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—Artemyces 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
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 Cheongnam-myeon, 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 µL containing 10 pmol of each primer and 1 µL of
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-
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

| Fungal classification | No. | Specimen No. | Tropism | Sampling season |
|-----------------------|-----|--------------|---------|-----------------|
| Agaricomycetes        |     |              |         |                 |
| Agaricales            |     |              |         |                 |
| Leucoagaricus rubrotinctus | 1 | SFC20120821-93 | S       | Summer          |
| Leucocoprinus sp.     | 1   | SFC20120821-45 | S       | Summer          |
| Russulaceae           |     |              |         |                 |
| Amanita alboflavescens | 1  | SFC20120725-51 | EM      | Summer          |
| Amanita ceciliae      | 1   | SFC20120821-92 | EM      | Summer          |
| Amanita imaeziki      | 1   | SFC20120725-42 | EM      | Summer          |
| Amanita pantherina    | 2   | SFC20120725-50 | EM      | Summer          |
| Amanita virosa        | 1   | SFC20120821-52 | EM      | Summer          |
| Corinariaceae         |     |              |         |                 |
| Hebeloma sp.          | 1   | SFC20120725-49 | S       | Summer          |
| Crepidotaceae         |     |              |         |                 |
| Crepidotus sulphurinus| 1   | SFC20120821-77 | WR      | Summer          |
| Lycoperdaceae         |     |              |         |                 |
| Lycoperon perlatum    | 2   | SFC20120821-50 | S       | Summer          |
| Marasmiaceae          |     |              |         |                 |
| Marasmiellus candidus | 1   | SFC20120821-43 | S       | Summer          |
| Marasmiellus koreanus | 1   | SFC20120821-84 | S       | Summer          |
| Marasmiellus sp.      | 1   | SFC20120821-83 | S       | Summer          |
| Marasmius sp.         | 1   | SFC20120821-42 | S       | Summer          |
| Physalacriaceae       |     |              |         |                 |
| Armillaria mellea     | 1   | SFC20120601-09 | WR      | Spring          |
| Cyptotrama asprata    | 2   | SFC20120725-46 | WR      | Summer          |
| Hymenopellis raphanipes | 1 | SFC20120821-44 | S       | Summer          |
| Psathyrellaceae       |     |              |         |                 |
| Psathyrella candolleana| 2  | SFC20120725-39 | S       | Summer          |
| Strophariaceae        |     |              |         |                 |
| Hypholoma sublateritium| 1  | SFC20121009-29 | WR      | Fall            |
| Pholiota abietis      | 1   | SFC20121009-35 | WR      | Fall            |
| Tricholomataceae      |     |              |         |                 |
| Collybia conflua ns   | 1   | SFC20120821-46 | S       | Summer          |
| Gymnoporus omphalodes | 1   | SFC20120821-57 | S       | Summer          |
| Gymnoporus polygrammus| 1   | SFC20120821-64 | S       | Summer          |
| Laccaria vinaceavellanea | 1 | SFC20120821-41 | EM      | Summer          |
| Mycena chlorophos     | 1   | SFC20120821-60 | S       | Summer          |
| Panellus stipitus     | 3   | SFC20120409-14 | WR      | Spring, summer, fall |
| Rhodocollybia butyracea| 1  | SFC20120821-75 | S       | Summer          |
| Tricholomopsis rutlians | 1 | SFC20120821-48 | EM      | Summer          |
| Auriculariales        |     |              |         |                 |
| Auriculariaceae       |     |              |         |                 |
| Heterochaete delicata | 1   | SFC20120409-16 | WR      | Spring          |
# Table 1. Continued

| Fungal classification | No. | Specimen No. | Tropism | Sampling season |
|-----------------------|-----|--------------|---------|-----------------|
| Boletales             |     |              |         |                 |
| Boletaceae            |     |              |         |                 |
| *Boletellus chrysenteroides* | 1   | SFC20120821-063 | EM      | Summer          |
| *Phylloporus brunneiceps* | 1   | SFC20120726-02 | EM      | Summer          |
| *Xerocomus sp.*       | 1   | SFC20120725-43 | EM      | Summer          |
| Corticiaceae          |     |              |         |                 |
| *Basidiobasidium radula* | 1   | SFC20120821-67 | WR      | Summer          |
| *Hyphoderma setigerum* | 1   | SFC20121009-31 | WR      | Fall            |
| *Phanerochaete sordida* | 3   | SFC20120821-79 | WR      | Summer          |
| *Rhizochaete filamentosa* | 1   | SFC20120601-08 | WR      | Spring          |
| *Schizopora paradoxa*  | 5   | SFC20120409-11 | WR      | Spring, summer, fall |
| Hymenochaetales       |     |              |         |                 |
| *Fuscoporia gelva*    | 2   | SFC20120409-12 | WR      | Spring, fall    |
| *Hydnochaete tabacina* | 4   | SFC20120409-09 | WR      | Spring, summer  |
| Polyporales           |     |              |         |                 |
| Auriscalpiaceae       |     |              |         |                 |
| *Artomyces microsporus* | 1   | SFC20120821-88 | WR      | Summer          |
| Coriolaceae           |     |              |         |                 |
| *Antrodia alba*       | 1   | SFC20120409-02 | WR      | Spring          |
| *Antrodia xantha*     | 1   | SFC20120601-10 | WR      | Spring          |
| *Daedalea dickinsii*  | 3   | SFC20120726-05 | WR      | Summer          |
| *Daedaleopsis confagosa* | 2   | SFC20120409-19 | WR      | Spring, fall    |
| *Daedaleopsis tricolor* | 2   | SFC20120409-20 | WR      | Spring, fall    |
| *Lenzites styracina*  | 3   | SFC20120409-01 | WR      | Spring, summer  |
| *Trametes versicolor* | 3   | SFC20120409-05 | WR      | Spring, summer  |
| *Tyromyces borealis* | 1   | SFC20120821-91 | WR      | Summer          |
| *Tyromyces chioneus*  | 2   | SFC20120821-78 | WR      | Summer          |
| Hapalopilaceae        |     |              |         |                 |
| *Trametopsis cervina* | 1   | SFC20120409-03 | WR      | Spring          |
| Meruliaceae           |     |              |         |                 |
| *Bjerkandera adusta*  | 1   | SFC20120409-08 | WR      | Spring          |
| *Irpepx lacteus*      | 1   | SFC20120821-51 | WR      | Summer          |
| *Junghuhnia nitida*   | 1   | SFC20120409-06 | WR      | Spring          |
| Phanerochaetaceae     |     |              |         |                 |
| *Terana caerulea*     | 1   | SFC20120821-68 | WR      | Summer          |
| Polyporaceae          |     |              |         |                 |
| *Cerrena consors*     | 1   | SFC20120821-89 | WR      | Summer          |
| *Cystidiophorus castaneus* | 1   | SFC20121009-27 | WR      | Fall            |
| *Polyporin varius*    | 1   | SFC20120821-59 | WR      | Summer          |
| *Porodiscula orientalis* | 1   | SFC20120409-04 | WR      | Spring          |
| *Skeletocutis nivea*   | 1   | SFC20120409-07 | WR      | Spring          |
| *Trichaptum abietinum*| 1   | SFC20120409-17 | WR      | Spring          |
| Russulales            |     |              |         |                 |
| Russulaceae           |     |              |         |                 |
| *Lactarius acris*     | 1   | SFC20120725-47 | EM      | Summer          |
| *Lactarius controversus* | 1   | SFC20120821-70 | EM      | Summer          |
| *Lactarius quietus*   | 1   | SFC20120725-41 | EM      | Summer          |
| *Lactarius sp.*       | 1   | SFC20120821-40 | EM      | Summer          |
| *Russula alboareolata* | 5   | SFC20120725-48 | EM      | Summer          |
| *Russula cerolens*    | 1   | SFC20120725-52 | EM      | Summer          |
| *Russula foetens*     | 1   | SFC20120821-73 | EM      | Summer          |
| *Russula mariae*      | 2   | SFC20120725-45 | EM      | Summer          |
| *Russula sp.1*        | 1   | SFC20120725-44 | EM      | Summer          |
| *Russula sp.2*        | 1   | SFC20120821-49 | EM      | Summer          |
| *Russula sp.3*        | 1   | SFC20120821-58 | EM      | Summer          |
| *Russula sp.4*        | 1   | SFC20120821-80 | EM      | Summer          |
| *Russula violeipes*   | 1   | SFC20120821-71 | EM      | Summer          |
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 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 subglobe to ellipsoid, 15.4~17.7 × 11.4~13.7 µm, Q = 1.2~1.4. Specimen examined, SFC20120821-88. Habitat on the bark of Pinus densiflora.

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, 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. Gills yellow, attached to stem. Stipe 50~70 mm long, 7~30~40 mm thick, olive yellow, margin slightly curved inward. This work was supported by the Korea National Arboretum (Project No. KNA 1-1-10, 12-3).

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