

A Checklist of the Basidiomycetous Macrofungi and a Record of Five New Species from Mt. Oseo in Korea

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Abstract Basidiomycetous macrofungi play important roles in maintaining forest ecosystems via carbon cycling and the mobilization of nitrogen and phosphorus. To understand the impact of human activity on macrofungi, an ongoing project at the Korea National Arboretum is focused on surveying the macrofungi in unexploited areas. Mt. Oseo was targeted in this survey because the number of visitors to this destination has been steadily increasing, and management and conservation plans for this destination are urgently required. Through 5 field surveys of Mt. Oseo from April to October 2012, 116 specimens of basidiomycetous macrofungi were collected and classified. The specimens were identified to the species level by analyzing their morphological characteristics and their DNA sequence data. A total of 80 species belonging to 57 genera and 25 families were identified. To the best of our knowledge, this is the first study to identify five of these species—*Artomyces microsporus*, *Hymenopellis raphanipes*, *Pholiota abietis*, *Phylloporus brunneiceps*, and *Sirobasidium magnum*—in Korea.

Keywords Basidiomycetous macrofungi, DNA barcoding, Fungal flora, Internal transcribed spacer, Mt. Oseo, Unrecorded species

Macrofungi are fungal species that produce fruiting bodies visible to the naked eye [1]. Most macrofungi are members of Basidiomycota, but some belong to Ascomycota. Basidiomycetous macrofungi, such as bracket fungi, mushrooms, and puffballs, play essential roles in maintaining forest ecosystems. Saprobic macrofungi contribute to carbon cycling by decomposing woody debris on the forest floor [2], while mycorrhizal macrofungi aid the survival of other forest species by mobilizing nitrogen and phosphorus [3]. Additionally, fruiting bodies of basidiomycetous macrofungi can be important sources of food [4, 5] and medicine [6-10] for humans.

Macrofungi have traditionally been identified based on

their fruiting body morphologies and microscopic features. Some macrofungi do not have unique characters, and therefore, it is difficult to distinguish them to the species level using morphology alone. Many of these problems can be overcome by using DNA barcoding [11]. For the kingdom Fungi, the internal transcribed spacer (ITS) region has been formally proposed as the primary fungal barcoding gene, because its high sequence variation resolves closely related species across the fungal tree of life [12]. Coupling morphological data with ITS sequences increases the accuracy of species identification and provides more detailed information on fungal flora. For example, several *Amanita* species were confirmed as new records in Korea based on their morphological characteristics and ITS and large subunit rDNA sequence data [13, 14]. In addition, 2 similar Korean species of the genus *Russula* were delimited based on their morphology and ITS sequences [15].

In 2012, the Korea National Arboretum initiated a survey of macrofungi in the unexploited areas of Korea. One of the areas targeted in the survey was Mt. Oseo, located in Chungcheongnam-do, South Korea. In recent years, the number of visitors to Mt. Oseo has steadily increased, and the fear that human activity is damaging the ecosystem is increasing. Management and conservation measures, which rely on baseline data from biodiversity surveys, are needed. Although many flora and fauna surveys have recently been conducted at Mt. Oseo [16-21], studies on fungal flora have been rare; the only fungal data

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available are from a single report based on a survey in August 2010 [22].

The goal of our study was to investigate and catalog the basidiomycetous macrofungi of Mt. Oseo. In addition to building a checklist of the basidiomycetous macrofungi of Mt. Oseo, we discovered 5 species previously not recorded in Korea.

MATERIALS AND METHODS

Study area. Mt. Oseo (36°27' N, 126°40' W), the highest peak of the Geumbuk Mountains, is located in Cheongnamyeon, Boryeong-si, Chungcheongnam-do, South Korea. The mountain range runs north to south for 12 km and east to west for 18 km and has an average altitude of 790.7 m [23]. The surveys were conducted from April to October 2012 in a region of Mt. Oseo that includes the Natural Recreation Forest (shaded region in Fig. 1). In 2012, the prevailing climate of the area was moderate, with an annual mean temperature of 12.2°C and temperatures ranging from -1.4°C (mean minimum temperature in December) to 26.8°C (mean maximum temperature in August). Precipitation during the spring, summer, and fall of 2012 was 160.3 mm, 681.3 mm, and 272.7 mm, respectively. All the meteorological data were collected by the Korea Meteorological Administration at the Boryeong meteorological observatory: http://www.kma.go.kr/weather/climate/data_monthly.jsp (accessed March 2014).

Fungal survey and processing. Five surveys were conducted throughout 2012 (April, June, July, August, and October). The surveys spanned spring, summer, and fall—

a period during which the macrofungi produce fruiting bodies. In the field, color photographs were obtained and information about the collecting site, habitat, host, substrate, and fruiting body was recorded for each specimen. Samples were placed in specially prepared paper boxes and brought to the laboratory for identification. After being dried in air-vented ovens at 55°C for 3~4 days, the specimens were deposited in the Seoul National University Fungus Collection (SFC).

The specimens were initially identified on the basis of their macro- and microscopic features according to published descriptions [24-30]. Taxonomic classification of species was as per the Fungal Tree of Life (<http://aftol.org>), and the nomenclature was based on Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>).

For the new records in Korea, microscopic structures of the specimens suspended in 3% (w/v) KOH, 1% (w/v) phloxine, and Melzer's reagent (IKI) [31] and mounted on slides were measured using a Nikon SMZ1500 dissecting microscope and a Nikon Eclipse 80i optical microscope (Nikon, Tokyo, Japan). Quotient (Q) was the ratio of the difference in the mean basidiospore length to the mean spore width of the specimens studied. The 'Methuen Handbook of Colour' [32] was used as the color standard.

DNA sequence-based identification. Genomic DNA was extracted using a modified CTAB extraction protocol [33]. The ITS region was amplified using the primer set ITS1F/ITS4B [34]. PCR was performed in a C1000 thermal cycler (Bio-Rad, Hercules, CA, USA) using the AccuPower PCR PreMix (Bioneer, Daejeon, Korea) in a final volume of 20 μ L containing 10 pmol of each primer and 1 μ L of

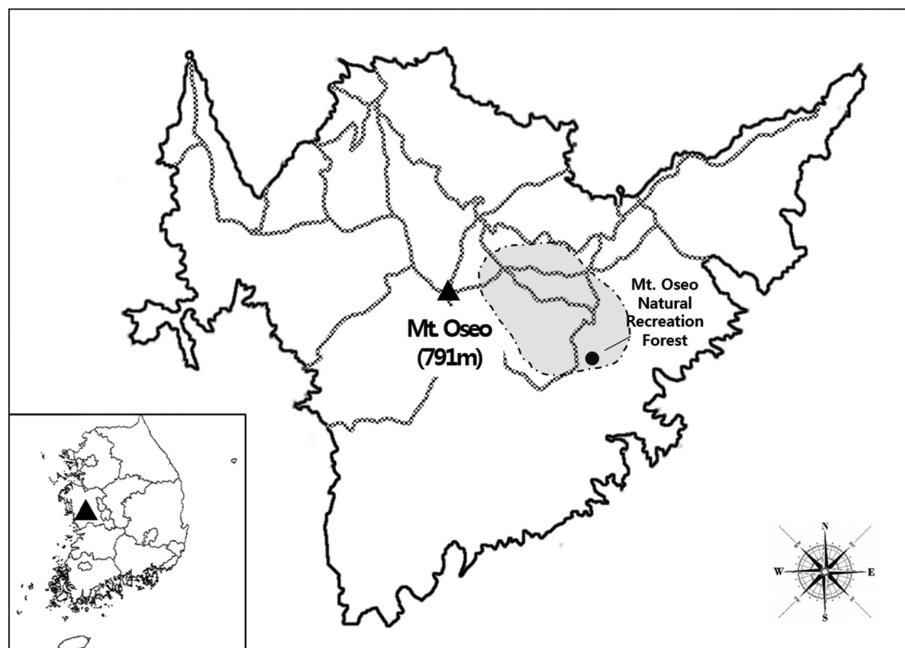


Fig. 1. Geographical location and sampling regions of Mt. Oseo (Chungcheongnam-do, South Korea). The area surveyed in this study is denoted by the shaded region, which includes the Mt. Oseo Natural Recreation Forest.

DNA (10 ng/ μ L) by the method of Park *et al.* [15]. The PCR products were electrophoresed on 1% agarose gel with LoadingSTAR (Dyne Bio, Seoul, Korea) and purified using the Expin PCR SV purification kit (GeneAll Biotechnology, Seoul, Korea), according to the manufacturer's instructions. Sequencing was performed in the reverse direction using the ITS4B primer. DNA sequencing was performed at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea), by using an ABI3700 automated DNA sequencer. All the sequences have been deposited in GenBank (accession Nos. KJ609155~KJ609174).

For molecular identification, each sequence was compared with reference sequences in GenBank by using BLAST [35]. Sequences were edited and aligned using MEGA5 [36]. A neighbor-joining phylogenetic analysis was also performed in MEGA5 using the Kimura 2-parameter model of evolution for tree inference and 500 bootstrap replicates.

RESULTS AND DISCUSSION

A total of 116 specimens of basidiomycetous macrofungi were collected during the surveys. Based on the macromorphological features alone, we were able to confidently identify 60 species. For the remaining specimens, DNA sequence analysis was performed because the morphological identification was inconclusive. For these unidentified specimens, a preliminary identification was performed using the ITS sequence analysis, and the identification was verified by comparing the observed morphological details with the published data. An additional 20 species were identified using the molecular data (Fig. 2). The morphological features and ITS sequences from 2 specimens did not match with the taxonomic information or sequences available in GenBank, and therefore, identification was limited to the genus level: *Xerocomus* sp. (SFC20120725-43) and *Marasmiellus* sp. (SFC20120821-

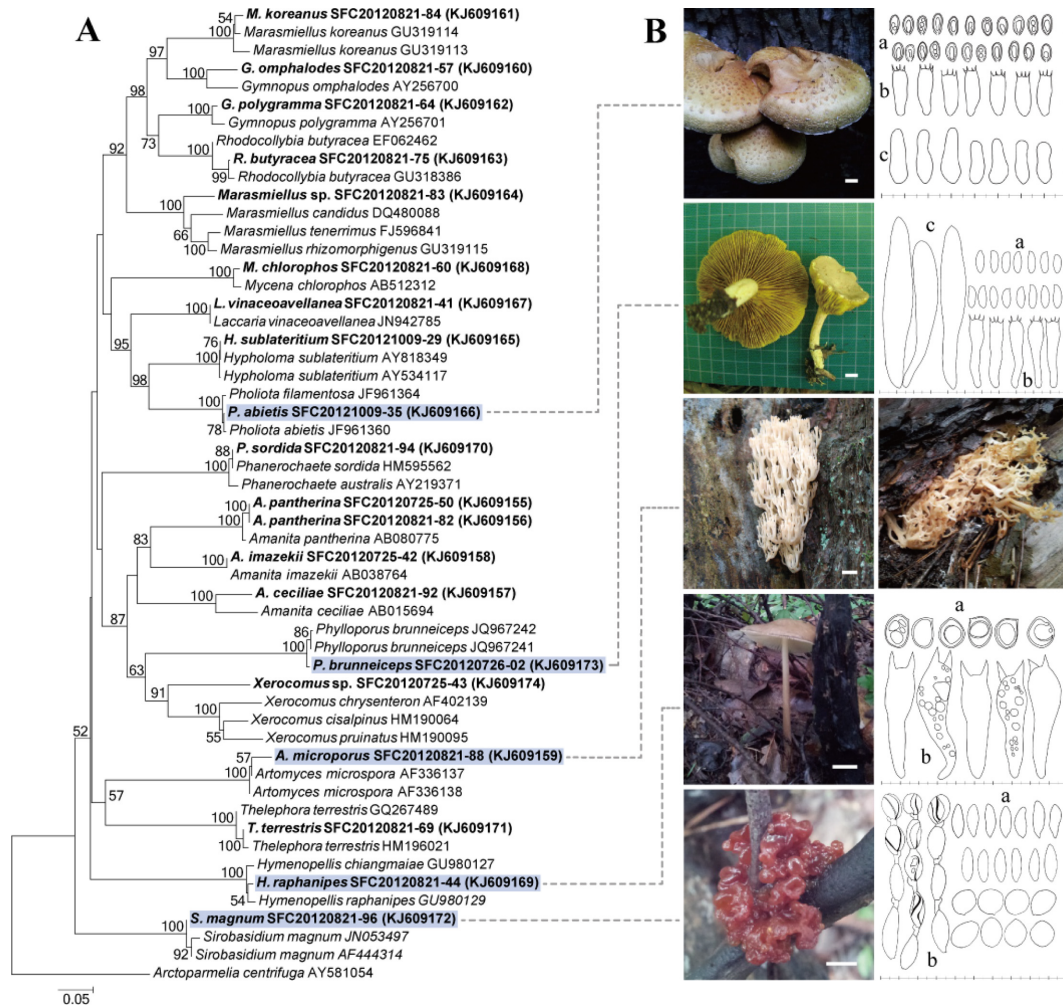


Fig. 2. Phylogenetic tree and morphologies of 5 new records in Korea. A, Phylogenetic tree inferred from internal transcribed spacer sequences. The topological structure is from neighbor-joining analysis. Bootstrap values exceeding 50% for nodes are indicated. Samples from our study are in bold font and the 5 new records in Korea are highlighted; B, Photographs of basidiocarps, and drawings of microscopic features (a, spores; b, basidia; c, cystidia) for the 5 new records in Korea. The scale bar is 1 cm in the basidiocarp images and 10 μ m in the microscopic images.

83). Additionally, 5 species previously not recorded in Korea were identified from Mt. Oseo: *Artomyces microsporus*, *Hymenopellis raphanipes*, *Pholiota abietis*, *Phylloporus brunneiceps*, and *Sirobasidium magnum* (Fig. 2). Microscopic features of *A. microsporus* could not be observed because the specimen was immature.

In total, 80 species belonging to 57 genera and 25 families were identified from Mt. Oseo (Table 1). The most common genera were *Russula* (9 species), *Amanita* (5), and *Lactarius* (5). The families that contained the most number

of species were Russulaceae (14 species), Coriolaceae (9), Tricholomataceae (8), Polyporaceae (6), and Amanitaceae (5), which together accounted for about 52% of the total fungal diversity in the collected specimens. The distribution of species by trophic group indicated a dominance of the saprotrophic species (68%), while parasitic species and symbionts were found in lower numbers (32%).

Species diversity and the type of tropism varied with the changing seasons. During the spring season, mostly wood-rotting fungi were observed. The distribution of the

Table 1. Checklist of basidiomycetous macrofungi from Mt. Oseo

Fungal classification	No. ^a	Specimen No. ^b	Tropism	Sampling season
Agaricomycetes				
Agaricales				
Agaricaceae				
<i>Leucoagaricus rubrotinctus</i>	1	SFC20120821-93	S	Summer
<i>Leucocoprinus</i> sp.	1	SFC20120821-45	S	Summer
Amanitaceae				
<i>Amanita alboflavescens</i>	1	SFC20120725-51	EM	Summer
<i>Amanita ceciliae</i>	1	SFC20120821-92	EM	Summer
<i>Amanita imazekii</i>	1	SFC20120725-42	EM	Summer
<i>Amanita pantherina</i>	2	SFC20120725-50	EM	Summer
<i>Amanita virosa</i>	1	SFC20120821-52	EM	Summer
Cortinariaceae				
<i>Hebeloma</i> sp.	1	SFC20120725-49	S	Summer
Crepidotaceae				
<i>Crepidotus sulphurinus</i>	1	SFC20120821-77	WR	Summer
Lycoperdaceae				
<i>Lycoperdon perlatum</i>	2	SFC20120821-50	S	Summer
Marasmiaceae				
<i>Marasmiellus candidus</i>	2	SFC20120821-43	S	Summer
<i>Marasmiellus koreanus</i>	1	SFC20120821-84	S	Summer
<i>Marasmiellus</i> sp.	1	SFC20120821-83	S	Summer
<i>Marasmius</i> sp.	1	SFC20120821-42	S	Summer
Physalacriaceae				
<i>Armillaria mellea</i>	1	SFC20120601-09	WR	Spring
<i>Cyptotrama asprata</i>	2	SFC20120725-46	WR	Summer
<i>Hymenopellis raphanipes</i>	1	SFC20120821-44	S	Summer
Psathyrellaceae				
<i>Psathyrella candolleana</i>	2	SFC20120725-39	S	Summer
Strophariaceae				
<i>Hypholoma sublateritium</i>	1	SFC20121009-29	WR	Fall
<i>Pholiota abietis</i>	1	SFC20121009-35	WR	Fall
Tricholomataceae				
<i>Collybia confluens</i>	1	SFC20120821-46	S	Summer
<i>Gymnopus omphalodes</i>	1	SFC20120821-57	S	Summer
<i>Gymnopus polygrammus</i>	1	SFC20120821-64	S	Summer
<i>Laccaria vinaceoavellanea</i>	1	SFC20120821-41	EM	Summer
<i>Mycena chlorophos</i>	1	SFC20120821-60	S	Summer
<i>Panellus stipticus</i>	3	SFC20120409-14	WR	Spring, summer, fall
<i>Rhodocollybia butyracea</i>	1	SFC20120821-75	S	Summer
<i>Tricholomopsis rutilans</i>	1	SFC20120821-48	EM	Summer
Auriculariales				
Auriculariaceae				
<i>Auricularia polytricha</i>	1	SFC20120726-04	WR	Summer
Exidiaceae				
<i>Heterochaete delicata</i>	1	SFC20120409-16	WR	Spring

Table 1. Continued

Fungal classification	No. ^a	Specimen No. ^b	Tropism	Sampling season
Boletales				
Boletaceae				
<i>Boletellus chrysenteroides</i>	1	SFC20120821-63	EM	Summer
<i>Phylloporus brunneiceps</i>	1	SFC20120726-02	EM	Summer
<i>Xeroconus</i> sp.	1	SFC20120725-43	EM	Summer
Corticiales				
Corticiaceae				
<i>Basidioradulum radula</i>	1	SFC20120821-67	WR	Summer
<i>Hyphoderma setigerum</i>	1	SFC20121009-31	WR	Fall
<i>Phanerochaete sordida</i>	3	SFC20120821-79	WR	Summer
<i>Rhizochaete filamentosa</i>	1	SFC20120601-08	WR	Spring
<i>Schizopora paradoxa</i>	5	SFC20120409-11	WR	Spring, summer, fall
Hymenochaetales				
Hymenochaetaceae				
<i>Fuscoporia gilva</i>	2	SFC20120409-12	WR	Spring, fall
<i>Hydnochaete tabacina</i>	4	SFC20120409-09	WR	Spring, summer
Polyporales				
Auriscalpiaceae				
<i>Artomyces microsporus</i>	1	SFC20120821-88	WR	Summer
Coriolaceae				
<i>Antrodia albida</i>	1	SFC20120409-02	WR	Spring
<i>Antrodia xantha</i>	1	SFC20120601-10	WR	Spring
<i>Daedalea dickinsii</i>	3	SFC20120726-05	WR	Summer
<i>Daedaleopsis confragosa</i>	2	SFC20120409-19	WR	Spring, fall
<i>Daedaleopsis tricolor</i>	2	SFC20120409-20	WR	Spring, fall
<i>Lenzites styracina</i>	3	SFC20120409-01	WR	Spring, summer
<i>Trametes versicolor</i>	3	SFC20120409-05	WR	Spring, summer
<i>Tyromyces borealis</i>	1	SFC20120821-91	WR	Summer
<i>Tyromyces chioneus</i>	2	SFC20120821-78	WR	Summer
Hapalopilaceae				
<i>Trametopsis cervina</i>	1	SFC20120409-03	WR	Spring
Meruliaceae				
<i>Bjerkandera adusta</i>	1	SFC20120409-08	WR	Spring
<i>Irpex lacteus</i>	1	SFC20120821-51	WR	Summer
<i>Junghuhnia nitida</i>	1	SFC20120409-06	WR	Spring
Phanerochaetaceae				
<i>Terana caerulea</i>	1	SFC20120821-68	WR	Summer
Polyporaceae				
<i>Cerrena consors</i>	1	SFC20120821-89	WR	Summer
<i>Cystidiophorus castaneus</i>	1	SFC20121009-27	WR	Fall
<i>Polyporus varius</i>	1	SFC20120821-59	WR	Summer
<i>Porodisculus orientalis</i>	1	SFC20120409-04	WR	Spring
<i>Skeletocutis nivea</i>	1	SFC20120409-07	WR	Spring
<i>Trichaptum abietinum</i>	1	SFC20120409-17	WR	Spring
Russulales				
Russulaceae				
<i>Lactarius acris</i>	1	SFC20120725-47	EM	Summer
<i>Lactarius controversus</i>	1	SFC20120821-70	EM	Summer
<i>Lactarius quietus</i>	1	SFC20120725-41	EM	Summer
<i>Lactarius</i> sp.	1	SFC20120821-40	EM	Summer
<i>Russula alboareolata</i>	5	SFC20120725-48	EM	Summer
<i>Russula cerolens</i>	1	SFC20120725-52	EM	Summer
<i>Russula foetens</i>	1	SFC20120821-73	EM	Summer
<i>Russula mariae</i>	2	SFC20120725-45	EM	Summer
<i>Russula</i> sp.1	1	SFC20120725-44	EM	Summer
<i>Russula</i> sp.2	1	SFC20120821-49	EM	Summer
<i>Russula</i> sp.3	1	SFC20120821-58	EM	Summer
<i>Russula</i> sp.4	1	SFC20120821-80	EM	Summer
<i>Russula violeipes</i>	1	SFC20120821-71	EM	Summer

Table 1. Continued

Fungal classification	No. ^a	Specimen No. ^b	Tropism	Sampling season
Stereaceae				
<i>Lopharia mirabilis</i>	1	SFC20120601-06	WR	Spring
<i>Stereum peculiare</i>	4	SFC20120409-10	WR	Spring, fall
<i>Stereum subtomentosum</i>	4	SFC20120409-13	WR	Spring, summer, fall
Thelephorales				
Thelephoraceae				
<i>Thelephora terrestris</i>	1	SFC20120821-69	S	Summer
Tremellomycetes				
Tremellales				
Sirobasidiaceae				
<i>Sirobasidium magnum</i>	1	SFC20120821-96	WR	Summer

S, saprophyte; EM, ectomycorrhiza; WR, wood rotter.

^aNo. of specimens collected.

^bHerbarium number for representative specimen.

species in the genera was as follows: *Antrodia* (2 species), *Daedaleopsis* (2), and *Stereum* (2). During summer, we identified 59 species belonging to 40 genera and 21 families, which were mostly gilled fungi. The distribution of the species in the genera was as follows: *Russula* (9 species), *Amanita* (5), *Lactarius* (4), and *Marasmiellus* (3). Specimens collected during summer accounted for 75% of the total diversity and 66% of the total number of specimens. During fall, 11 species belonging to 9 genera and 7 families were identified, most of which were wood-rotting fungi. The most common genera were *Daedaleopsis* (2 species) and *Stereum* (2).

In a fungal survey of Mt. Oseo in 2010, 98 species belonging to 62 genera and 32 families were reported [22]. Although the 2010 study was limited to the summer season, it focused on different regions of Mt. Oseo than we did in our study. Comparing the results of our study with the results of the 2010 survey, we found that the majority of fungal diversity was unique to each survey, and only 8 species were determined in both the surveys. This shows that even with fungi that can be seen with the naked eye, new and not recorded macrofungal species may be waiting for discovery at Mt. Oseo. Precipitation patterns in 2012 were unique: spring and summer were drier and fall was wetter than usual. Rainfall during late summer and early fall was relatively high owing to 3 typhoons (Sanba, Bolaven, and Tembin). The lower summertime fungal diversity observed in 2012 compared with that in 2010 may be a result of these abnormal precipitation patterns.

Herein, we provide a checklist of the basidiomycetous macrofungi found in surveys of Mt. Oseo in 2012 as well as detailed descriptions of 5 new species recorded in Korea. In addition to exemplifying the fungal diversity of Mt. Oseo, our checklist provides baseline data for future comparisons [37]. Regular surveys of the fungal diversity in the area can provide valuable information on the effects of increased human activity and may be an adequate indicator of regional climate change, including global

warming [38, 39]. These fungal species play essential roles in maintaining forest ecosystems and biodiversity [40, 41], and understanding these changes will aid in developing better strategies for management and conservation of these ecosystems [41, 42].

Artomyces microsporus (Qiu X. Wu & R. H. Petersen) Lickey, in Lickey, Hughes & Petersen, *Sydowia* 55: 227 (2003) [43].

Basidiocarp coral-like on crack in pine bark, beige to light ochraceous buff when fresh, 90 mm in length, repeatedly branched, 2~4 branches per node, branches with crown-like shape and 2 mm diameter. Specimen examined, SFC20120821-88. Habitat on the bark of *Pinus densiflora*.

Hymenopellis raphanipes (Berk.) R. H. Petersen, in Petersen & Hughes, *Beih. Nova Hedwigia* 137: 213 (2010) [44].

Cap convex or plane, 50-mm wide, surface viscid, light brown. Gills adnexed, distant, white to cream when fresh and brown when dry. Stipe slightly cylindrical with swollen base, 65 × 5 mm, white to cream. Basidia clavate, 60.7~71.8 × 14.6~17.3 μm, with 2 sterigmata. Basidiospore subglobose to ellipsoid, 15.4~17.7 × 11.4~13.7 μm, Q = 1.2~1.4. Specimen examined, SFC20120821-44.

Pholiota abietis A. H. Sm. & Hesler, *The North American species of Pholiota*: 176 (1968) [45].

Cap broad, convex or plane, warts on surface, 40~98 mm in diameter, brown in center and yellow around the edge, margin inrolled. Gills close and brown, connected to stipe. Stipe more densely scaled at base, 50 mm long, 20 mm thick, white to brown. Basidia clavate, 18.8~21.7 × 6.1~6.8 μm. Basidiospore 7.4~9.1 × 4.0~4.8 μm, Q = 1.6~1.9. Cheilocystidia capitulate or obclavate, 17~23.2 × 4.5~7.3 μm. Specimen examined, SFC20121009-35. Habitat on oak tree.

Phylloporus brunneiceps N. K. Zeng, Zhu L. Yang & L. P. Tang, *Fungal Divers.* 58: 82 (2013) [46].

Cap convex, slightly depressed, 50~90 mm in diameter, 30~40 mm thick, olive yellow, margin slightly curved inward. Gills yellow, attached to stem. Stipe 50~70 mm long, 7~10 mm thick, surface yellow to brown. Basidia clavate, 34.7~41.5 × 7.8~9.1 μm. Basidiospore cylindrical, 11.4~13.1 × 3.8~4.5 μm, Q = 2.3~2.9. Pleurocystidia abundant, fusiform, 78.5~97.6 × 10.4~16.7 μm. Specimen examined, SFC20120726-02.

Sirobasidium magnum Boedijn, Bull. Jard. Bot. Buitenzorg, 3 Sér. 13: 266 (1934) [47].

Basidiocarps foliose, gelatinous when fresh, 1~4 cm in diameter, reddish brown. Basidia subglobose to oval, 2~6 basidia in chains, 11.4~16.5 × 10.8~11.8 μm, Q = 1.1~1.6, very short sterigmata. Basidiospore fusiform, 15.9~19.4 × 5.1~5.7 μm, Q = 2.5~3.4. Specimen examined, SFC20120821-96.

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