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이학석사 학위논문

**Taxonomic and phylogenetic analysis of
Korean *Suillus* using three molecular markers**

분자마커를 이용한 한국 비단그물버섯속의 계통연구

2014년 2월

서울대학교 대학원
생명과학부

민영주

**Taxonomic and phylogenetic analysis of
Korean *Suillus* using three molecular markers**

A Thesis Presented

By

YoungJu Min

Advised by Asst. Prof. Young Woon Lim, Ph. D.

**In partial fulfillment of the requirements for the degree of
Master of Science, Biological Sciences**

Graduate School of Biological Sciences

Seoul National University

Seoul, Korea

February 2014

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지도교수 임 영 운

**이 논문을 이학석사 학위논문으로 제출함
2014년 2월**

**서울대학교 대학원
생명과학부
민 영 주**

**민 영 주의 이학석사 학위논문을 인준함
2014년 2월**

위 원 장 _____ (인)

부 위 원 장 _____ (인)

위 원 _____ (인)

**Taxonomic and phylogenetic analysis of Korean *Suillus*
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**YoungJu Min, Master of Science
Graduate School of Biological Sciences
Seoul National University, December 2013
Young Woon Lim, Advisor**

ABSTRACT

The genus *Suillus* Micheli ex S.F. Gray, well-known edible fungi, plays on important roles in coniferous forest as ectomycorrhizal fungi. While this genus is easily characterized with fleshy pileus, glutinous or tomentose cap and small glandular dots on the stipe, it is still difficult to identify species based solely on fruiting body morphology. Currently, around 98 *Suillus* species have been reported worldwide and 13 species have been reported from Korea. The present study focuses on confirming the phylogenetic relationships and to refine the confused taxonomic state of Korean *Suillus* using a

combination of morphological features and a multi gene analysis of the internal transcribed spacer (ITS), large subunit (LSU) rRNA, RNA polymerase II gene (RPB2).

On the basis of molecular identification using the ITS sequences, 11 species based on morphological identification were re-identified as nine species: *S. americanus*, *S. bovinus*, *S. granulatus*, *S. grevillei*, *S. luteus*, *S. pictus*, *S. placidus*, *S. viscidus* and the previously unrecorded *S. subaureus*. Out of 118 specimens used in this study, 51 specimens were misidentified (43% misidentification rate) and *Suillus granulatus* presented the highest misidentification rate. Also, micro-morphological features were insufficient to differentiate the similar *Suillus* species.

Molecular phylogenetic results using the three markers showed that *Suillus* species were divided into seven clades, six of which were supported with more than 70% bootstrap values on Neighbor-Joining analysis. However, Molecular phylogenetic patterns do not follow taxonomical classification suggested by previous taxonomist and *Suillus* species in the same section are divided into separated clades. The ITS and RPB2 phylogenies show clearer relationship among *Suillus* species, while the LSU phylogeny was less resolved. The consensus tree of three regions was similar to the ITS results and showed clearer differentiation between Korean *Suillus* species.

Furthermore, results of maximum intraspecific dissimilarity analyses of *Suillus* species in the each of the sections showed that ITS1 sequences of six *Suillus* species have higher variability compared to the other two fragment of ITS (ITS2, ITS1-5.8S-ITS2), while two *Suillus* species (*S. americanus* and *S. grevillei*) showed higher intraspecific dissimilarity of ITS2. *Suillus viscidus* showed the highest intraspecific

divergence (3.87- 5.37) among Korean *Suillus* species. In general, interspecific variation of *Suillus* species in each clade showed a clear barcoding gap between closely related species. ITS sequence provides useful data to distinguish the morphologically variable species and to determine the interrelationships among the *Suillus* species.

Key words: Korean *Suillus*, ectomycorrhizal fungi, ITS, RPB2, interspecific variation, barcoding gap

Student Number: 2011-23249

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1. Introduction

1.1. Taxonomic history

The genus *Suillus* Micheli ex S.F. Gray is a common edible fungi with a fleshy pileus, glutinous or tomentose cap, with or without a veil, and small glandular dots on the stipe. *Suillus* species are important ectomycorrhizal fungi associated primarily with coniferous trees in northern temperate and boreal regions (Smith and Thiers 1964; Smith and Thiers 1971; Singer 1986). Currently, around 98 *Suillus* species have been reported worldwide (Kirk and Cooper 2010) and *Suillus luteus* is known as type species in this genus.

Suillus was first used as a generic name by Micheli, applying it to *Boleti* (Micheli 1729). At that point of time, scientists showed strong differences in opinion in using the name *Suillus*. Afterwards, *Suillus* as generic name was suggested by Adanson and it has been used as official name to date (Adanson 1763). For this reason, nomenclature of *Suillus* was known as *Suillus* Micheli ex. Adans at that time (Hansen and Seberg 1984). However, Adanson used the name *Suillus* for the *boletes* while Gray differentiated two different genera, using *Suillus* for only *S. luteus* and *Polypores* for *Boletus* (Gray 1821). Henceforward, Palm and Stewart amended the typification of *Suillus* Micheli ex. Adans to *Suillus* Micheli ex. S. F. Gray (Palm and Stewart 1984) and *S. luteus* (L.:Fr.) S. F. Gray was settled as type species of *Suillus* (Gray 1821).

On the other hand, Smith and Thiers divided the genus *Suillus* into three sections

including *Paragyrodon*, *Boletinus*, and *Suillus* (Smith and Thiers 1971). *Paragyrodon* has distinguishing features of subglobose spores and a thick veil, whereas *Boletinus* and *Suillus* sections featured elliptic, oblong, or subfusoid spores. However, characteristics of the stipe and pileus separate these two sections that have similar spore shape. Typically, the section *Suillus* has features of a slimy pileus, stipe with glandular dots, and the absence or presence of veil and annulus. The *Boletinus* group has a fibrillose pileus with veil and no glandular dots (Smith and Thiers 1971). However, *Boletinus* section was reclassified as a new genus according to the presence of clamp connections and *Suillus cavipes* was transferred to genus *Boletinus* rather than section *Boletinus* of genus *Suillus* (Singer et al. 1963; Moser et al. 1983; Singer 1986). Moreover, Singer separated both sections of *Paragyrodon* and *Boletinus* defined by Smith and Thiers from genus *Suillus* (Singer 1986).

The genus *Suillus* were re-established to have five sections: *Solidipedes*, *Grandulosi*, *Larigni*, *Suillus* and *Fungosi*. Each section presented distinct features such as gelatinous or fibrillose pileus, presence or absence of veil and annulus, and mycorrhizal relationships with specific plant. *Solidipedes* has a pileus covered with non-slimy fibrils and a stipe without glandular dots. The section *Larigni* has a stipe with an annulus and no glandular dots, and glabrous and glutinous pileus, unlike the squamulose pileus of *Solidipedes*. *Larigini* mainly form a mycorrhizal relationship with *Larix*, and on rare occasion with *Pinus*. The section *Glandulosi* features a viscous pileus or stipe with annulus, but the stipe does not have glandular dots. Also, *Glandulosi* is mostly associated

with conifers rather than *Larix*. The section *Suillus* presents typical features of the genus *Suillus* and therefore most species occupy this section. The pileus is glutinous and glandular dots scattered in the stipe. The presence or absence of a membranous veil with an annulus hanging from the margin. In addition, species in the section *Suillus* are strictly associated with *Pinus*. The last section, *Fungosi*, has a stipe without glandular dots, variegatic acid but no veil, and forms a mycorrhiza with *Pinus*. In the present study, morphological characteristics of *Suillus* followed taxonomical classification suggested by Singer, Moser, Smith and Thiers.

1.2. Taxonomic studies of Korean *Suillus*

Since the first report of *Suillus*, which were first recorded in the genus *Boletus*, 13 species of *Suillus* have been reported in Korea through many taxonomic studies. According to the studies before the liberation of South Korea, many Korean fungi were reported by Japanese taxonomist. At that time, *Boletus bovinus* and *Boletus luteus*, which are assumed to be in the genus *Suillus*, were recorded in the report “鮮滿實用林業便覽” (Kaburagi 1940). After few years, Lee and Lee reported *Suillus bovinus* as genus *Boletus* through study of “A list of the Korean fungi part (I)” (Lee and Lee 1957) and continuously *Suillus pictus* was reported as genus *Boletinus* in version (II) (Lee and Lee 1958).

In “Taxonomic Investigations on Korean Higher Fungi (I)” including *S. bovinus*

and *S. luteus* (Lim and Kim 1972), *Suillus* were presented as its own generic name. Lee and Cho reported *Suillus grevillei*, which are identified according to the Imazaki-Hongo's classification key through the list of basidiomycetes in Mt. Sobaek areas (Lee and Cho 1976). At that time *Suillus cavipes* was classified in the genus *Boletinus*. Lee, Kim, and Cho reported *Suillus viscidus*, which was called "*Suillus aeruginascens*" at that time, in "Notes on Korean Higher Fungi (4)". This report contained descriptions and illustrations of microscopic features and field photos (Lee et al. 1978).

In the Korean Mycological Society suggested standard Korean names for mushroom in Korea to avoid confusion of species names (Kim et al. 1978). The list specified 586 species of Korean fungi with scientific name and subjective synonyms including six species in *Suillus*. *Suillus bovinus*, *S. granulatus*, *S. grevillei*, *S. luteus*, *S. viscidus*, *S. piperatus* were listed with Korean name but *S. piperatus* was transferred to another other genus. Also, two species in *Suillus* were classified to the genus *Boletinus*: *B. cavipes* and *B. pictus*. The next year, Kim reported the list of edible mushrooms collected in Korea including one species of *Suillus* in "Floral investigation of Higher Fungi in Korea" (Kim 1979). In 1982, the report of the fungal flora of Mt. Gyeryong National Park classified Basidiomycetes into 19 families, 53 genera, and 93 species, including two species in *Suillus*: *S. granulatus* and *S. pictus* (Park and Shin 1982).

According to the study of ectomycorrhizal fungi associated with pure stands of 12 tree species in Korea, seven species in *Suillus* were reported under *Pinaceae*, and it was confirmed that *S. grevillei*, which was collected under *Larix leptolepis*, has high host

specificity (Lee and Kim 1985). Also, *S. sibiricus* was presented in Lee and Kim (1985) even though presence of this species has not been found since. Since then, a total of nine species in *Suillus* were included by the full list of recorded mushrooms in Korea (Lee 1990). This report was based on the published references of taxonomic studies. *Suillus placidus* was added to the list. However, of these, *Suillus cavipes* was still classified as being in the genus *Boletinus* and *Suillus piperatus* was reclassified and re-named as *Chalciporus piperatus*. According to a master's thesis study on the taxonomy of genera *Suillus* and *Tylopilus* in Korea, Kim collected the *Suillus* specimens from 1984 to 1992 and identified eight species in the genus *Suillus* including a previously unrecorded species, *Suillus americanus* (Kim 1993). This study was performed based on system of classification suggested by Moser, Singer, and Hongo and also presented a key to the species of *Suillus*, including microscopic descriptions and figures.

Afterward, *Suillus viscidipes* was recorded in a study of higher fungi in Korea with description of microscopic features and illustrations (Seok et al. 1995). In 2000, two species in *Suillus* were newly reported through rearranged list of recorded mushrooms in Korea: *S. tomentosus* and *S. subluteus* (Lee and Lee 2000). Also, *S. tomentosus* was described in the study of the diversity of ectomycorrhizal mushrooms in NaejangSan National Park (Jang 2006).

Reviewing the previous studies in Korea, all of the reports were based on taxonomic observation by morphological features. Up to the present, 13 species of the genus of *Suillus* have been reported from Korea: *S. americanus*, *S. bovinus*, *S. cavipes*, *S.*

granulatus, *S. grevillei*, *S. luteus*, *S. pictus*, *S. placidus*, *S. viscidipes*, *S. viscidus*, *S. tomentosus*, *S. subluteus*, and *S. variegatus*.

1.3. Phylogenetic studies of *Suillus*

In the initial stage of studies, classification of *Suillus* is a debatable issue because some species of genus *Suillus* were previously recognized as other genera (Pomerleau and Smith 1962; Smith and Thiers 1971; Sutara 1987; Kretzer et al. 1996). However, most of these species had been reclassified in *Suillus* through DNA sequencing (Baura et al. 1992; Kretzer et al. 1996; Kretzer and Bruns 1997). Phylogenetic analysis based on the internal transcribed spacer (ITS) region was first suggested in order to clarify the relationships between the 38 species of *Suillus* at the species level (Kretzer et al. 1996). This study confirmed the divergence of intraspecific sequence among the closely related species and proposed to amend the controversial taxa such as *Boletinus* and *Fuscoboletinus* into *Suillus*.

In addition, phylogenetic study suggested that there is a close relationship between species from North America and Asia (Wu et al. 2000). Furthermore, host specificity in *Suillus* species implies the possibility of cospeciation with between *Suillus* and *Pinus* (Wu et al. 2000). In spite of morphological similarity, molecular methods based on ITS sequences are beneficial to verify the phylogenetic relationship and genetic diversity of genus (Manian et al. 2001).

As former studies mentioned, the ITS region has been generally used for sequence-based mycological studies at the species level including species identification as a barcode region in fungi because ITS1 and ITS2 spacer regions are variable and high resolution for intra- and interspecific variability (Nilsson et al. 2009; Begerow et al. 2010; Schoch et al. 2012). However, the ITS region alone is not sufficient for phylogenetic study of all fungi (Seifert 2009). Recently, the RNA polymerase II (RPB2) gene has been applied to phylogenetic and systematic studies for mushroom forming fungi (Wang et al. 2004; Frøslev et al. 2005; Matheny 2005; Sheedy et al. 2013). Variable regions of RPB2 produced high resolution in the combination with previous used markers for Basidiomycota (Matheny and Bougher 2006; Matheny et al. 2007).

Although the D1/D2 domains of the nuclear large subunit rDNA (LSU) region are less variable than ITS region sequence, it is useful for some fungi as a DNA barcode (Seifert 2009). Yeast are more variable in D1/D2 domain of LSU (Peterson and Kurtzman 1991; Kurtzman and Robnett 1998) and LSU sequences has been adopted in confirming identification and diversity of arbuscular mycorrhizal (AM) fungi due to the high resolution (Kjøller and Rosendahl 2001; Stockinger et al. 2010). For these reasons, LSU region was also selected as molecular marker used for the standard for identification. In general, analyses of both ITS and LSU markers are implemented because LSU has less resolution than ITS (Seifert 2009; Eberhardt 2010). In the present study, a multi-gene analysis of genus *Suillus* using ITS, LSU, and RPB2 is performed.

1.4. Objective of this study

The genus *Suillus* is easily distinguished by distinctive morphological features, but morphology of the fruiting body does not provide trustworthy identification between species. For this reason, species need to be verified using molecular methods. The present study focuses on re-evaluating Korean *Suillus* using a combination of micro-morphological features and a multi-gene analysis to confirm the phylogenetic relationships and clarify the ambiguous taxonomic state. Furthermore, intra- and interspecific dissimilarity of Korean *Suillus* sections were compared to examine the genetic variation of ITS sequences and to verify its usefulness as a barcoding marker.

2. Materials and Methods

2.1. Collection of *Suillus* specimens

A total of 118 *Suillus* specimens were used in this study. Specimens were obtained from the Herbarium Conservation Center of National Academy of Agricultural Sciences (HCCN), Seoul National University Fungal Collection (SFC), and Korea National Arboretum (KA). Specimens have been collected from all over South Korea from 1988 to 2013. Specimens were initially identified based on morphology as *S. americanus* (10 specimens), *S. bovinus* (19 specimens), *S. granulatus* (13 specimens), *S. grevillei* (14 specimens), *S. luteus* (17 specimens), *S. pictus* (11 specimens), *S. placidus* (5 specimens), *S. subluteus* (2 specimens), *S. tomentosus* (3 specimens), *S. viscidipes* (3 specimens), *S. viscidus* (1 specimen), unidentified *Suillus* species (15 specimens), and an unrecorded species *S. sibiricus* (5 specimens), (Table 1).

Table 1. List of *Suillus* specimens, identification history, final identification using the ITS sequence, Korean name, and distribution information used in this study

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN02685	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Yangsan-si, Gyeongnam, Korea	1989-07-07	I
HCCN00116	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Wonju-si, Gangwon-do, Korea	1993-07-08	I, L
HCCN01032	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Yangsan-si, Gyeongnam, Korea	1994-05-29	I
HCCN01223	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Yangsan-si, Gyeongnam, Korea	1994-06-29	I
HCCN02079	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Haenam-gun, Jeonnam, Korea	1995-05-22	I
HCCN02400	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Wonju-si, Gangwon-do, Korea	1995-07-12	I
HCCN07942	<i>S. sibiricus</i>	<i>S. americanus</i>	노란대비단그물버섯	Busan, Korea	1999-10-05	I
HCCN10003	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Gurye-gun, Gyeongnam, Korea	2002-07-10	I
HCCN11268	<i>S. bovinus</i>	<i>S. americanus</i>	노란대비단그물버섯	Geoje-si, Gyeongnam, Korea	2003-07-09	I
HCCN11796	<i>S. tomentosus</i>	<i>S. americanus</i>	노란대비단그물버섯	Wonju-si, Gangwon-do, Korea	2003-09-30	I
HCCN13203	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Guri-si, Gyeonggi-do, Korea	2005-09-30	I
HCCN15955	<i>S. sibiricus</i>	<i>S. americanus</i>	노란대비단그물버섯	Namwon-si, Gyeongbuk, Korea	2007-10-12	I, R
HCCN16006	<i>S. sibiricus</i>	<i>S. americanus</i>	노란대비단그물버섯	Uiwang-si, Gyeonggi-do, Korea	2007-10-28	I, R
HCCN18804	<i>S. sibiricus</i>	<i>S. americanus</i>	노란대비단그물버섯	Gwacheon-si, Gyeonggi-do, Korea	2009-09-14	I, R
HCCN18949	<i>S. subluteus</i>	<i>S. americanus</i>	노란대비단그물버섯	Yongin-si, Gyeonggi-do, Korea	2009-09-23	I
HCCN19197	<i>Suillus</i> sp.	<i>S. americanus</i>	노란대비단그물버섯	Cheonan-si, Chungnam, Korea	2009-10-26	I
HCCN20678	<i>Suillus</i> sp.	<i>S. americanus</i>	노란대비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2010-10-03	I

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN20820	<i>S. sibiricus</i>	<i>S. americanus</i>	노란대비단그물버섯	Guri-si, Gyeonggi-do, Korea	2010-10-09	I, L
HCCN20839	<i>S. tomentosus</i>	<i>S. americanus</i>	노란대비단그물버섯	Gangdong-gu, Seoul, Korea	2010-10-12	I
HCCN23777	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Wonju-si, Gangwon-do, Korea	2012-09-14	I, L, R
SFC20120919-28	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-19	I
SFC20120919-30	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-19	I, L, R
HCCN23971	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-21	I
HCCN24079	<i>S. grevillei</i>	<i>S. americanus</i>	노란대비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-23	I
HCCN24092	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-25	I
SFC20120929-04	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Seo-gu, Daejeon, Korea	2012-09-29	I, L, R
SFC20120929-07	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Seo-gu, Daejeon, Korea	2012-09-29	I
SFC20121001-02	<i>S. americanus</i>	<i>S. americanus</i>	노란대비단그물버섯	Yuseong-gu, Daejeon, Korea	2012-10-01	I
HCCN13678	<i>Suillus</i> sp.	<i>S. bovinus</i>	황소비단그물버섯	Muju-gun, Jeonbuk, Korea	2006-07-06	I
HCCN13747	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Muju-gun, Jeonbuk, Korea	2006-07-06	I
HCCN16489	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Cheonan-si, Chungnam, Korea	2008-07-05	I
HCCN18059	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2009-06-26	I
HCCN18065	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2009-06-26	I
HCCN18985	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Inje-gun, Gangwon-do, Korea	2009-09-24	I, L, R
HCCN20536	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Gwanak-gu, Seoul, Korea	2010-09-18	I, L, R

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN20632	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Namhae-gun, Gyeongnam, Korea	2010-09-29	I, L, R
HCCN24060	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-23	I
KA12-1590	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Boeun-gun, Chungbuk, Korea	2012-09-27	I, L, R
SFC20121001-04	<i>S. luteus</i>	<i>S. bovinus</i>	황소비단그물버섯	Yuseong-gu, Daejeon, Korea	2012-10-01	I, L, R
SFC20130926-03	<i>S. bovinus</i>	<i>S. bovinus</i>	황소비단그물버섯	Hongcheon-gun, Gangwon-do, Korea	2013-09-26	I
HCCN03171	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Boeun-gun, Chungbuk, Korea	1995-10-06	I
HCCN03694	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	1996-10-05	I
HCCN05963	<i>S. pictus</i>	<i>S. granulatus</i>	젓비단그물버섯	Goesan-gun, Chungbuk, Korea	1996-10-09	I
HCCN10888	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2002-10-10	I
HCCN10903	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Cheongyang-gun, Chungnam, Korea	2002-10-23	I
HCCN11671	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2003-09-20	I
HCCN13033	<i>S. placidus</i>	<i>S. granulatus</i>	젓비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2005-08-20	I, L
HCCN14034	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Gimje-si, Jeonbuk, Korea	2006-07-14	I
HCCN14350	<i>S. placidus</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2006-08-27	I
HCCN14451	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Inje-gun, Gangwon-do, Korea	2006-08-29	I
HCCN14511	<i>S. grevillei</i>	<i>S. granulatus</i>	젓비단그물버섯	Inje-gun, Gangwon-do, Korea	2006-08-30	I
HCCN14827	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2006-09-20	I
HCCN18079	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Pocheon-si, Gyeonggi-do, Korea	2009-06-28	I

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN18130	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2009-07-13	I
HCCN18461	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2009-07-30	I
HCCN20187	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2010-08-20	I
SFC20110818-20	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2011-08-18	I
SFC20110818-69	<i>S. placidus</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2011-08-18	I
SFC20120719-07	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gyeongju-si, Gyeongbuk, Korea	2012-07-19	I, L, R
SFC20120727-02	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-07-27	I
SFC20120814-15	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Ulleung-gun, Gyeongbuk, Korea	2012-08-14	I, L, R
SFC20120827-06	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-08-27	I
HCCN23305	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2012-08-29	I
KA12-1193	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Geumsan-gun, Chungnam, Korea	2012-08-29	I, L, R
HCCN23311	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Chuncheon-si, Gangwon-do, Korea	2012-08-31	I
HCCN23389	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Wonju-si, Gangwon-do, Korea	2012-09-02	I
HCCN23433	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2012-09-03	I
HCCN23453	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2012-09-03	I
SFC20120904-03	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-04	I, L, R
SFC20120904-04	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-04	I
SFC20120905-18	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-05	I

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN23596	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Yeongwol-gun, Gangwon-do, Korea	2012-09-08	I
SFC20120915-22	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Yeongdeungpo-gu, Seoul, Korea	2012-09-15	I
SFC20120918-07	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-18	I
SFC20120922-10	<i>S. luteus</i>	<i>S. granulatus</i>	젓비단그물버섯	Gwacheon-si, Gyeonggi-do, Korea	2012-09-22	I
HCCN24004	<i>Suillus</i> sp.	<i>S. granulatus</i>	젓비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2012-09-22	I
SFC20120930-01	<i>S. granulatus</i>	<i>S. granulatus</i>	젓비단그물버섯	Miryang-gun, Gyeongnam, Korea	2012-09-30	I, L, R
HCCN19126	<i>Suillus</i> sp.	<i>S. grevillei</i>	큰비단그물버섯	Gangdong-gu, Seoul, Korea	2009-09-30	I, L, R
KA12-1537	<i>S. grevillei</i>	<i>S. grevillei</i>	큰비단그물버섯	Yeongdong-gun, Chungbuk, Korea	2012-09-24	I, L, R
HCCN24140	<i>Suillus</i> sp.	<i>S. grevillei</i>	큰비단그물버섯	Inje-gun, Gangwon-do, Korea	2012-09-25	I, L, R
KA12-1709	<i>S. grevillei</i>	<i>S. grevillei</i>	큰비단그물버섯	Ulleung-gun, Gyeongbuk, Korea	2012-10-16	I, L, R
SFC20130917-34	<i>S. grevillei</i>	<i>S. grevillei</i>	큰비단그물버섯	Yecheon-gun, Gyeongbuk, Korea	2013-09-17	I
HCCN13739	<i>S. grevillei</i>	<i>S. luteus</i>	비단그물버섯	Muju-gun, Jeonbuk, Korea	2006-07-06	I
HCCN15711	<i>S. grevillei</i>	<i>S. luteus</i>	비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2007-09-17	I
HCCN18238	<i>S. granulatus</i>	<i>S. luteus</i>	비단그물버섯	Suwon-si, Gyeonggi-do, Korea	2009-07-20	I
HCCN18952	<i>S. grevillei</i>	<i>S. luteus</i>	비단그물버섯	Yongin-si, Gyeonggi-do, Korea	2009-09-23	I, L, R
HCCN18972	<i>Suillus</i> sp.	<i>S. luteus</i>	비단그물버섯	Inje-gun, Gangwon-do, Korea	2009-09-24	I, L, R
HCCN20830	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Uiwang-si, Gyeonggi-do, Korea	2010-10-12	I
HCCN21008	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Mapo-gu, Seoul, Korea	2010-10-22	I

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
HCCN23289	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Pyeongchang-gun, Gangwon-do, Korea	2012-08-29	I
HCCN23928	<i>S. grevillei</i>	<i>S. luteus</i>	비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-21	I, L, R
KA12-1569	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Geumsan-gun, Chungnam, Korea	2012-09-26	I, L, R
HCCN24248	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Seogwipo-si, Jeju-do, Korea	2012-10-09	I, L, R
SFC20121031-01	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Gwanak-gu, Seoul, Korea	2012-10-31	I, L, R
SFC20130926-19	<i>S. luteus</i>	<i>S. luteus</i>	비단그물버섯	Hongcheon-gun, Gangwon-do, Korea	2013-09-26	I
HCCN02278	<i>S. placidus</i>	<i>S. placidus</i>	평원비단그물버섯	Jeju-do, Korea	1988-05-24	I, L
HCCN23560	<i>Suillus</i> sp.	<i>S. placidus</i>	평원비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2012-09-07	I, L, R
HCCN24084	<i>S. granulatus</i>	<i>S. placidus</i>	평원비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-25	I, L, R
HCCN03600	<i>S. placidus</i>	<i>S. pictus</i>	붉은비단그물버섯	Hongcheon-gun, Gangwon-do, Korea	1990-09-14	I, L
HCCN03758	<i>S. bovinus</i>	<i>S. pictus</i>	붉은비단그물버섯	Cheongyang-gun, Chungnam, Korea	1991-06-27	I, L
HCCN06311	<i>S. viscidipes</i>	<i>S. pictus</i>	붉은비단그물버섯	Yangsan-si, Gyeongnam, Korea	1997-07-03	I
HCCN07034	<i>S. viscidipes</i>	<i>S. pictus</i>	붉은비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	1998-07-05	I
HCCN13635	<i>S. tomentosus</i>	<i>S. pictus</i>	붉은비단그물버섯	Seogwipo-si, Jeju-do, Korea	2006-07-01	I, L
HCCN23401	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2012-09-03	I
HCCN23554	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Yangpyeong-gun, Gyeonggi-do, Korea	2012-09-07	I
HCCN23759	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Wonju-si, Gangwon-do, Korea	2012-09-11	I
HCCN23750	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Yongin-si, Gyeonggi-do, Korea	2012-09-11	I, L, R

Table 1. Continued

Collection No. ^a	Original ID	Molecular ID	Korean name	Site	Date	Seq. ^b
SFC20120919-31	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-19	I
HCCN23933	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-21	I
SFC20120922-06	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Dongdaemun-gu, Seoul,	2012-09-22	I
HCCN24076	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-23	I
SFC20120925-11	<i>S. pictus</i>	<i>S. pictus</i>	붉은비단그물버섯	Gwanak-gu, Seoul, Korea	2012-09-25	I, L, R
HCCN24109	<i>S. americanus</i>	<i>S. pictus</i>	붉은비단그물버섯	Guri-si, Gyeonggi-do, Korea	2012-09-25	I
HCCN13812	<i>S. viscidus</i>	<i>S. viscidus</i>	녹슬은비단그물버섯	Muju-gun, Jeonbuk, Korea	2006-07-07	I
HCCN16543	<i>Suillus</i> sp.	<i>S. viscidus</i>	녹슬은비단그물버섯	Goesan-gun, Chungbuk, Korea	2008-07-10	I, L, R
HCCN24143	<i>S. pictus</i>	<i>S. viscidus</i>	녹슬은비단그물버섯	Inje-gun, Gangwon-do, Korea	2012-09-25	I, L, R
HCCN10904	<i>Suillus</i> sp.	<i>S. viscidus</i>	녹슬은비단그물버섯	Cheongyang-gun, Chungnam, Korea	2002-10-23	I
HCCN18050	<i>S. subluteus</i>	<i>S. subaureus</i>		Pyeongchang-gun, Gangwon-do, Korea	2009-06-25	I, L, R

^aHCCN-Herbarium Conservation Center of National Academy of Agricultural Sciences; SFC-Seoul National University Fungus Collection; KA-Korea National Arboretum.

^bSequence data used molecular analysis, I: ITS, L: LSU, R: RPB2.

2.2. DNA extraction, PCR amplification and sequencing

Tissue from dried voucher specimens and fresh fruiting bodies were placed in 2X CTAB buffer. Genomic DNA was extracted using a modified CTAB extraction protocol (Rogers and Bendich 1994). In order to avoid amplification of undesirable species from contamination, *Suillus* specific primer was newly designed in this study. The ITS region was amplified using primers ITS1F or ITS5 (White et al. 1990) with a newly designed primers SI1R or SI2R (Table 2). The LSU region was amplified using primer ITS1 and LR5 (Vilgalys and Hester 1990) and the RPB2 was amplified using primer fRPB2-5F or bRPB2-6F1 and bRPB2-8.2R or bRPB2-7.1R (Liu et al. 1999; Brandon Matheny et al. 2007)(Table 2, Fig. 1).

PCR reactions were performed on a T-Professional thermocycler (Biometra, Gottingen, Germany) using the Accupower PCR PreMix (Bioneer, Seoul, Korea) in a final volume of 20 μ l containing 10 pmol of each primer and 1 μ l of DNA. The PCR conditions used were 95°C for 5 min, followed by 35 cycles of 95°C for 40 sec, 55°C for 40 sec and 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were electrophoresed through a 1% agarose gel stained with EcoDye DNA staining solution (Solgent, Seoul, Korea) and purified using the ExpinTM PCR Purification Kit (GeneAll Biotechnology, Korea) according to the manufacturer's instructions. Sequencing was done in a reverse direction for each sample using the PCR primers. The DNA sequencing was performed at the DNA synthesis and Sequencing

Facility, Macrogen (Seoul, Korea), using an ABI3700 automated DNA sequencer.

Table 2. Information of the primer used for PCR and sequencing in this study

Primer		Sequence (5' to 3')	Use ^a	Reference	
rDNA	Forward	ITS1F	CTTGGTCATTTAGAGGAAGTAA	A	White et al. (1990)
		ITS5	GGAAGTAAAAGTCGTAACAAGG	A	White et al. (1990)
		ITS1	TCCGTAGGTGAACCTGCGG	A	Vilgalys and Hester (1990)
	Reverse	SI1R	TACACGGTCCAGCGCGGAAG	A, S	This study
		SI2R	CATCACAYAGCMCTGGBMGTC	A	This study
		LR5	TCCTGAGGGAAACTTCG	A, S	Vilgalys and Hester (1990)
		LR7	TACTACCACCAAGATCT	A	Vilgalys and Hester (1990)
RNA polymerase II	Forward	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	A	Liu et al. (1999)
		bRPB2-6F1	CACAAYCANCAYTGGGGWATGGT	A	Liu et al. (1999)
	Reverse	bRPB2-8.2R	CTNCGGAANAGRCCRCGRTC	A	Brandon Matheny et al. (2007)
		bRPB2-7.1R	CCCATRGCYTGYYTMCCCATDGC	A, S	Matheny (2005)

^aA: PCR amplification, S: sequencing

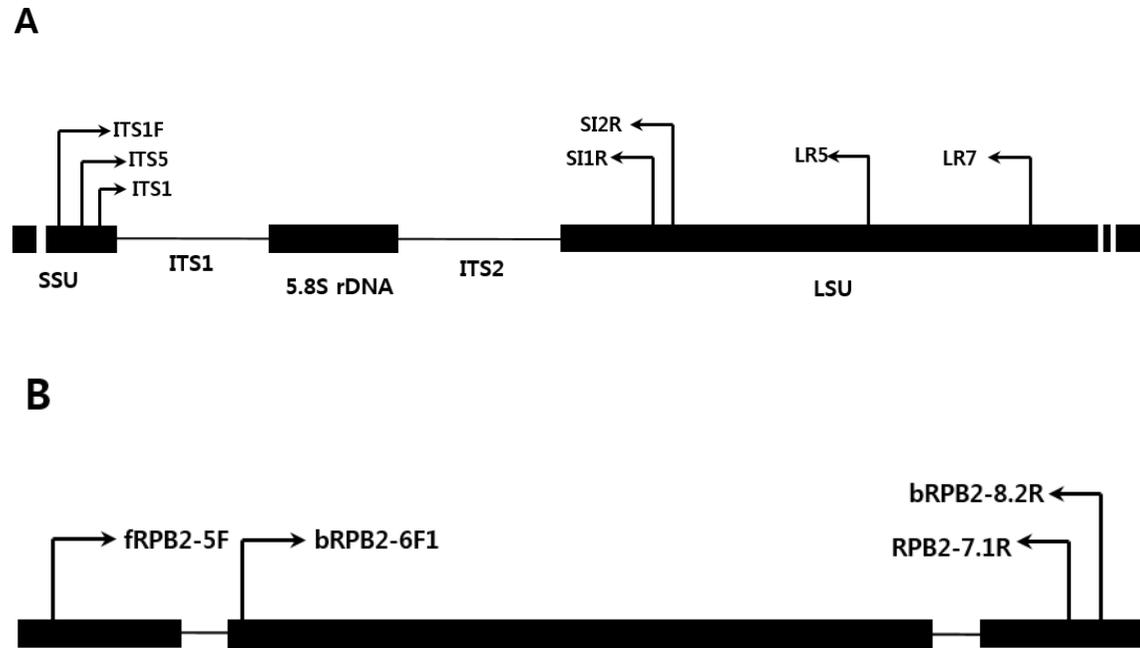


Figure 1. Location of primers used for PCR amplification. A) rDNA region, B) RPB2 region.

2.3. Sequence analysis

Sequences were assembled, aligned, and edited using MEGA5.2 (Tamura et al. 2011). Reference sequences were selected for each marker based on previous phylogenetic studies of the *Suillus* genus and available data in GenBank (Kretzer et al. 1996; Manian et al. 2001).

Multiple alignments were performed using the default settings of MAFFT version 7.122 (Kato and Standley 2013). Ambiguously aligned positions were checked manually. Neighbor-joining (NJ) was conducted in this study. NJ phylogenetic analysis was used with MEGA5.2 (Kumar et al. 2008) with 1,000 bootstrap replicates.

Table 3. List of the reference sequence data used in this study

Species	Type	Origin	Seq. region	Organization	Acc. no.
<i>Suillus amabilis</i>	isolate	USA	ITS	Genbank	AF166499
<i>Suillus americanus</i>	isolate	Japan	ITS	Genbank	AB284437
<i>Suillus bellinii</i>	isolate	Spain	ITS	EMBL	AY898621
<i>Suillus bovinus</i>	voucher	Sweden	ITS	Genbank	EF493250
<i>Suillus bovinus</i>	isolate	Sweden	ITS	EMBL	L54077
<i>Suillus bovinus</i>	isolate	UK	ITS	EMBL	AJ272401
<i>Suillus bovinus</i>	isolate	Europe	ITS	EMBL	AJ272403
<i>Suillus brevipes</i>	voucher	USA	ITS	Genbank	GQ249388
<i>Suillus caeruleus</i>	voucher	Canada	ITS	Genbank	EU486453
<i>Suillus cavipes</i>	isolate	China	ITS	EMBL	AF166505
<i>Suillus cavipes</i>	voucher	UK	ITS	Genbank	JN021096
<i>Suillus collinitus</i>	isolate	Spain	ITS	Genbank	DQ440570
<i>Suillus collinitus</i>	isolate	France	ITS	EMBL	L54089
<i>Suillus cothurnatus</i>	isolate	Spain	ITS	Genbank	AJ419217
<i>Suillus decipiens</i>	isolate	USA	ITS	Genbank	AF166508
<i>Suillus flavidus</i>	voucher	UK	ITS	Genbank	JQ888208
<i>Suillus fluryi</i>	isolate	UK	ITS	Genbank	AJ272407
<i>Suillus granulatus</i>	isolate	UK	ITS	EMBL	AJ272409
<i>Suillus granulatus</i>	voucher	Sweden	ITS	EMBL	EF493252
<i>Suillus granulatus</i>	isolate	Sweden	ITS	EMBL	L54076
<i>Suillus granulatus</i>	isolate	USA	ITS	EMBL	L54113
<i>Suillus grevillei</i>	voucher	Sweden	ITS	Genbank	EF493260
<i>Suillus intermedius</i>	voucher	USA	ITS	Genbank	GU188434
<i>Suillus lakei</i>	voucher	Canada	ITS	Genbank	HQ650767
<i>Suillus luteus</i>	isolate	Austria	ITS	EMBL	AJ272414
<i>Suillus luteus</i>	isolate	USA	ITS	EMBL	L54110
<i>Suillus luteus</i>	isolate	Europe	ITS	EMBL	AJ272415
<i>Suillus luteus</i>	isolate	UK	ITS	EMBL	AJ272413
<i>Suillus luteus</i>	isolate	Germany	ITS	EMBL	L54083
<i>Suillus luteus</i>	voucher	USA	ITS	Genbank	JF908725
<i>Suillus mediterraneensis</i>	isolate	Spain	ITS	Genbank	AY935512
<i>Suillus megaporinus</i>	voucher	USA	ITS	Genbank	GQ249400
<i>Suillus neoalbidipes</i>	isolate	USA	ITS	Genbank	AY880940
<i>Suillus occidentalis</i>	voucher	USA	ITS	Genbank	GQ249394
<i>Suillus pictus</i>	isolate	China	ITS	Genbank	AF166521
<i>Suillus placidus</i>	isolate	Japan	ITS	Genbank	AB284443
<i>Suillus plorans</i>	isolate	Europe	ITS	EMBL	AJ272417
<i>Suillus ponderosus</i>	voucher	USA	ITS	Genbank	JQ958319
<i>Suillus pseudobrevipes</i>	isolate	USA	ITS	Genbank	AY880938
<i>Suillus pungens</i>	isolate	USA	ITS	Genbank	JN858071
<i>Suillus quiescens</i>	voucher	USA	ITS	Genbank	GQ249390
<i>Suillus sibiricus</i>	isolate	China	ITS	Genbank	AF166515

Table 3. Continued

Species	Type	Origin	Seq-region	Organization	Acc. no.
<i>Suillus subaureus</i>	isolate	USA	ITS	Genbank	L54109
<i>Suillus subluteus</i>	isolate	USA	ITS	Genbank	AB284445
<i>Suillus tridentinus</i>	isolate	Germany	ITS	Genbank	AJ419220
<i>Suillus umbonatus</i>	isolate	USA	ITS	Genbank	AF166526
<i>Suillus variegatus</i>	isolate	Sweden	ITS	EMBL	AJ272419
<i>Suillus variegatus</i>	voucher	UK	ITS	Genbank	JQ888210
<i>Suillus variegatus</i>	isolate	UK	ITS	EMBL	AJ272421
<i>Suillus variegatus</i>	isolate	Germany	ITS	EMBL	L54081
<i>Suillus viscidus</i>	isolate	UK	ITS	EMBL	AJ272400
<i>Suillus viscidus</i>	isolate	Japan	ITS	Genbank	AB284440
<i>Suillus viscidus</i>	isolate	USA	ITS	EMBL	L54099
<i>Suillus viscidus</i>	voucher	Sweden	ITS	Genbank	EF493254
<i>Suillus volcanalis</i>	voucher	USA	ITS	Genbank	GQ249398
<i>Suillus tomentosus</i>	isolate	Canada	ITS	Genbank	JQ711905
<i>Suillus tomentosus</i>	voucher	UK	ITS	Genbank	JN021100
<i>Suillus americanus</i>	voucher	USA	LSU	Genbank	AY612829
<i>Suillus cavipes</i>	isolate	USA	LSU	Genbank	AF071535
<i>Suillus hirtellus</i>	voucher	USA	LSU	Genbank	AY612828
<i>Suillus sinuspaulianus</i>	isolate	USA	LSU	Genbank	AF071536
<i>Suillus punctipes</i>	voucher	USA	LSU	Genbank	AY612826
<i>Suillus pictus</i>	isolate	USA	LSU	Genbank	NG027637
<i>Suillus pictus</i>	voucher	USA	LSU	Genbank	AY612830
<i>Suillus luteus</i>	isolate	Sweden	LSU	Genbank	AY586715
<i>Suillus grevillei</i>	voucher	USA	LSU	Genbank	AY612827
<i>Suillus viscidus</i>	isolate	Germany	LSU	Genbank	AF098396
<i>Suillus viscidus</i>	voucher	Germany	LSU	Genbank	AF462350
<i>Suillus pictus</i>	isolate	USA	RPB2	Genbank	AY786066

2.4. Genetic diversity

To inquire the genetic variation of *Suillus* species through ITS region, maximum intraspecific sequence dissimilarity was calculated for each species in the four *Suillus* sections belong to Korean species. ITS divided into three fragment (ITS1, ITS2, ITS1-5.8S-ITS2) were analyzed separately. Maximum intraspecific sequence dissimilarities were calculated using PHYDIT version 3.2 (Chun 1995). Additionally, boxplots were created using R (R Development Core Team, 2007) to compare intra- and interspecific dissimilarity among the sections of *Suillus*.

2.5. Microscopic observation

Suillus specimens that were re-identified by sequence analysis were verified by their microscopic characters: basidia, basidiospores and cystidia. Dried tissue from voucher specimens was rehydrated in 3% KOH, mounted in Congo red solution and observed under a light microscope (Nikon 80i). Up to 20 basidiospores, 10 basidia and cystidia were measured per specimen. Collections were assigned a species designation by identification against the macro- and micro-morphological characters described in three studies (Smith and Thiers 1971; Moser et al. 1983; Singer 1986).

3. Result

3.1. Re-identification of Korean *Suillus* using ITS sequence and morphological verification

One of the problems for PCR amplification of archived specimens after long-term herbarium storage is acquiring the desirable sequence. Depending on the environment of storage or condition of specimens, the sample may be contaminated by mold or the DNA degraded. To maximize our success of PCR amplification, new *Suillus* specific primers were developed (Fig.2).

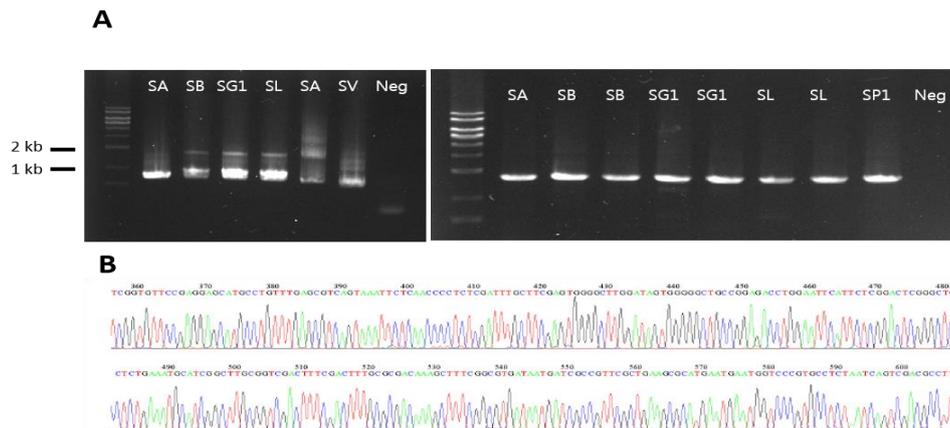


Figure 2. Agarose gel electrophoresis of the PCR amplified rDNA ITS

region. A) Results of PCR amplification using universal primer (ITS5, ITS4) (left) and using *Suillus* specific reverse primer (ITS5, SI1R) (right). B) sequence data of *Suillus* specimen using *Suillus* specific primers.

The ITS (635 bp) region was amplified and sequenced for all specimens. On the NJ tree, the 118 specimens were divided into nine clades (Fig. 3). Each clade was identified by BLAST search and further phylogenetic analysis. The 118 specimens were identified as nine species including one unrecorded species (Fig. 3).

Of the 28 specimens identified as *S. americanus*, 19 specimens were previously misidentified as *S. bovinus* (8), *S. sibiricus* (5), *S. tomentosus* (2), *S. grevillei* (1), *S. subluteus* (1), and unidentified species (2), while there were correctly identified *S. americanus* specimens (9). Most *S. bovinus* specimens (10) were in accord with the morphological identification, but two specimens were added this group (Fig. 4). In the case of *S. granulatus*, 37 specimens were identified as this species and 25 specimens were misidentified based on morphological characters. Among these specimens identified as *S. granulatus*, nine specimens previously belonged to *S. luteus*, six specimens from *S. grevillei*, three specimens from *S. placidus*, one specimen from *S. pictus*, and six previously unidentified specimens.

Also, some *S. luteus* and *S. placidus* specimens were misidentified as each other. Of the 13 specimens of *S. luteus*, seven specimens were correctly identified as *S. luteus*, while the others were determined to be *S. grevillei* (4), *S. granulatus* (1) and unidentified species (1) were determined based on molecular analysis. Also, of the three *S. placidus* specimens, one specimen had same identification result, but two specimens were previously misidentified as *S. granulatus* and unidentified species. In other case, three *S. grevillei* specimens were correctly identified as previous species and two unidentified

specimens were added in this species.

Suillus viscidus comprised of four specimens which were previously identified as one *S. viscidus*, one *S. pictus*, and two unidentified species. Lastly, in the case of *S. pictus*, molecular analysis of nine specimens was identical to previous morphological identification except for that six specimens of 15 total specimens were misidentified as other species. One specimen each of *S. americanus*, *S. bovinus*, *S. placidus*, and *S. tomentosus* were transferred to the *S. pictus* group, and two specimens identified morphologically as *S. viscidipes* were amended to this species. Additionally, one specimen was moved from *S. subluteus* to the unrecorded species *S. subaureus* based on sequence analysis.

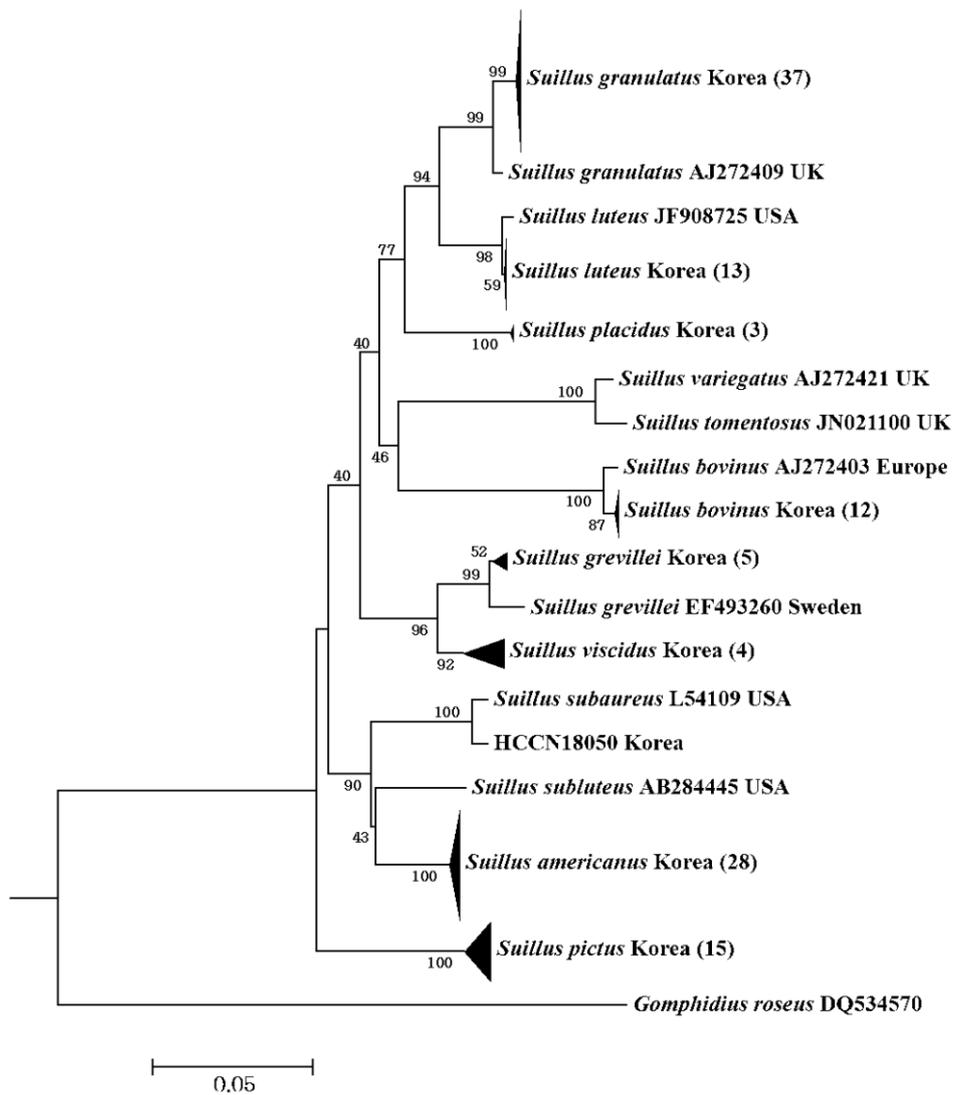


Figure 3. The neighbor-joining tree of the ITS region for identification of 118 Korean *Suillus* specimens. Numbers above the branches are frequencies (%) of statistical occurrence based on 1000 bootstrap replicates. *Gomphidius roseus* (DQ534570) was used as the outgroup.

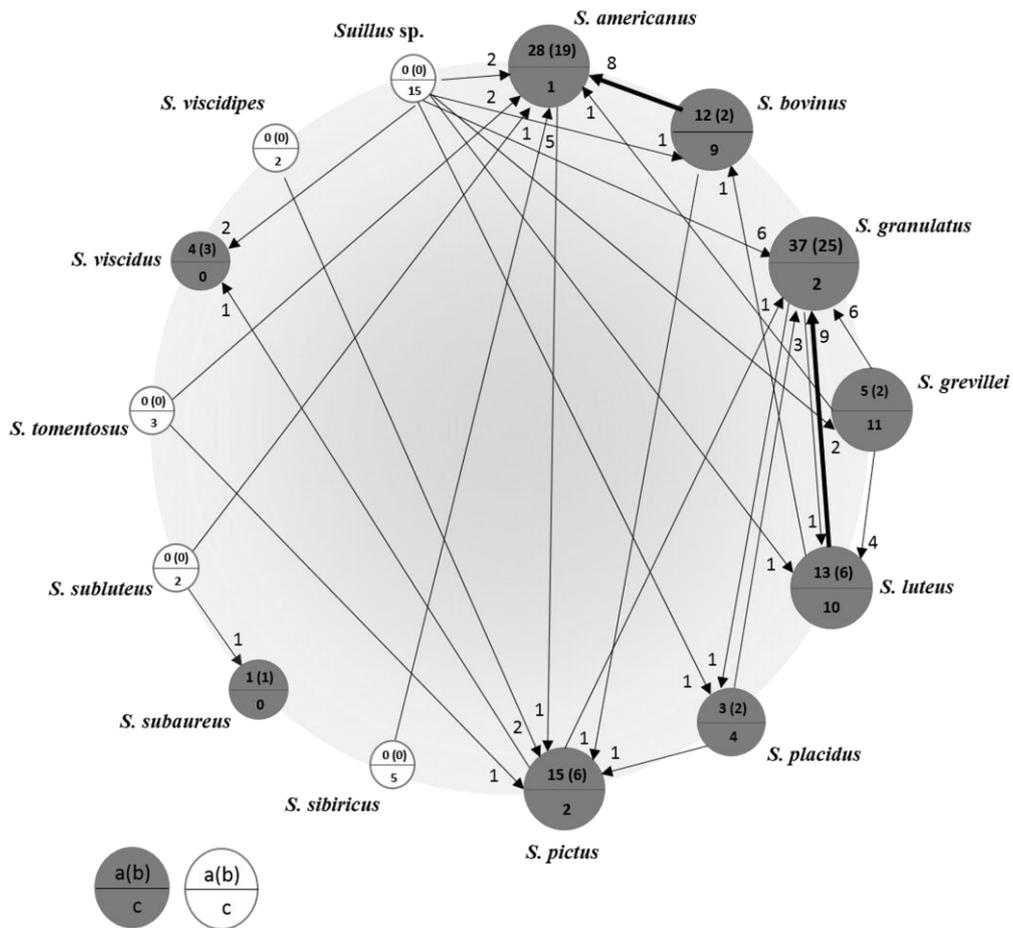


Figure 4. A flow of *Suillus* species identification. Gray circle shows the presence of this species. Species represented by white circle were previously recorded in Korea but not verified in this study (with the exception of *S. sibiricus*). a) number based on final molecular identification, b) number of specimens added based on molecular identification, c) number of misidentified specimens.

To verify ITS based identification, morphological characters such as basidia, basidiospore and pleurocystidia were compared with previous references (Smith and Thiers 1971; Moser et al. 1983) (Table 4). Typically, basidiospores of *Suillus* are elliptical or globose and the smooth surface is yellowish color. Basidia are clavate with four spores and pleurocystidia are also generally clavate to subcylindrical, often coloring in KOH when fresh and cystidia often in clusters (Smith and Thiers 1971). In this study, almost all species share similar characters, but some species presented size variation of basidia and basidiospores.

Suillus viscidus has basidia and basidiospores of bigger size in comparison with other species. Basidia size of *S. luteus* match up with Moser's description, while pleurocystidia size is similar to Smith & Thiers's description (Table 4). On the other hand, the basidia size of *S. americanus* is longer than reference data. Unrecorded species, *S. subaureus*, is also observed and compared to published data. Basidia, basidiospores, and pleurocystidia of Korea *S. subaureus* are $19.2-26.6 \times 5.9-7.7 \mu\text{m}$, $6.2-9.2 \times 2.2-4 \mu\text{m}$ and $39.8-53.7 \times 4.2-8.8 \mu\text{m}$, respectively (Table 4). This observation matches closely with *S. subaureus* reported by Smith & Thiers (1971). Overall, microscopic characters of Korean *Suillus* species are within the range of published data.

Table 4. Microscopic characteristics of eight Korean *Suillus* species, including one previously unrecorded species

Species	Reference	Basidia*(μm)	Basidiospore*(μm)	Pleurocystidia*(μm)
<i>S. americanus</i> (Peck) Snell	Palm and Stewart (1986)	21-25 \times 5.5-6	8-9.5 \times 3.5-5	32-58 \times 6-10
	Korean species	21.7-33 \times 5.5-10.3	7.4-11.2 \times 3-4.2	33.1-62.2 \times 4.3-8.7
<i>S. bovinus</i> (L.) Roussel	Moser (1983)	20-25 \times 4.5-6	7-11 \times 3-5	30-60 \times 4.5-6
	Korean species	19.4-25.8 \times 5.6-7.9	7.2-10.2 \times 3-4.4	28.3-54.4 \times 3.7-6.9
<i>S. granulatus</i> (L.) Roussel	Moser (1983)	20-30 \times 5-6.5	8-11 \times 3-4.5	36-50 \times 7-9
	Korean species	21.2-28.1 \times 5.4-7.5	6-10.6 \times 2.4-3.8	32.1-54.6 \times 4.7-8.7
<i>S. grevilleii</i> (Klotzsch) Singer	Moser (1983)	20-24 \times 5-6.5	8-11(14) \times 3-5	30-45 \times 4.5-6.5
	Korean species	19.5-28.1 \times 4.9-8.9	7.8-10.6 \times 3.4-5.4	33.3-53.9 \times 4.1-8.1
<i>S. luteus</i> (L.) Roussel	Moser (1983)	20-24 \times 4.5-5.5	7-9 \times 2.5-3.5	no data
	Smith & Thiers (1971)	14-18 \times 4-5	7-9 \times 2.5-3	20-35 \times 5-7
	Korean species	20-24 \times 4.4-7.7	6.4-9 \times 2.8-4.6	24.8-50 \times 4.7-9.1
<i>S. subaureus</i> (Peck) Snell	Smith & Thiers (1971)	23-28 \times 5-7	7-10 \times 2.7-3.5	40-50 \times 4-6
	Korean species	19.2-26.6 \times 5.9-7.7	6.2-9.2 \times 2.2-4	39.8-53.7 \times 4.2-8.8
<i>S. placidus</i> (Bonord.) Singer	Moser (1983)	20-26 \times 5-6	7-10.5 \times 2.5-3.5	35-65 \times 9-23
	Korean species	19.9-25.9 \times 5.4-8.7	6-8.4 \times 2.8-4	34.8-63.4 \times 5.2-11.3
<i>S. pictus</i> (Peck) A.H. Sm. & Thiers	Smith & Thiers (1971)	20-26 \times 6-8	8.5-10.5 \times 3.5-4.5	30-75 \times 7-12
	Korean species	22.7-30.3 \times 5.5-9.2	7.2-10.4 \times 3-4.8	32.1-80.4 \times 6.3-10.5
<i>S. viscidus</i> (L.) Roussel	Moser (1983)	28-35 \times 7-9	9.7-12.9 \times 4.2-5.4	35-65 \times 5-10
	Korean species	24.2-36.2 \times 6.7-10.8	8.8-12.4 \times 3.6-4.8	41.4-72 \times 4.1-10.9

*Data obtained from up to 20 basidiospores, 10 basidia and cystidia. Measurements are given as length range \times width range.

On the basis of microscopic features and molecular identification using the ITS sequences, 118 specimens were re-identified as nine species. In the end, *S. americanus* (28 specimens), *S. bovinus* (12 specimens), *S. granulatus* (37 specimens), *S. grevillei* (5 specimens), *S. luteus* (13 specimens), *S. pictus* (15 specimens), *S. placidus* (3 specimens), *S. viscidus* (4 specimen) and a previously unrecorded species *S. subaureus* (1 specimens) were used in this study (Table 1 and Figure 4).

3.2. Phylogenetic analyses using ITS, LSU and RPB2

To infer the phylogenetic position of *Suillus* species, the ITS (635 bp) regions was amplified and sequenced for all specimens. More than two specimens of each species, based on ITS, were sequenced for LSU (835 bp) and RPB2 (653 bp).

For the NJ, the 118 specimens were divided into seven clades, six which were supported with more than 70% bootstrap values (Fig. 5). Although the relationship among the seven clades is uncertain, groups of species in each clade were strongly supported. Clade A including three Korean species, *Suillus granulatus*, *Suillus luteus* and *Suillus placidus*, were weakly supported because their interrelationships were unstable. Clades D, E, and G formed well supported groups with over 90% bootstrap values. Section *Suillus* was not monophyletic, and was divided into clade A, B, and E. Section *Solidipedes* is also divided into three independent clades, whereas clade B is in the section *Fungosi* and clade F is in the section *Larigni*.

In contrast with the ITS phylogeny, LSU was not well-resolved and recovered different positions of clades (Fig. 6). There was no clear pattern in the relationship among *Suillus* species of LSU region because reference sequences were limited (Fig. 6). RPB2 analysis inferred similar results as the ITS phylogeny (Fig. 7). However, *Suillus placidus* in clade A was still in an unclear in position with low bootstrap value. Finally, the consensus tree of three regions was similar to ITS analysis and also showed more obvious relationship between Korean *Suillus* species (Fig. 8)

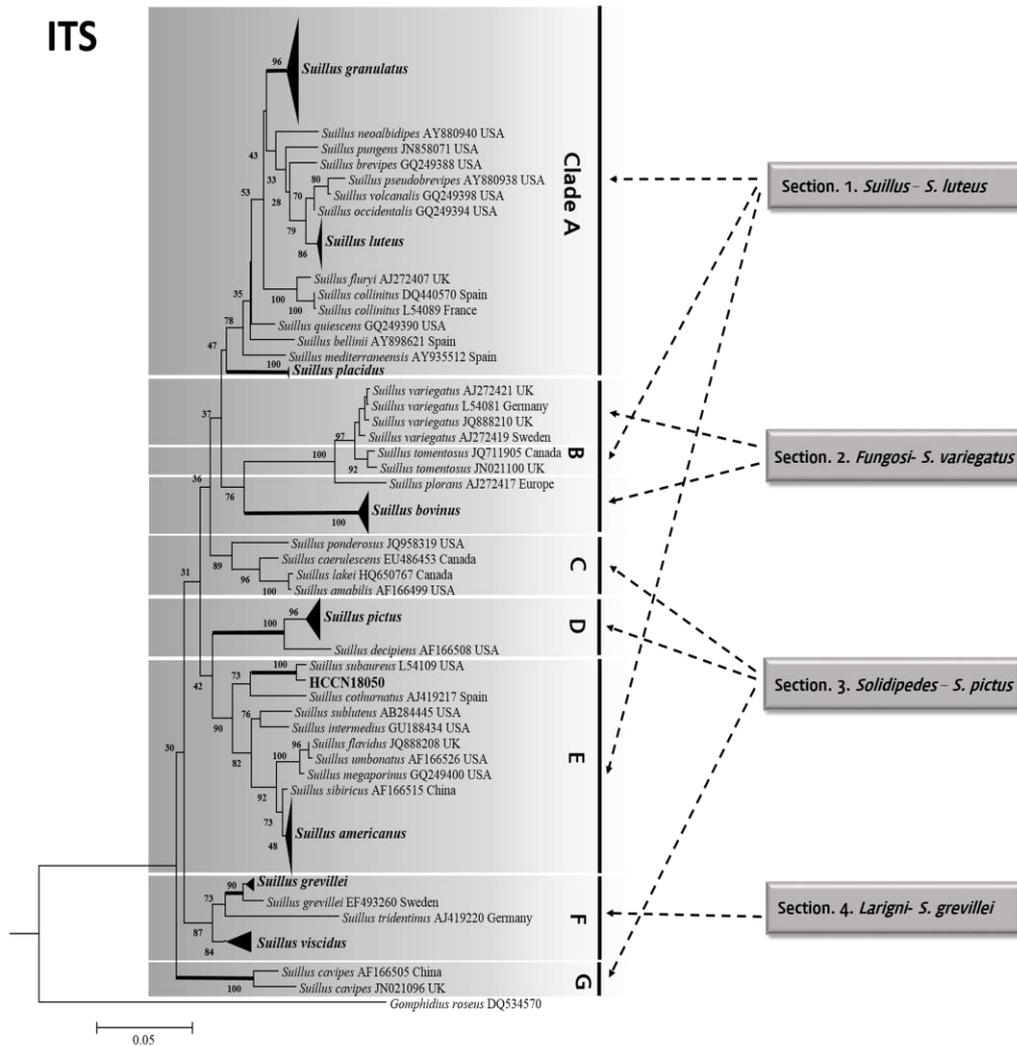


Figure 5. The neighbor-joining tree of *Suillus* species including 118 specimens based on ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). *Gomphidius roseus* (DQ534570) was used as the outgroup.

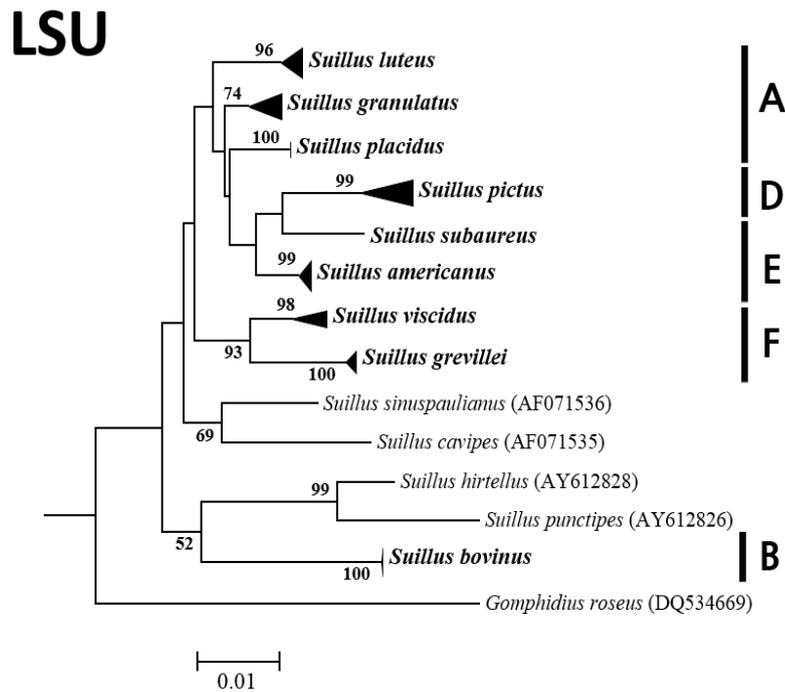


Figure 6. The neighbor-joining tree of *Suillus* species including 37 specimens based on LSU region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). *Gomphidius roseus* (DQ534570) was used as the outgroup.

RPB2

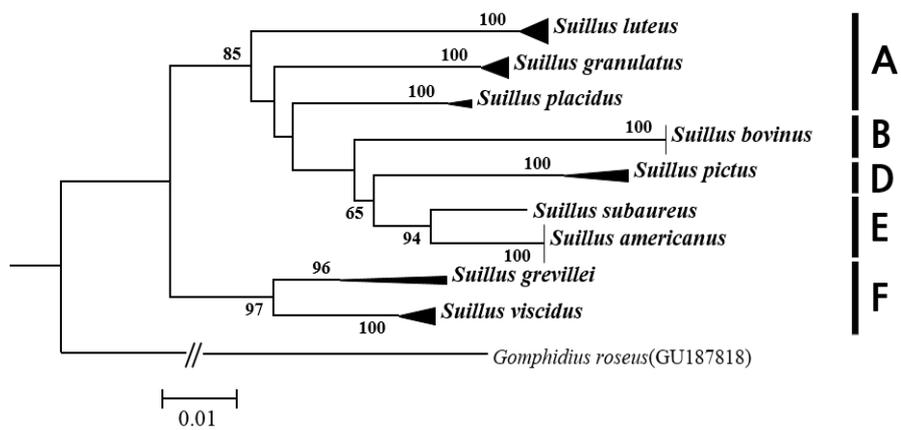


Figure 7. The neighbor-joining tree of *Suillus* species including 34 specimens based on RPB2 region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). *Gomphidius roseus* (DQ534570) was used as the outgroup.

ITS+LSU+RPB2

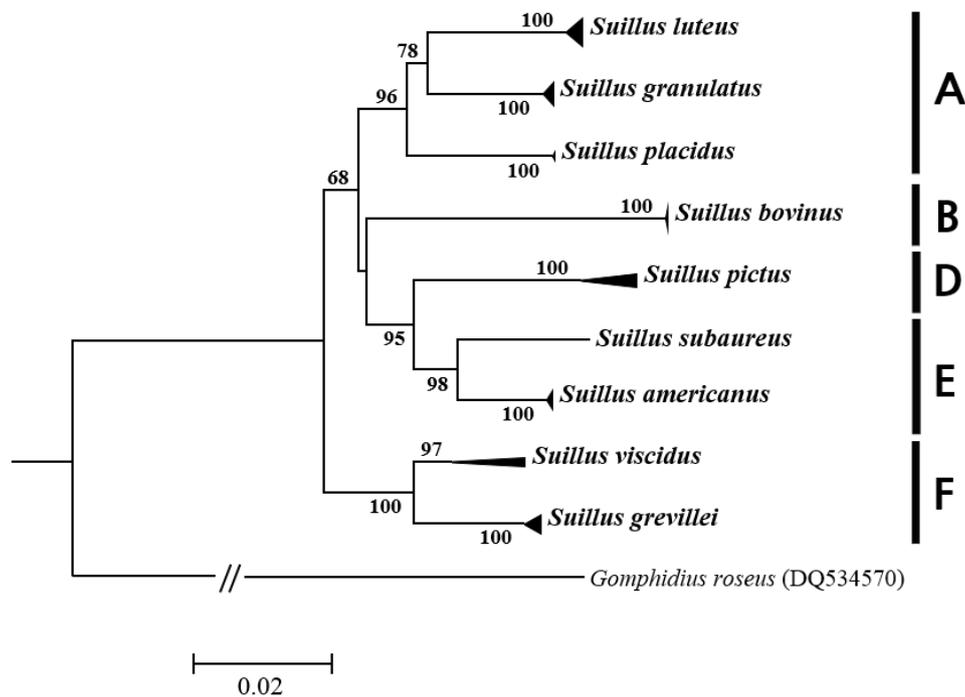


Figure 8. The neighbor-joining consensus tree of *Suillus* species including 34 specimens based on the three molecular markers (ITS, LSU and **RPB2**). Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). *Gomphidius roseus* (DQ534570) was used as the outgroup.

For the ITS phylogeny, *Suillus* species were divided into seven clades and Korean samples were present in five clades. In general, clade B is classified into *Suillus* section *Fungosi* which contains the type species *S. variegatus*. Clade F belong to *Suillus* section *Larigni*. Clades A and E belong to *Suillus* section *Suillus* and clade C, D and G belong to *Suillus* section *Solidipedes*. Exceptionally, *Suillus tomentosus* is closely related to section *Fungosi*, although this species belongs to section *Suillus* (Fig. 5). This result shows that molecular phylogeny of genus *Suillus* does not follow the taxonomical relationships previous reported.

In clade A, Korean *S. granulatus* group was monophyletic with European *S. granulatus*. *S. luteus* group is monophyletic with reference sequences from United States, but cluster together with other reference sequences (Fig. 9). On the other hand, the relationship of *S. placidus* group to other species is unclear but this group solely clustered together including the reference sequence.

Clade B is divided into four main species: *S. bovinus*, *S. plorans*, *S. tomentosus*, *S. variegatus* (Fig. 10). Korean and European *S. bovinus* are monophyletic with strong support. On the other hand, *S. tomentosus*, *S. variegatus*, and *S. plorans* clustered together with 100% bootstrap value and a *S. plorans* and *S. variegatus* group rendered paraphyletic by *S. tomentosus*.

Clade D includes only one Korean species (Fig. 11). The Korean *S. pictus* group clustered with conspecifics, but this group showed high sequence variation. Korean *Suillus pictus* is closely related *Suillus* reference sequences from China.

Subsequently, clade E included two Korean species and one unrecorded species (Fig. 12). The *S. americanus* group clustered together with reference sequence from Japan and this group has a close relationship with *S. sibiricus*. In addition, one Korean species was clustered with *Suillus subaureus*, which was previously unknown in Korea.

Clade F included two Korean species: *S. viscidus* and *S. grevillei* (Fig. 13). Although these species had a small number of specimens, sequence analysis presented a clear relationship among the other species. Five specimens of *S. grevillei* have monophyletic relationship with reference sequences of *S. grevillei* from Sweden. However, *S. viscidus* from Japan is monophyletic with one Korean specimen and species from United Kingdom is also monophyletic with one Korean specimen. Besides, *S. viscidus* from Japan is monophyletic with one Korean specimen and two Korean *S. viscidus* are rendered paraphyletic by this reference sequence.

Clade A

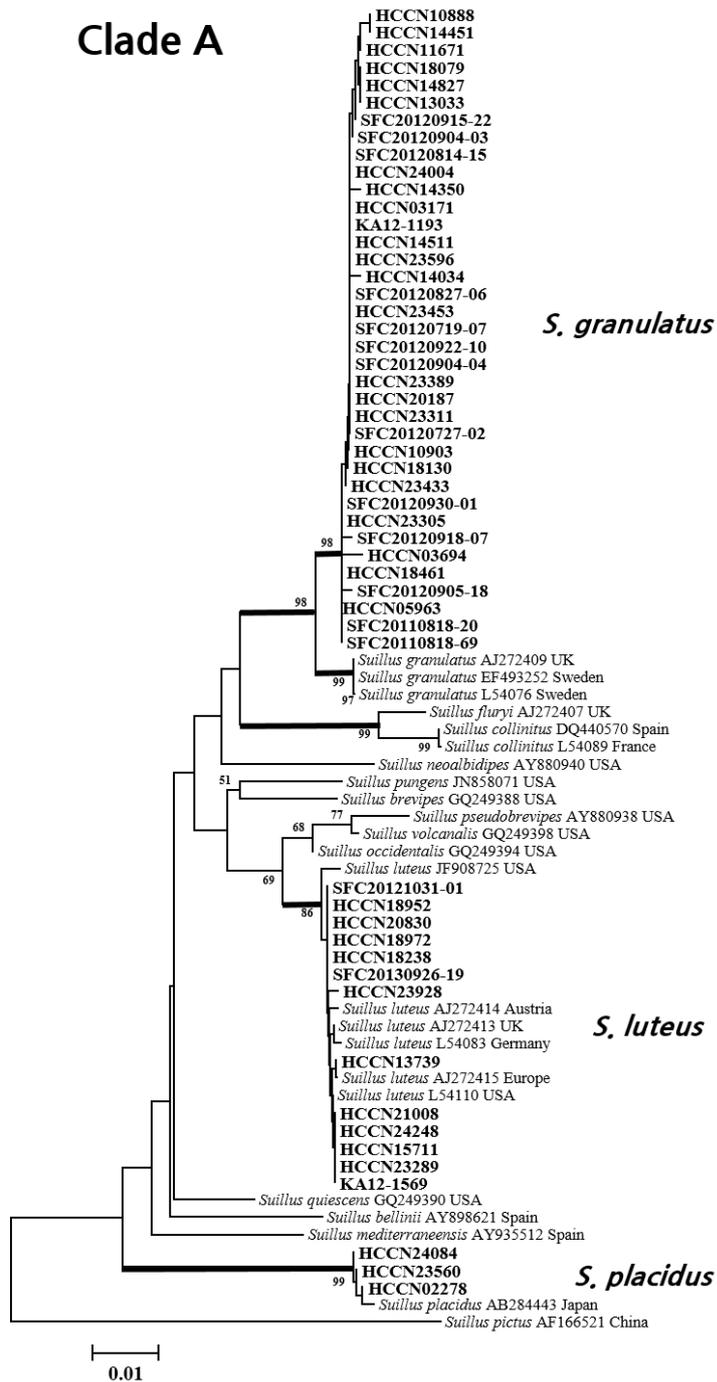


Figure 9. Phylogenetic analysis of clade A in section. *Suillus* inferred from the ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). Korean species is divided into three groups (*S. granulatus*, *S. luteus*, *S. placidus*).

Clade B

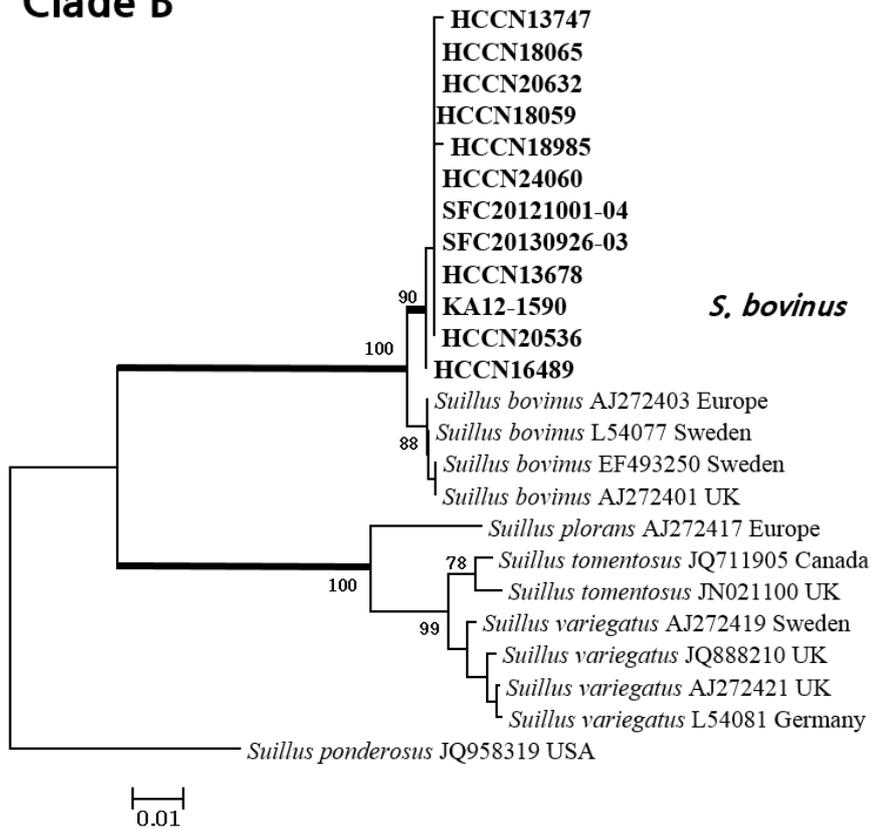


Figure 10. Phylogenetic analysis of clade B in section. *Fungosi* and *Suillus* inferred from the ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). Korean species is divided into three groups (*S. bovinus*, *S. tomentosus*, *S. variegatus*). Only *S. bovinus* was included in this study.

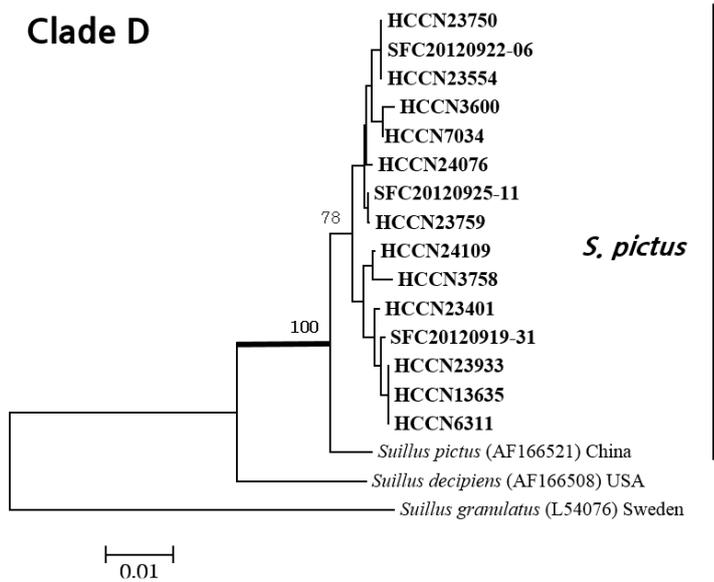


Figure 11. Phylogenetic analysis of clade D in section. *Solidipedes* inferred from the ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). Only *S. pictus* was included in this study.

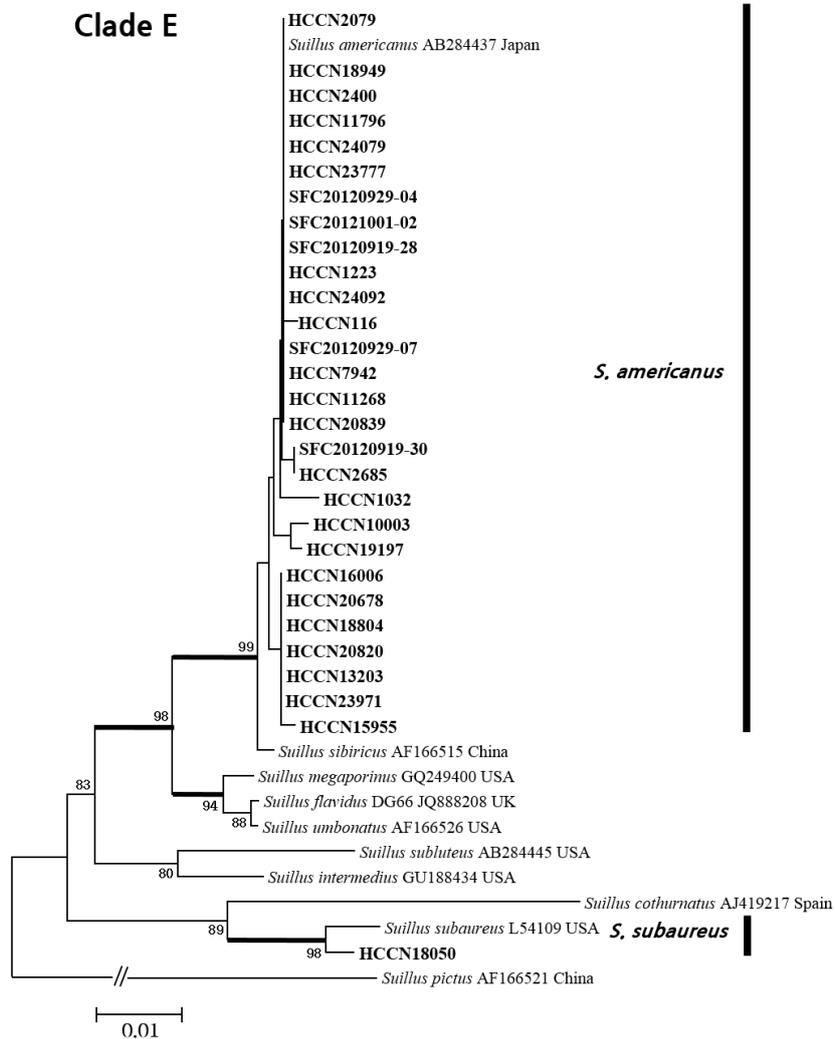


Figure 12. Phylogenetic analysis of clade E in section. *Suillus* inferred from the ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). Korean species are divided into *S. americanus* and *S. subaureus* groups. *Suillus subaureus* was a previously unrecorded species in Korea.

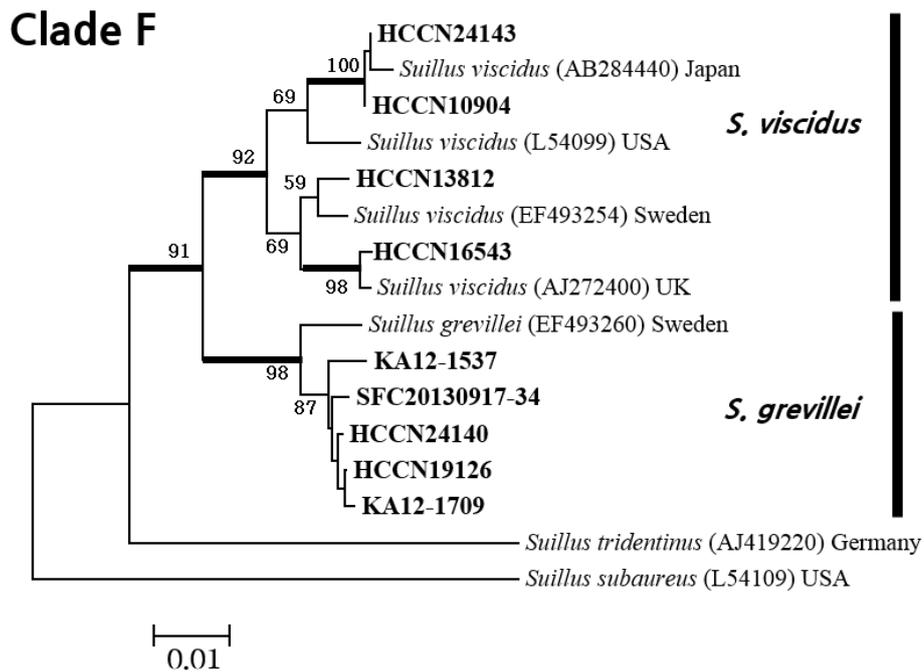


Figure 13. Phylogenetic analysis of clade F in section *Larigni* inferred from the ITS region. Numbers above the branches are frequencies (%) of statistical occurrence based on one hundred bootstrap replicates (values < 50% are not shown). Korean species include *S. viscidus* and *S. grevillei*.

3.3. Intra- and inter specific variation of ITS region

To define genetic diversity in detail, the pairwise dissimilarity was calculated using ITS region for the eight *Suillus* species. Maximum intraspecific dissimilarity was determined for each species, including reference sequences in each group (Table 5). The nucleotide sequence of ITS regions were analyzed and compared for three fragments (ITS1, ITS2 and ITS1-5.8S-ITS2). Among the *Suillus* species, maximum intraspecific dissimilarity of the ITS1 region ranged from 0.94 to 5.37 and the level of ITS2 region ranged from 0.95 to 5.22. The level of ITS1-5.8S-ITS2 has lower values than two smaller ITS fragments. All species, except *S. americanus* and *S. grevillei*, showed greater differences in the ITS1 region compared to ITS2. *S. granulatus*, *S. placidus*, *S. pictus* showed a clear intraspecific dissimilarity in ITS1. *S. viscidus* had the highest value among the *Suillus* species. In general, *Suillus* species showed higher intraspecific variation levels in order with all three regions.

Table 5. Maximum intraspecific dissimilarity of ITS of *Suillus* species

Species	Maximum intraspecific dissimilarity (%)		
	ITS1	ITS2	ITS1-5.8S-ITS2
<i>Suillus americanus</i>	0.94	2.33	0.96
<i>Suillus bovinus</i>	1.89	1.58	1.26
<i>Suillus granulatus</i>	2.35	1.62	1.45
<i>Suillus grevillei</i>	1.38	3.69	1.69
<i>Suillus luteus</i>	1.1	0.95	0.55
<i>Suillus placidus</i>	2.35	0.98	1.13
<i>Suillus pictus</i>	2.73	1.96	1.58
<i>Suillus viscidus</i>	5.37	5.22	3.87

Along with the intraspecific sequence variation, interspecific dissimilarity of *Suillus* species for ITS region was studied to compare the genetic differences between each *Suillus* species (Fig. 14). In box plot, clade A and E belonging to section *Suillus* showed a clear pattern of interspecific dissimilarity for ITS. In other words, there is no overlap the range of intraspecific variation with the range of interspecific variation. Clade D and G also showed a non-overlapping pattern between *S. pictus* and *S. cavipes* and clade B has obvious interspecific dissimilarity, except for a comparison of *S. tomentosus* with *S. variegatus*. On the other hand, clade F presented a pattern of overlapping of intra- and interspecific variation.

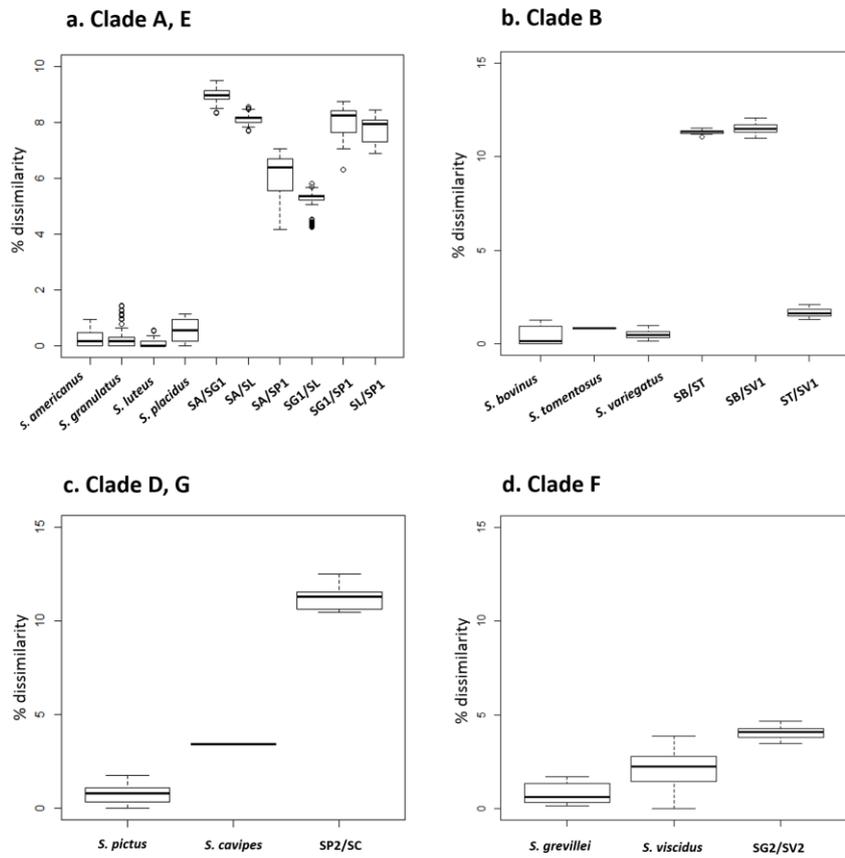


Figure 14. Boxplots of intra- and interspecific variation of *Suillus* species.

Pair-wise comparisons using ITS gene are made of % dissimilarity among the species belonged to each clade (a) Clade A, E (b) Clade B (c) Clade D, G (d) Clade F. SA: *Suillus americanus*, SB: *Suillus bovinus*, SG1: *Suillus granulatus*, SG2: *Suillus grevillei*, SL: *Suillus luteus*, SP1: *Suillus placidus*, SP2: *Suillus pictus*, ST: *Suillus tomentosus*, SV1: *Suillus variegatus*, SV2: *Suillus viscidus*.

4. Discussion

4.1. Korean *Suillus*

Korean *Suillus* species were re-evaluated using DNA sequence analysis and microscopic characteristics. Since first report of *Suillus*, 13 total species have been reported from Korea (Lim and Kim 1972; Lee 1990; Kim 1993; Seok et al. 1995; Lee and Lee 2000; Park et al. 2010). Based on microscopic features and ITS, some specimens of *Suillus* were re-identified as different species. Even though some species are easily distinguished though their distinctive morphological features, it is difficult to identify some species belonging to same section because of shared characters.

Out of 118 specimens used in this study, 51 specimens were misidentified (43% misidentification rate). *S. granulatus* specimens were misidentified as *S. grevillei* (6), *S. luteus* (9), *S. placidus* (3), *S. pictus* (1) and previously unidentified specimens. *Suillus luteus* is also prone to be misidentified as *S. grevillei* (4). There are classification keys to differentiate these species such as the presence/absence of a ring and the presence of an exudate when young, but these characters are not reliable as the ring can be detached from the stem or droplets might not be flow out depending on ecological environment. DNA sequence analysis has been suggested to reduce morphological misidentification. At the onset of this study, 11 species based on morphological identification were used in this study, later being amended to nine species with molecular analysis, including a

previously unrecorded species. However, the rest of the previously recorded species in Korea could not be confirmed due to absence of specimens.

Based on molecular identification, microscopic features of each species were observed to confirm taxonomical details. As a result, microscopic characters of Korean *Suillus* species are covered by published data. However, there are no clear differences in size and shapes of basidia, spores, and cystidia among species. Consequently, this result indicates that molecular analysis with micro-morphological observation is suggested for more accurate identification because microscopic features are insufficient to compare the confusable species.

4.2. Phylogeny of Korean *Suillus*

Molecular phylogenetic results of the three molecular markers (ITS, LSU, RPB2) presented in this study are in agreement with *Suillus* phylogeny as suggested by Kretzer et al (1996). Based on ITS dataset, 118 *Suillus* species were divided into seven clades (Fig. 5). Even though phylogenetic relationships among all included species is weakly supported by bootstrap values, each clade formed a clear relationship.

According to the Agaricales in the modern taxonomy by Singer (1986), the genus *Suillus* was largely classified into five sections: *Solidipedes*, *Glandulosi*, *Larigni*, *Suillus* and *Fungosi* (Singer 1986). Besides, each section was divided into several subsections. Section *Suillus* include two clades (clade A, E) but these clade are not closely clustered.

Clade A including *S. granulatus*, *S. luteus* and *S. placidus* is classified as subsection *Suillus* within section *Suillus* (Fig. 9).

Within this group, *S. granulatus* is separated into two distinctive groups of Korea and Europe. This result implies that genetic difference can be attributable to geographic separation. Previous studies observed that allopatric population of *S. granulatus* presented high genetic divergence and suggested the probability of presence of several taxa (Manian et al. 2001). Also, heterogeneity among *S. granulatus* isolates revealed physiological variation such as mating patterns and difference of host specificity (Fries and Neumann 1990; Jacobson and Miller Jr 1992). The *S. luteus* group has no regional differences, while Korean *S. placidus* are closely related to Japanese *S. placidus*, but the phylogenetic relationships among the group of subsection is unclear. Typically, the pileus of subsection *Suillus* is somewhat viscid, spore print brownish cinnamon, pores usually smaller than 1 mm. However, clade E belonging to same section is divided into three subsections (Fig. 12).

Of these, Korean *S. americanus* belong to subsection *Latiporini*. This subsection distinguishing from subsection *Suillus* (clade A) features wider pore size than 1 mm and a cinnamon colored spore print without an olive tint. Korean *S. americanus* are clustered with Japanese *S. americanus* and they are closely related to Chinese *S. sibiricus* sequence. In this study, 28 specimens of *S. americanus* were identified but five specimens were originally identified as *S. sibiricus* based on morphology. Phylogenetic patterns between *S. americanus* and *S. sibiricus* from Asia and U.S appeared to have a closer relationship

by a high bootstrap value (Wu et al. 2000). Likewise, the phylogenetic relationship between Korean *S. americanus* and Chinese *S. sibiricus* are also monophyletic by 99% bootstrap values (Fig. 12). Also, additional confirmation in the RPB2 phylogeny showed that the specimens misidentified as *S. sibiricus* are clustered with *S. americanus*. In clade E, there is an unrecorded species which was previously suspected in Korea. A specimen, HCCN18050, was close related to *S. subaureus* from United States by 98% bootstrap value. This species belongs to the subsection *Hirtellini* within the section *Suillus*. Microscopic characteristics of this species comes with description by Smith and Thiers (1971).

Clade B, based on the ITS phylogeny, is divided into two clear groups which include *S. bovinus* and *S. variegatus*. A group of *S. bovinus* shows geographic variation with European *S. bovinus*. Even though *S. tomentosus* are not included in the same section as other species within clade B, *S. variegatus*, *S. tomentosus*, and *S. plorans* appear to have close relationships supported by strong bootstrap values (Fig. 10). Previous studies presented that genetic divergence between *S. variegatus* and *S. plorans* is low (Kretzer et al. 1996; Manian et al. 2001). However, since there were no specimens in Korea, molecular and microscopic features could not be observed.

The ITS phylogenetic analysis presents different pattern compared to morphological characteristics. Section *Solidipedes* was divided into three separate parts (Fig. 5). A group of Korean *S. pictus* is monophyletic with *S. pictus* from China and also appears to have a closer relationship with *S. decipiens* from United States (Fig. 5, 11). It is difficult

to differentiate between *S. pictus* from Asian and *S. decipiens* from United States because they have similar morphological features (Wu et al. 2000). Some specimens morphologically identified as *S. viscidipes* belong to this *S. pictus* group. Even though these specimens were re-identified based on ITS sequences, LSU and RPB2 sequences of these specimens could not be amplified because the condition of specimens were not good. Since the reference sequences of *S. viscidipes* did not exist in the NCBI database, further studies should be examined by comparison with foreign isolates or vouchers.

On the other hand, clade F including section *Larigni* forms a strong supported clade by high bootstrap value (Fig. 13). *Suillus grevillei* is monophyletic with *S. grevillei* from Sweden while *S. viscidus* showed genetic variation among Asian, U.S and European species (Fig. 13). These species belong to different subsections. *Suillus viscidus*, which belong to subsection *Megasporini*, is distinguished by wider pores, basidia and pleurocytidia compared to *S. grevillei* within subsection *Leptoporini* (Table 4).

Korean *Suillus* species, in the RPB2 phylogeny, showed a similar pattern as ITS phylogeny while the LSU phylogeny provided little resolution, but the phylogenetic tree could not clarify relationships among *Suillus* species due to scarcity of sequence data. Thus, to fully understand the genetic divergence among the *Suillus* species, follow up studies are needed to confirm using more RPB2 sequences.

4.3. The maximum intraspecific dissimilarity

Intra- and interspecific variations among all samples in this study were analyzed for ITS. Maximum intraspecific dissimilarity of LSU and RPB2 were not calculated because there were too few reference sequences. In comparing the intraspecific dissimilarity of ITS regions, each fragment of ITS region showed fine distinctions depending on the species (Table 5). Overall, ITS1 sequences among the *Suillus* species had higher variability than the other two fragments of ITS. The intraspecific variability of ITS1 in fungi is more variable because of evolving rapidly while ITS2 is relatively slow (Narutaki et al. 2002; Hinrikson et al. 2005). On the contrary, two *Suillus* species, *S. americanus* and *S. grevillei*, appear to have higher intraspecific dissimilarity of ITS2 (Table 5). Previous studies suggest that ITS1 is always not the most variable part of the ITS region in some groups of fungi (Leaw et al. 2006; Nilsson et al. 2008). Also, *S. viscidus* in this result showed the highest intraspecific divergence (3.87- 5.37) among Korean *Suillus* species (Table 5). These results mean that it may be difficult to identify some species that have similar morphology on the species level. However, there is no doubt that intraspecific divergence based on ITS sequence data determine to defining the taxonomy of the genus (Manian et al. 2001).

4.4. Comparisons of interspecific variation

Korean *Suillus* species confirmed in this study are divided into four groups. A gap between intra- and interspecific variation verify genetic differences among similar species. Mostly Korean *Suillus* species are members of the section *Suillus* including three frequently confused species. Clade A and E belonging to section *Suillus* have low intraspecific variations and also showed an obvious gaps between the intra- and interspecific dissimilarities in the boxplots (Fig. 14a). They are clearly distinguished by ITS sequence variation even though they are members of the same section. In other words, ITS sequence provides useful data to distinguish the morphologically variable species and to determine the interrelationships among the *Suillus* species (Kretzer et al. 1996; Wu et al. 2000; Manian et al. 2001).

On the other hand, *S. tomentosus* and *S. variegatus* are not in same section, but interspecific variation is very low between these species (Fig. 14b). In case of clade F, ITS sequences provide insufficient information to recognize the genetic distinction (Fig. 14d). For this reasons, LSU and RPB2 sequence analysis are suggested to make up for the weak points between confused species by additional marker (Matheny 2005; Eberhardt 2010).

5. Conclusions

Based on DNA sequence analysis and microscopic features, 11 *Suillus* species based on morphology were re-identified as eight species and one previously unrecorded species in Korea. Some species of genus *Suillus* are usually mistaken as different species in same section. Molecular markers are beneficial to distinguish these species and to supplement shortcomings of the identification based on morphological characteristics. Through ITS, LSU, and RPB2 markers, phylogenetic relationships among *Suillus* species was confirmed and compared with the morphological classification. Additionally, intra- and interspecific variation of ITS sequences showed the clear distinctions among the species belonging to same section, although some species could not be confirmed in this study.

6. References

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7. Taxonomic study of *Suillus* in Korea

Genus *Suillus* Micheli ex S.F. Gray

Nat. Arr. Brit. Pl. 1: 646. 1821; em. Snell (1942)

Characteristics Pileus glabrous or fibrils or scales; surface dry or glutinous; hymenophore tubulose; pores small or wide; stipe central to eccentric; with or without glandular dots on stipe; veil present or absent; annulus present or absent in the margin of the pileus; clamps connections present or very rare; elongate, elliptical spores; large and clustered cystidia; mostly mycorrhizal relations with *Pinaceae*.

Type species: *Suillus luteus* (L. ex Fr.) S. F. Gray.

Distribution: Across the northern hemisphere where *Pinaceae* can be found, but mostly distributed in temperate areas.

Remarks: Hymenophore not strongly decurrent at stipe unlike other genus in *Boletaceae*. Cap usually greasy except for tomentose species while stipe dry and sometimes has glandular dots. A veil is present or absent. *Suillus* species are closely related with genus *Gomphidius*.

Key to Korean *Suillus* species

1. Cap dry, surface tomentose, reddish brown scale..... *S. pictus*
1. Cap slimy and glabrous..... 2
2. Mycorrhizal association with *Populus*.....*S. subaureus*
2. Mycorrhizal association with conifers.....3
3. Mycorrhizal association with *Larix*.....4
3. Mycorrhizal association with *Pinus*.....5
4. Cap greyish to brownish, pores greyish..... *S. viscidus*
4. Cap yellow to brownish orange, pores pale yellow..... *S. grevillei*
5. Mycorrhizal association with *Pinus strobus*.....6
5. Mycorrhizal association with *Pinus densiflora*.....7
6. Cap yellowish, light yellow patches along the cap margin*S. americanus*
6. Cap whitish yellow to brown, pores white, pinkish droplets..... *S. placidus*
7. Stipe with membranous annulus..... *S. luteus*
7. Stipe without annulus..... 8
8. Tubes beaded with milky droplets when young..... *S. granulatus*
8. Tubes not beaded with milky droplets, cap concolorous with stipe..... *S. bovinus*

Suillus americanus (Peck) Snell

Lloydia 7:39. 1944

Synonym: *Boletus americanus* Peck, Bull. N.Y. St. Mus. nat. Hist. 1 (no. 2): 62 (1887); *Ixocomus americanus* (Peck) E.-J. Gilbert, Les Livres du Mycologue Tome I-IV, Tom. III: Les Bolets: 93 (1931).

Basidiocarps Cap 40-68 mm wide, subconic to convex with incurved margin, hanging down a light yellow veil at the margin, surface of cap yellow to brownish and slimy, with distributed adhered patch debris; Context mustard yellow or pale apricot color; Stipe 40-75 mm length, 10 mm thick, frequently cylindrical and crooked, scattered with brownish glandular dots, downy surface with membranous ring; Tube yellow or greenish-yellow but brown in age, subdecurrent, pores large and radially arranged, angular shape, around 1-2 mm width.

Spore print dull cinnamon to yellowish-brown

Basidia clavate with 4 sterigmata, 21.7-33 × 5.5-10.3 μm

Basidiospores elliptic and smooth surface, 7.4-11.2 × 3-4.2 μm

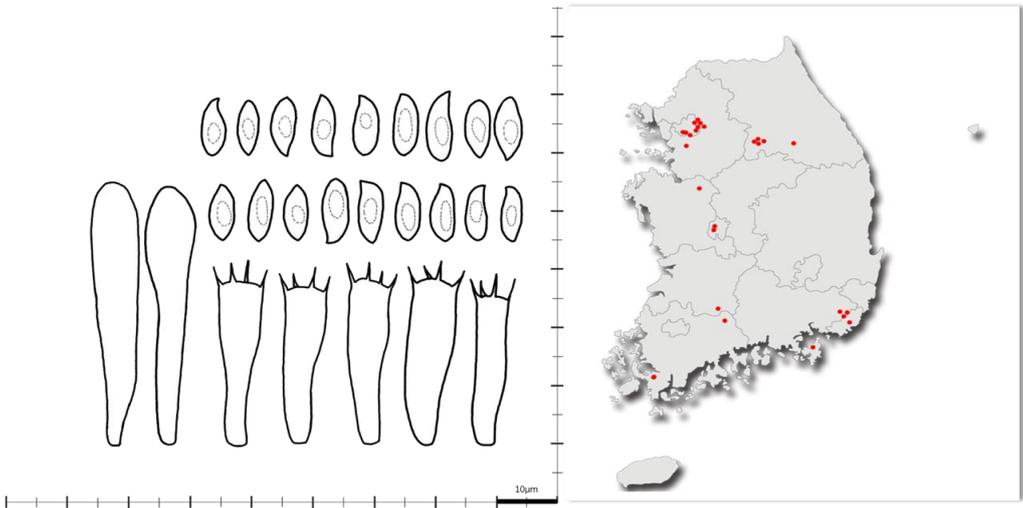
Pleurocystidia cylindrical to clavate, 33.1-62.2 × 4.3-8.7 μm

Microscopic observed specimens HCCN23777, HCCN20820

Distribution The conifer zone and/or temperate area. Korea, North America, Japan.

Habitat in Korea mycorrhizal association with *Pinus strobus*, solitary or gregarious, late summer to fall.

Remarks mustard yellowish colored cap and pores, cottony patches along the cap margin when young and presence of epicutis in the cap when old. This species is commonly confused with *S. sibiricus*, but stipe is more slender than *S. sibiricus*.



Suillus bovinus (L.:Fr.) O. Kuntze

Synonym: *Boletus bovinus* L., Sp. pl. 2: 1177 (1753); *Agaricus bovinus* (L.) Lam., Encycl. Méth. Bot. 1(1): 52 (1783); *Viscipellis bovina* (L.) Quél., Enchir. fung. (Paris): 157 (1886); *Ixocomus bovinus* (L.) Quél., Fl. mycol. France (Paris): 413 (1888); *Mariaella bovina* (L.) Šutara, Česká Mykol. 41(2): 76 (1987); *Boletus congoensis* (Beeli) Heinem., Bull. Jard. Bot. État 21: 331 (1951).

Basidiocarps Cap 30-100 mm wide, convex with incurved margin when young, plane when old, glabrate and smooth surface, little viscid, brownish-orange to reddish-brown, context whitish to yellowish-white, solid, unchanging when cut; Stipe 30-75 length, 5-10 mm thick, cylindrical and crooked in the middle or upper part, brownish-yellow or concolorous with the cap, slightly pinstripe and reticulate fibrillose at base; Tubes subdecurrent, grayish-yellow or concolorous with the cap, pores radially arranged and irregularly various angular shape.

Spore print greenish-brown

Basidia clavate with 4 sterigmata, 19.4-25.8 × 5.6-7.9 μm

Basidiospores elliptic and smooth surface, 7.2-10.2 × 3-4.4 μm

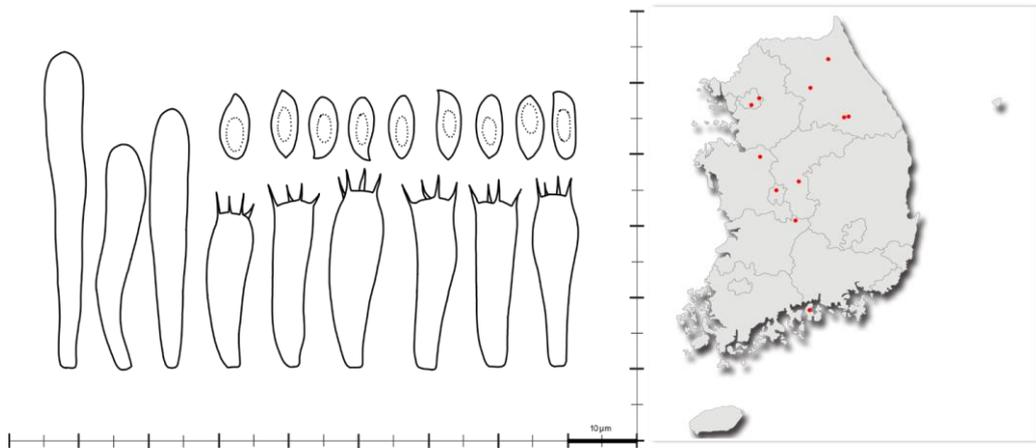
Pleurocystidia cylindrical to clavate, 28.3-54.4 × 3.7-6.9 μm

Microscopic observed specimens HCCN20536, KA12-1590

Distribution the conifer zone and/or temperate area. Korea, Japan, Europe, Australia.

Habitat in Korea mycorrhizal association with *Pinus densiflora*, often solitary or gregarious, summer to fall.

Remarks color of stipe and pores similar with the cap, sometimes confused with *S. granulatus*, but without secretion of milky droplets. This species is strongly associated with *Pinus densiflora* in Korea and also closely related with genus *Gomphidius roseus*.



Suillus granulatus (Fr.) Kuntze

Rev. Gen Pl. 32:535.1898

Synonym: *Boletus granulatus* L., Sp. pl. 2: 1177 (1753); *Rostkovites granulatus* (L.) P. Karst., Revue mycol., Toulouse 3(no. 9): 16 (1881); *Viscipellis granulata* (L.) Quél., Enchir. fung. (Paris): 156 (1886); *Ixocomus granulatus* (L.) Quél., Fl. mycol. France (Paris): 412 (1888); *Suillus lactifluus* (With.) A.H. Sm. & Thiers, Michigan Bot. 7: 16 (1968).

Basidiocarps Cap 40-110 mm wide, hemispherical or convex, surface slimy or glabrous, incurved margin, color variation but typically brownish-orange, light brown, sometimes pinkish in center and yellowish-white in edge when young, but pinkish-brown when it matures, wave patterned or spotted with reddish-brown or cinnamon color; Context soft and plumpy, whitish to yellow, unchanging when cut; Stipe 50-80 length, 10-20 thick, solid, scattered with brownish glandular dots, white or yellowish flesh; Tubes subdecurrent, pale yellow or light yellow, pores around 1 mm width, often beaded with milky droplets when young.

Spore print yellowish-brown

Basidia clavate with 4 sterigmata, 21.2-28.1 × 5.4-7.5 μm

Basidiospores elliptic and smooth surface, 6-10.6 × 2.4-3.8 μm

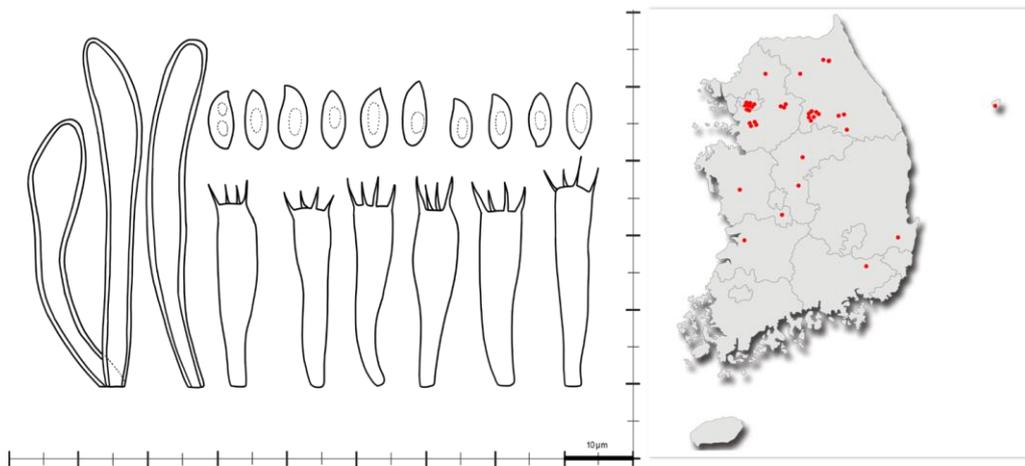
Pleurocystidia cylindrical to clavate, 32.1-54.6 × 4.7-8.7 μm

Microscopic observed specimens SFC20120814-15, KA12-1193

Distribution North hemisphere temperate area. Korea, Australia, New Zealand.

Habitat in Korea mycorrhizal association with *Pinus densiflora*, solitary or gregarious, summer to fall.

Remarks This species is distinguished from *S. luteus* by the absence of an annulus or glandular dots and secretion of the droplets when young.



Suillus grevillei (Klotzsch) Singer

Farlowia 2:259. 1945

Synonym: *Boletus grevillei* Klotzsch, Linnaea 7: 198 (1832); *Boletus elegans* var. *aureus* Fr., Epicr. syst. mycol. (Upsaliae): 409 (1838); *Boletus clintonianus* Peck, Ann. Rep. Reg. N.Y. St. Mus. 23: 128 (1872); *Ixocomus grevillei* (Klotzsch) Vassilkov, Compl. Fl. Champ. Supér. Maroc: 20 (1955); *Boletinus grevillei* (Klotzsch) Pomerl., Naturaliste Can. 107: 303 (1980)

Basidiocarps Cap 50-100 mm wide, hemispherical or convex to plane, surface viscid and glabrous, lemon yellow or brownish-orange; Context soft and thick, pale yellow; Stipe 75-100 mm length 10-15 mm thick and viscid, almost equal and cylindrical, yellowish-brown, reticulate above the annulus and pinstriped filamentous at base, yellowish annulus; Tubes adnate to decurrent, pale yellow but cinnamon colored when bruised or cut, pores angular, around 1-2 mm width.

Spore print yellowish-brown

Basidia clavate with 4 sterigmata, 19.5-28.1 × 4.9-8.9 μm

Basidiospores elliptic and smooth surface, 7.8-10.6 × 3.4-5.4 μm

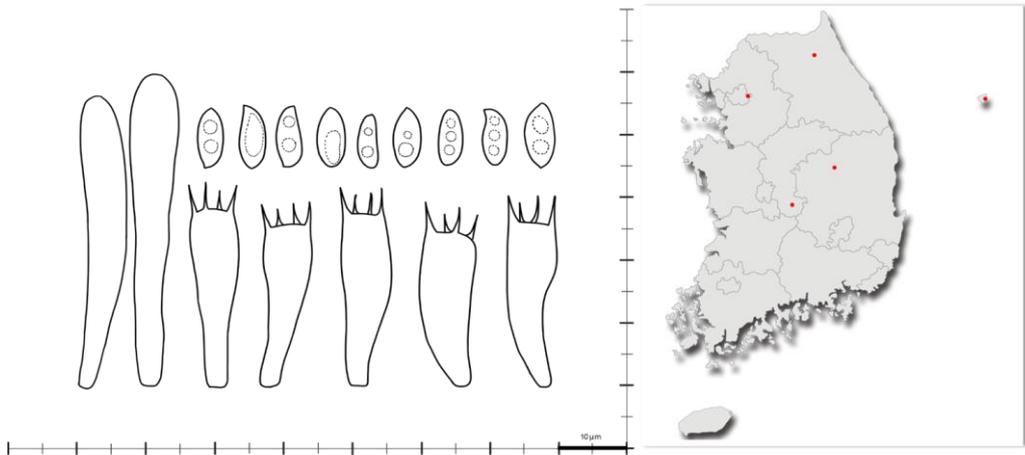
Pleurocystidia cylindrical to clavate, 33.3-53.9 × 4.1-8.1 μm

Microscopic observed specimens HCCN19126, KA12-1709

Distribution North hemisphere temperate area. Korea, Australia, New Zealand.

Habitat in Korea mycorrhizal association with *Larix*. gregarious and sometimes forming a fairy ring, late summer to fall.

Remarks This species has slimy floccose annulus and is commonly associated with *Larix* in Korea.



Suillus luteus (Fr.) S. F. Gray

Nat. Arr. Brit.Pl. 1:646. 1821

Synonym: *Boletus luteus* L., Sp. pl. 2: 1177 (1753); *Cricunopus luteus* (L.) P. Karst., Revue mycol., Toulouse 3(no. 9): 16 (1881); *Viscipellis luteus* (L.) Quél., Enchir. fung. (Paris): 155 (1886); *Ixocomus luteus* (L.) Quél., Fl. mycol. France (Paris): 414 (1888); *Boletopsis lutea* (L.) Henn., in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1**:
195 (1898)

Basidiocarps Cap 60-100 mm wide, hemispherical or convex to plane, surface viscid and glabrous, shiny when dry dark brown to reddish-brown, membranous veil hanging from the margin; Context soft, whitish-yellow, not changed when cut; Stipe 50-90 mm length, 10-20 mm thick, frequently cylindrical and crooked, pale yellow and scattered with brownish glandular dots above the annulus, typically covered by purplish-brown veil at base; Tubes 1 mm width, adnate and small, pale yellow or greyish-yellow, surface usually covered with partial veil.

Spore print reddish-brown

Basidia clavate with 4 sterigmata, 20-24 × 4.4-7.7 μm

Basidiospores elliptic and smooth surface, 6.4-9 × 2.8-4.6 μm

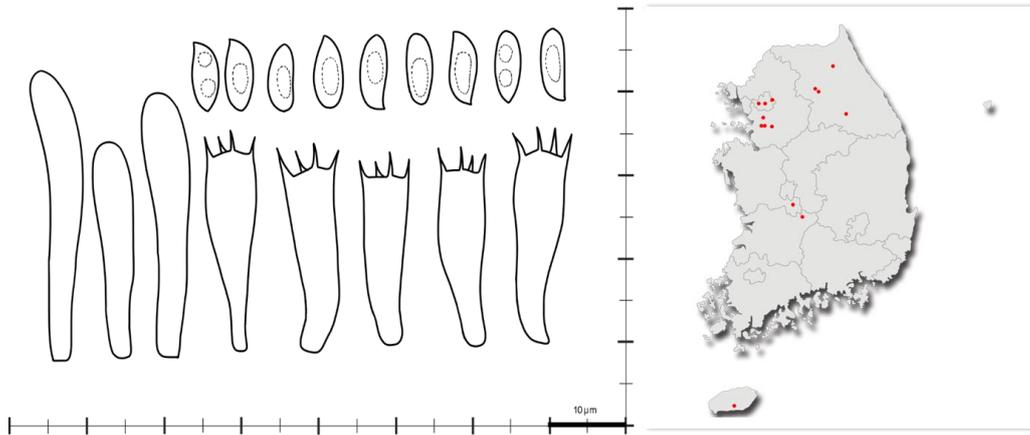
Pleurocystidia cylindrical to clavate, 24.8-50 × 4.7-9.1 μm

Microscopic observed specimens HCCN24248, HCCN18972

Distribution Northern hemisphere temperate area. Korea, Australia, New Zealand.

Habitat in Korea mycorrhizal association with *Pinus*, solitary or gregarious, summer to fall.

Remarks This species is easily recognized by the purplish-brown annulus and slimy surface of cap when moist.



Suillus pictus (Peck) A. H. Sm. & Thiers

Contrib. toward a Monograph of N. Amer. Sp. of *Suillus* p. 31.1964

Synonym: *Boletus pictus* Peck, Ann. Rep. Reg. N.Y. St. Mus. 23: 128 (1872); *Boletinus pictus* Peck, Bull. N.Y. St. Mus. nat. Hist. 2(no. 8): 77 (1889)

Basidiocarps Cap 40-80 mm wide, convex to plane, surface tomentose and dry, inrolled margin, fibrillose veil hanging from the margin, background yellow to orange yellow, mostly forming brownish-red or grayish-brown scales; Context soft and yellow; Tubes decurrent, light yellow, pores large and angular, changing reddish-brown when cut, covered with membranous veil; Stipe 40-80 mm length, equal or subclavate, 10-20 mm thick, coating similar to the fibrils of the cap, fine soft annulus but evanescent, context yellow to brownish and soft in the inside.

Spore print greenish-brown or yellowish-brown

Basidia clavate with 4 sterigmata, 22.7-30.3 × 5.5-9.2 μm

Basidiospores elliptic and smooth surface, 7.2-10.4 × 3-4.8 μm

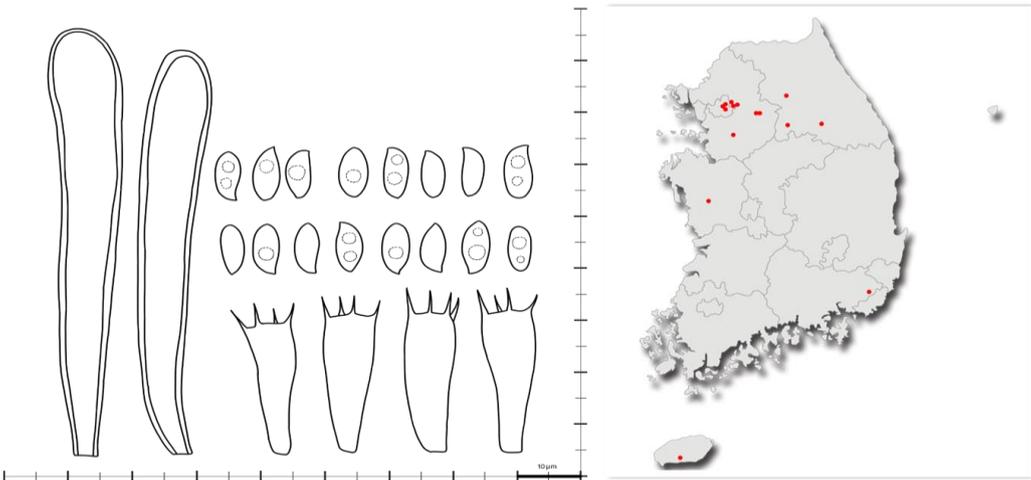
Pleurocystidia cylindrical to clavate, 32.1-80.4 × 6.3-10.5 μm

Microscopic observed specimens SFC20120925-11, HCCN23750

Distribution the conifer zone and/or temperate area. Korea, North America, Japan, China.

Habitat in Korea mycorrhizal association with *Pinus*, solitary or gregarious and sometimes forming a fairy ring, late summer to fall.

Remarks surface tomentose and presence of scales on the cap and stipe.



***Suillus subaureus* (Peck) Snell in Slipp & Snell**

Lloydia 7:30. 1944

Synonym: *Boletus subaureus* Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 39: 42 (1887); *Rostkovites subaureus* (Peck) Murrill, Mycologia 1(1): 13 (1909); *Ixocomus subaureus* (Peck) Singer, Revue Mycol., Paris 3: 45 (1938)

Basidia clavate with 4 sterigmata, $19.2-26.6 \times 5.9-7.7 \mu\text{m}$

Basidiospores elliptic and smooth surface, $6.2-9.2 \times 2.2-4 \mu\text{m}$

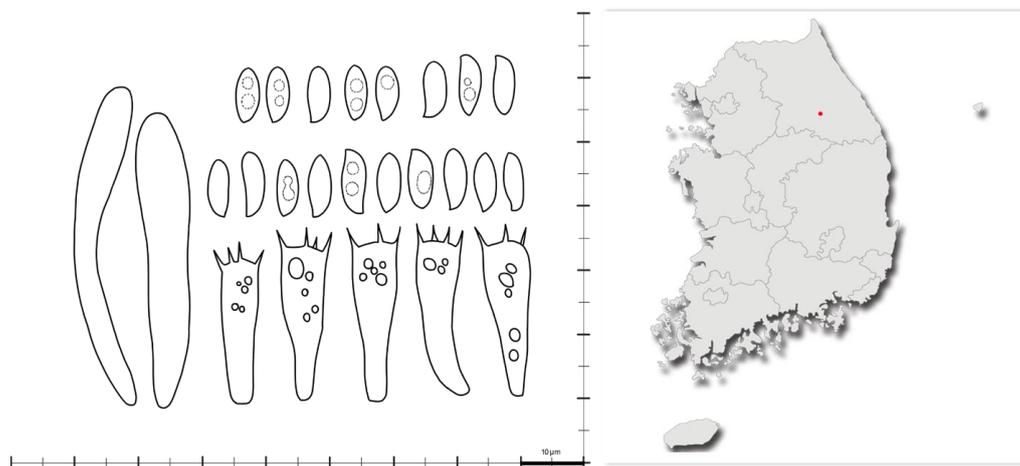
Pleurocystidia cylindrical to clavate, $39.8-53.7 \times 4.2-8.8 \mu\text{m}$

Microscopic observed specimens HCCN18050

Information of collecting region Pyeongchang-gun, Gangwon-do, Korea

Distribution the conifer zone and/or temperate area. Korea, North America, Japan, China.

Habitat in Korea mycorrhizal association with *Populus*, solitary or gregarious, summer to fall (Smith and Thiers 1971).



Abstract in Korean

비단그물버섯속은 외생균근으로서 침엽수 산림에 중요한 역할을 하는 식용 버섯으로 잘 알려져 있다. 이 버섯은 다육질이고 끈적거리거나 털이 밀생하는 갓을 가지며, 대에는 갈색의 점들이 분포되어 있는 특징으로 쉽게 식별되지만, 형태적인 특징만으로 종 수준의 동정은 쉽지 않다. 현재까지 전 세계적으로 약 98 종의 비단그물버섯이 존재한다고 보고되어 있으며, 한국에는 13 종이 보고되어 있다. 본 연구의 목적은 형태학적인 미세 특징과 ITS, LSU, RPB2 유전자 분석을 함께 수행함으로써 한국의 비단그물버섯의 정확한 분류체계를 재정립하고 계통유전학적인 유연관계를 확인하는 것이다.

ITS 시퀀스를 이용한 분자적인 동정을 기반으로 형태적으로 동정된 11 종은 9 종 노란대비단그물버섯(*S. americanus*), 황소비단그물버섯(*S. bovinus*), 젓비단그물버섯(*S. granulatus*), 큰비단그물버섯(*S. grevillei*), 비단그물버섯(*S. luteus*), 붉은비단그물버섯(*S. pictus*), 평원비단그물버섯(*S. placidus*), 녹슬은비단그물버섯(*S. viscidus*), 미기록종 (*S. subaureus*) 으로 재동정되었다. 본 연구에서 이용된 118 점의 표본 중에서 51 점이 오동정되었으며(43%의 오동정률), 젓비단그물버섯이 형태적으로 가장 높은 오동정률을 나타내었다. 또한 현미경 관찰에서는 종간의 명확한 차이점이 없어 미세형태학적인 특징만으로 유사종을 구별하는 것은 불충분하다는 것을 보여 주었다.

세 유전자 마커를 이용한 분자 계통학적인 결과에서 Neighbor-Joining 분석을 통해 비단그물버섯종이 7 개의 그룹으로 나누어졌으며, 이 중에서 6 개의 그룹은 70% 이상의 bootstrap 값으로 지지되었다. 그러나

분자 계통학적 패턴은 이전 분류학자에 의해 제안되었던 분류학적 체계와 다르게 같은 섹션에 속하는 비단그물버섯 종들이 서로 다른 그룹으로 나누어졌다.

LSU 기반 계통도보다 ITS 와 RPB2 기반의 계통도에서 비단그물버섯 종들간의 관계가 명확하였으며, 세 유전자의 유합 분석에서는 ITS 결과와 유사하였고 한국 비단그물버섯 종들의 구별이 좀 더 명확하였다.

각 섹션에 속하는 비단그물버섯 종의 최대 종내 변이를 분석한 결과 6 종의 비단그물버섯 종은 ITS1 영역이 ITS2 영역과 ITS 전체보다 높은 변이를 보였고, 나머지 두 종(*S. americanus*, *S. grevillei*)은 ITS2 에서 더 높은 종내 변이를 보였다. 녹슬은비단그물버섯(*S. viscidus*)은 한국의 비단그물버섯 종들 중에서 가장 높은 종내 변이를 보였다(3.87-5.37). 또한 각 그룹에 속하는 가깝게 연관된 비단그물버섯의 종간 변이는 명확한 바코딩 갭을 나타내었다. ITS 시퀀스는 형태적으로 다양한 종들을 구별하며 비단그물버섯 종들 간에 종간 관계를 결정하는데 유용한 정보를 제공한다.

주요어: 한국 비단그물버섯, 외생균근, ITS, RPB2, 종내 변이, 종간 변이, 바코딩 갭