidia, and marginal cells; cheilomacrocyttidia abundant, 32.5–71.5 × 7–9.5 µm, cylindrical to subclavate, with obtuse apex; marginal cells 6–20 × 2–4.5 µm, subcylindrical to clavate, 1–3 septate. *Pseudocystidia* 2–3.5 µm in diam., emergent, cylindrical to slightly tortuose, often branching. *Pileipellis* a hyphoepithelium; suprapellis 50–130 µm thick, consisting of thin, hyaline hyphae, 1.5–4 µm broad; subpellis cellular, consisting of globose to subglobose cells, 6–23.5 µm in diameter; dermatocystidia rather abundant in suprapellis, 4.5–6.5 µm broad, subcylindrical to cylindrical.

*Habitat:* Solitary or gregarious on soil in the *Quercus* forests or mixed forests dominated by *Quercus*.

*Additional studied material:* SOUTH KOREA: Gyeongsangbuk-do, Sangju-si, Mt. Noem, alt. 203 m, N36°26'39" E128°06'50", 8 Aug 2013, SFC20130808-37 (SFC); Jeollanam-do, Haenam-gun, Mt. Duryun, alt. 364 m, N34°28'59" E126°37'30", 1 Aug 2005, HCCN12866 (HCCN).

*Comments:* *Lactifluus viridilacteus* and *Lf. subviridilacteus* are the two Korean species in this section with greenish discoloring latex. *Lactifluus viridilacteus* furthermore characterized by the very crowded lamellae. *Lactifluus glaucescens*, distributed in Europe, is phylogenetically closely related to this species (Figure 4). However, the basidiospores of *Lf. glaucescens* (6.5–9.3 × 5.3–6.9) are larger than those of *Lf. viridilacteus* (38–45 × 7.5–9 µm) [42].

## 5. Discussion

Through a comprehensive morphological and multi-locus phylogenetic analysis of 199 *Lactifluus* specimens collected over 37 years, a total of 24 *Lactifluus* species were identified. Our study shows that *Lactifluus* is not only well distributed in tropical ecosystems and the Southern Hemisphere, but can also be diverse in temperate forest in the Northern Hemisphere. Surprisingly, only five species were previously described species, while at least 18 others were confirmed as new species (one putatively new species requires additional data). The five previously recognized species were all described from Asia: two species (*Lf. acicularis* and *Lf. pilosus*) were originally reported from Thailand [8,10] and three (*Lf.*

*holophyllus*, *Lf. luteolamellatus* and *Lf. pseudohygrophoroides*) were described from South Korea [23]. As expected, many specimens were previously misidentified, being assigned to morphologically similar species from Europe and North America. Our results strongly support that most Asian *Lactifluus* species are not conspecific with morphologically similar taxa from other continents [5,8]. Such was also the case for *Lactarius* species in Korea [47]. Therefore, European and North American species are not likely to be distributed in Asia, and most of the recorded species based on morphological surveys in Korea (and other parts of Asia) are likely incorrect. For example, *Lf. piperatus*, a European species was reported in Korea without detailed morphological descriptions [61], and in our study, sequence data of a European specimen (voucher 2001 08 19 68, France) did not match data from any Korean specimens (Figure 4). Specimens labeled as *Lf. piperatus* in Korea have been identified as one of three new species: *Lf. quercicola*, *Lf. subquercicola*, and *Lf. undulatus*.

Previously, 21 *Lactifluus* species were reported in Korea [22–24,62], yet only five were found in this study. Of the 16 remaining recorded species, only three of them were originally reported from Asia, while seven were described from Europe, and six from North America. The three Asian species recorded in Korea are *Lf. ochrogalactus* (Hashiya) X.H. Wang, *Lf. pinguis* (Van de Putte & Verbeken) Van de Putte, and *Lf. subpiperatus* Hongo. *Lactifluus ochrogalactus* and *Lf. pinguis* were recently reported in Korea [24,62], while the specimens labeled as *Lf. subpiperatus* turn out to be a new species in this study, *Lf. subviridilacteus*. Although we did not identify *Lf. subpiperatus* in our study, the existence of this species in Korea cannot be ruled out as it is present in Japan.

For infrageneric classification of Korean *Lactifluus*, color and color change of latex, pileipellis type, and cystidium type are useful characters. *Lactifluus* species show various latex discoloration patterns when exposed to air, and this informative to distinguish some groups. The morphological characters of the new species discovered in Korea correspond well with the species in the known clades of *Lactifluus* [5]. Korean species belonging to *Lf.* sect. *Lactifluus* and *Lf.* sect. *Gymnocarpi* have brownish discoloration of latex without exception. On the other hand, some groups have diverse patterns of latex discoloration. Most species in the *Lf.* sect. *Piperati* have greenish discoloration of latex, which is true for two Korean species, but the other four show no discoloration. Korean species of *Lf.* sect. *Pseudogymnocarpi* exhibit mixed patterns—two

species with brownish discoloration of white latex and one species with unchanging white latex.

In previous studies, the type of pileipellis was noted as an important character to delimit sections or subgenera within *Lactifluus* [7,44]. *Lactifluus* species in sect. *Lactifluus* and sect. *Pseudogymnocarpi* share the same type of pileipellis. However, various pileipellis types are observed in species of sect. *Albati* and sect. *Gerardii*. For species native to Korea, we found only one type of pileipellis for each section; lamprotrichoderm type of sect. *Albati* and palisade type of sect. *Gerardii*. The type of cystidia and presence/absence of cystidia is also useful to delimit some sections such as sect. *Lactifluus* (pleurolamprocyatidia) and sect. *Luteoli* (no true cystidia). The presence of only one type of cystidia for each section of Korean species highlights its informativeness for identification. Further work is needed to determine if cystidia characters are useful for species identification in other parts of the world. The characters of the context (color, discolouration, smell, and taste) are largely unknown in the Korean species, although in some groups they might reveal additional relevant differences. They certainly need more attention in future.

In addition to the three characters mentioned above (latex color and color change, pileipellis type, cystidia type), the shape of basidiomata also is an important character of *Lactifluus* species. All species of *Lactifluus* investigated in this study are agaricoid. So far, pleurotoid *Lactifluus* species are known in three *Lactifluus* subgenera: *Lactariopsis*, *Gymnocarpi*, and *Lactifluus*. While pleurotoid species are reported from all continents except Europe [11,41,63–68], they have thus far not been observed in Korea. Previous studies show that the pleurotoid basidiomata has multiple evolutionary origins [65,66]. As plerotooid species are reported from Japan and China [11,69], whether they exist in Korea requires further investigation.

Ecological information of ecotomycorrhizal fungi can be useful for their identification and forest management. Sequence data in GenBank may include information on species distribution and host. From the same sequence data with the new species found in this study, we could confirm that many new species are also distributed in other countries of Asia: *Lf. orientivellereus* from Japan [70], *Lf. betulicola* from Japan [9], *Lf. longistipes* from China and Far East Russia (GenBank), *Lf. quercicola* from China (GenBank) and Japan [71], *Lf. subquercicola* from China and Japan (GenBank), and *Lf. undulata* from Thailand and Vietnam [42]. Hosts that interacted with these species were similar to those studied in Korea (Table 1). An ITS sequence (AB568438) consistent with *Lf. quercicola* was obtained from the

root of orchid *Cephalanthera longibracteata*, Japan [72]. Thus, *Lf. quercicola* may be one of the mixotrophic hosts of *Cephalanthera longibracteata*.

In conclusion, we confirm the presence of 24 *Lactifluus* species in Korea, including at least 18 species new to science, based on morphological and multilocus phylogenetic approaches. Our results show that identification of *Lactifluus* species using morphological data alone can be unreliable due to considerable overlap of characters among species. In addition to morphological observation of defining characters, a molecular approach is necessary for infrageneric classification and species identification in Korean *Lactifluus*. Since many specimens used in this study were missing information regarding host plants, the ecological relevance of all Korean *Lactifluus* species could not be examined. Further investigation is necessary to better understand the general ecology of *Lactifluus* species.

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