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First Report of Six Macrofungi from Daecheongdo and Socheongdo Islands, Korea

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ABSTRACT
Daecheongdo and Socheongdo Islands are located in the West Sea of Korea, 210 km away from land, and are military border areas very close to North Korea, making them difficult to access. Although the ecosystem of the islands is relatively well preserved due to the lack of accessibility, research on fungi of the regions is insufficient. Therefore, we aimed to investigate indigenous fungi in these geographically and geopolitically constrained regions. A survey of the indigenous fungal diversity of the islands was conducted in 2018. All specimens were identified at the species level based on morphological and molecular analyses. Among them, six macrofungi—namely, *Agaricus menieri, Crepidotus praecipuus, Dichomitus squalens, Hortiboletus amygdalinus, Melanoleuca friesii,* and *Trametes lactinea*—were not previously reported in Korea. Considering that the proportion of unrecorded species is high in the survey area and period as well as the number of samples collected, similar research on adjacent islands may be necessary.

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1. Introduction

Daecheongdo and Socheongdo Islands are located at 37°46′–37°50′ north latitude and 124°40′–124°45′ east longitude. Both islands belong to Daecheong-myeon, Ongjin-gun, Incheon in South Korea, located approximately 210 km northwest of the city of Incheon on the South Korean mainland and approximately 40 km southwest of the Ongjin Peninsula in North Korea. The Daecheongdo and Socheongdo Islands have winter minimum temperatures that are 10 °C warmer, with 400 mm lesser precipitation annually, and 1.79 m/s higher wind speeds than inland at similar latitudes [1]. Natural vegetation in Daecheongdo and Socheongdo islands was destroyed before and after the Korean War, 1950. Since the 1970s, there has been no artificial afforestation, and secondary forests have been formed naturally with *Carpinus turczaninoxii* and *Quercus* sp. [2]. Pine trees (*Pinus densiflora*) are widely distributed across these islands, but *Carpinus turczaninovii* and *Camellia japonica* dominate high elevations. The geological properties of these islands are unique and have a simple hierarchical structure. The coastal dune vegetation is relatively well preserved because these islands have fewer tourists than other South Korean islands. Some floristic studies in these areas were conducted by Choi and Lee [3] and Yang et al. [4], but research on fungi has rarely been conducted. Vascular plant diversity and fungal diversity are closely related in that the higher diversity of vascular plants creates niches and microhabitats for fungi and the presence of many ecotones [5–6]. Through re-identification of the collected specimens, it has been confirmed that 398 species of plants inhabit Daecheongdo island [7]. Despite the relatively small area (12.75 km^2), the variety of inhabiting plant species can lead to the assumption that the fungal species will also vary. To secure, preserve, and manage the genetic biological resources of higher fungi in Korea, a research project was carried out on Daecheongdo and Socheongdo Islands with the support of the National Institute of Biological Resources (NIBR) of the Ministry of Environment. Through this study, six unrecorded fungal species in the Korean peninsula were discovered from these islands.

2. Materials and methods

To fully characterize the distribution of fungi on these islands, regular surveys were conducted between July and September 2018. Each specimen

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was photographed, and their location of collection, habitat, host, substrate and fruiting characteristics were recorded onsite. The specimens were dried at ~50 °C for several days and stored at the NIBR. The specimens were initially identified based on their macro- and microscopic features, according to published descriptions [8–16]. The taxonomic classification and nomenclature of the species were assigned using the Index Fungorum database (http://www.index-fungorum.org). Measurements and drawings were made using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). For molecular identification, genomic DNA was extracted from dried samples using the AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). The internal transcription spacer (ITS) and partial nuclear large subunit (nLSU) rDNA regions were amplified using the primers ITS5 [17] and LR3 [18] as described previously [19]. DNA sequencing was performed using an ABI 3730XL sequencer (Macrogen, Seoul, Korea). The resulting nucleotide sequences were proofread and edited using jPHYDIT software [20]. A neighbor-joining (NJ) phylogenetic analysis was implemented by PAUP 4.0b10 [21] using the Jukes-Cantor correction. The robustness of the inferred NJ topologies was tested using 1000 bootstrap replicates.

3. Results and discussion

The resulting nucleotide sequences were edited and deposited in GenBank (accession numbers MW578953–MW578964). Species identities were confirmed by comparison with GenBank reference sequences using BLASTn (Table 1), and an NJ phylogenetic analysis was conducted (Figures 1 and 2). Based on both morphological and phylogenetic analyses, 103 fungal taxa were enumerated and classified according to current taxonomy guidelines, among which 27 unique families were represented, consisting of 83 species within 51 genera. Among these taxa, there were six species (*Agaricus menieri, Crepidotus praecipuus, Dichomitus squalens, Hortiboletus amygdalinus, Melanoleuca friesii,* and *Trametes lactinea*) that have not been previously reported in Korea (Figure 3). *Agaricus menieri* is consumed in Saint-Brevin, France, but it is not tasty, and therefore not highly valued [22]. *Crepidotus praecipuus* is recorded as an endemic species of New Zealand, and this is the first time it has been recorded outside of New Zealand (https://inaturalist.nz). *Dichomitus squalens* is a white-rot basidiomycete that produces diverse extracellular enzymes for lignocellulose degradation and oxidative enzymes to act on lignin [23–25]. The extracts from cultivated *Trametes lactinea* significantly inhibit the activities of lipoxygenase and hyaluronidase [26].

Table 1. Closest GenBank matches of 6 undescribed species in this study.

		ITS rDNA			nLSU rDNA		
Species	Voucher No.	GenBank accession No.	The closest GenBank taxa	Identity (%)	GenBank accession No.	The closest GenBank taxa	Identity (%)
Agaricus menieri	NIBRFG0000502802	MW578953	*Agaricus menieri*	99	MW578959	*Agaricus bisporus*	98
Crepidotus praecipuus	NIBRFG0000502861	MW578958	*Crepidotus praecipuus*	97	MW578960	*Crepidotus tobolensis*	99
Dichomitus squalens	NIBRFG0000502804	MW578954	*Dichomitus squalens*	99	MW578961	*Dichomitus squalens*	99
Hortiboletus amygdalinus	NIBRFG0000502792	MW578955	*Hortiboletus rubellus*	89	MW578962	*Hortiboletus amygdalinus*	99
Melanoleuca friesii	NIBRFG0000502881	MW578956	*Melanoleuca friesii*	100	MW578963	*Melanoleuca friesii*	99
Trametes lactinea	NIBRFG0000502816	MW578957	*Trametes lactinea*	100	MW578964	*Trametes lactinea*	99

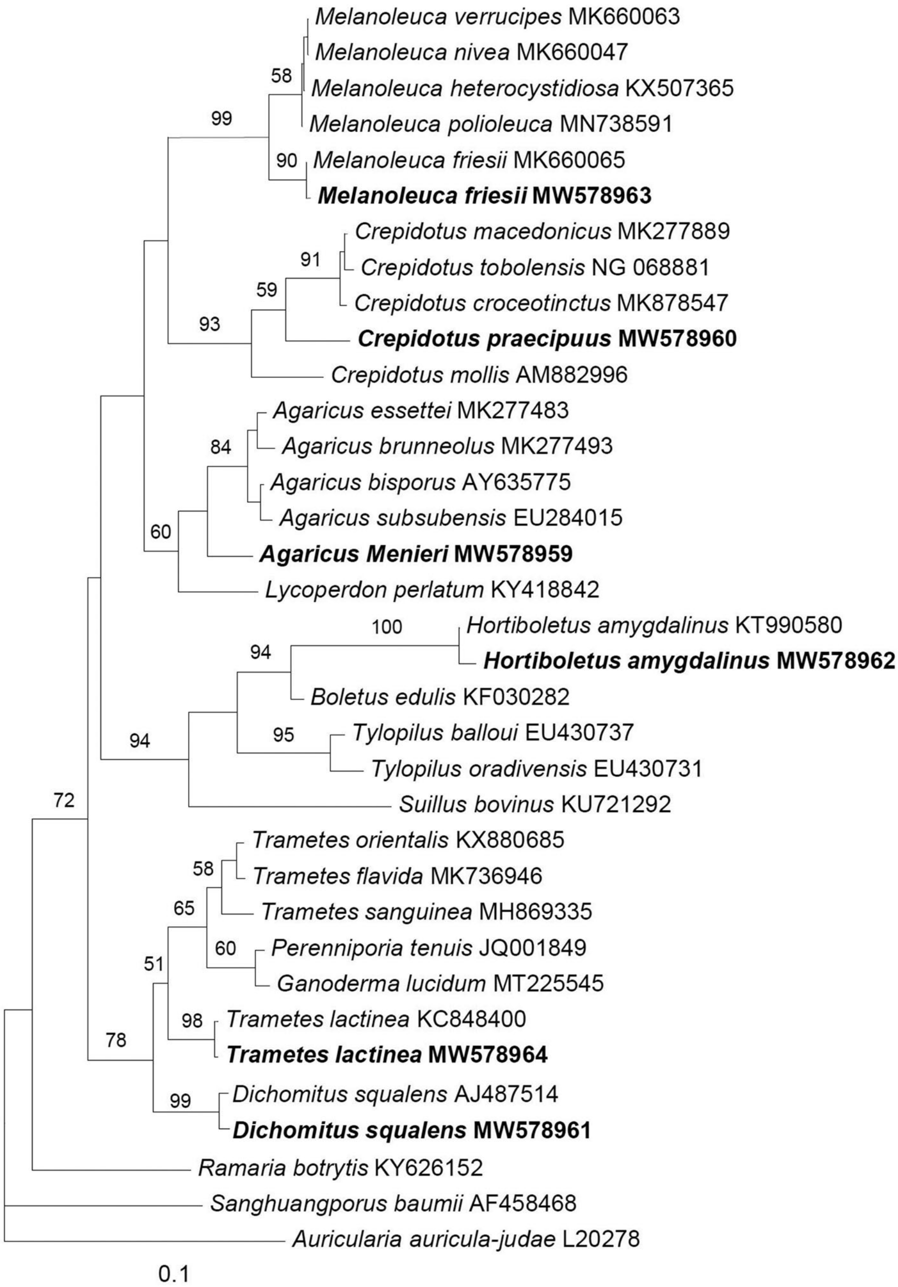

Figure 1. Neighbor-joining tree inferred from nuclear large subunit rDNA sequences of the six macrofungal species newly recorded in Korea. *Auricularia auricula-judae* (AAURG25S) was used as outgroup. Bootstrap values exceeding 50% for nodes are indicated. Samples from the present study are indicated in bold.

Also, Trametenolic acid B, one of the components in *T. lactinea*, is reported to possess cytotoxic activities, thrombin inhibiting effects, and the ability to inhibit gastric cancer cell viability [27]. *Melanoleuca* is a character-poor genus with similar macroscopic characters and a morphology strongly influenced by habitat conditions [28]. It is a taxonomically confused genus based on similar morphological factors. Three species (*M. arcuata, M. melaleuca,* and *M. verrucipes*) have been reported in Korea, and *M. friesii* is rarely reported worldwide. From the results of various previous studies, it seems necessary to study physiological activity about these species. As a result of the re-identification of plant specimens collected on Daecheongdo island, 398 taxa were confirmed [7]. The plant species diversity and fungal diversity are closely related, so higher plant diversity should positively affect fungal diversity. Considering that the proportion of unrecorded species is high given the survey area, survey period, and a number of specimens collected, similar research on adjacent islands of the West Sea may be necessary. Also, the results indicated that regional variables had a greater effect than the number of times and frequency of collection. This study showed that the selection and concentration of the research area can bring more diverse species diversity results.

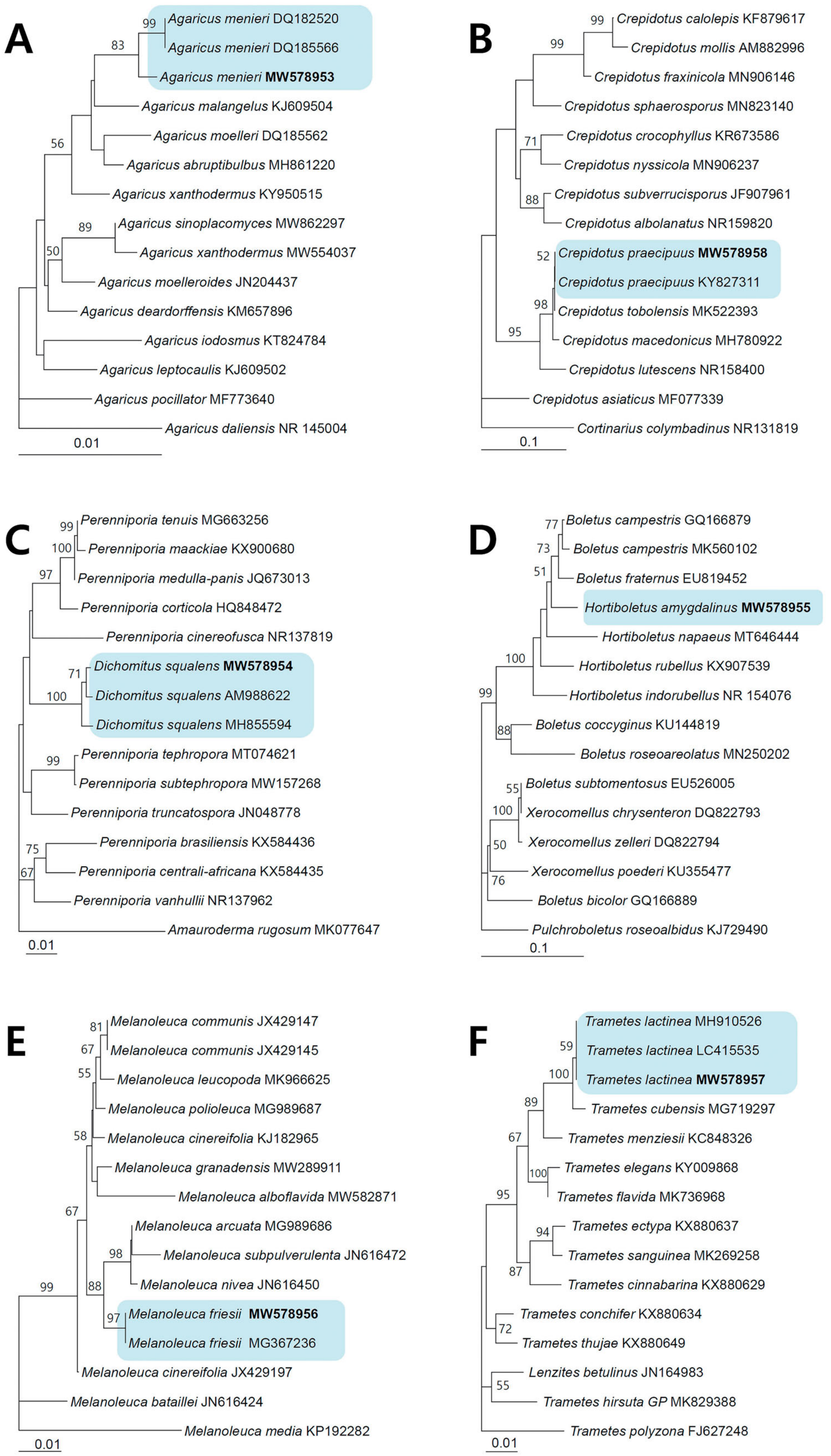

Figure 2. Neighbor-joining tree inferred from nuclear ITS sequences of six macrofungal species newly recorded in Korea. (A) *Agaricus menieri*; (B) *Crepidotus praecipuus*; (C) *Dichomitus squalens*; (D) *Hortiboletus amygdalinus*; (E) *Melanoleuca friesii*; (F) *Trametes lactinea*. Bootstrap values exceeding 50% for nodes are indicated.

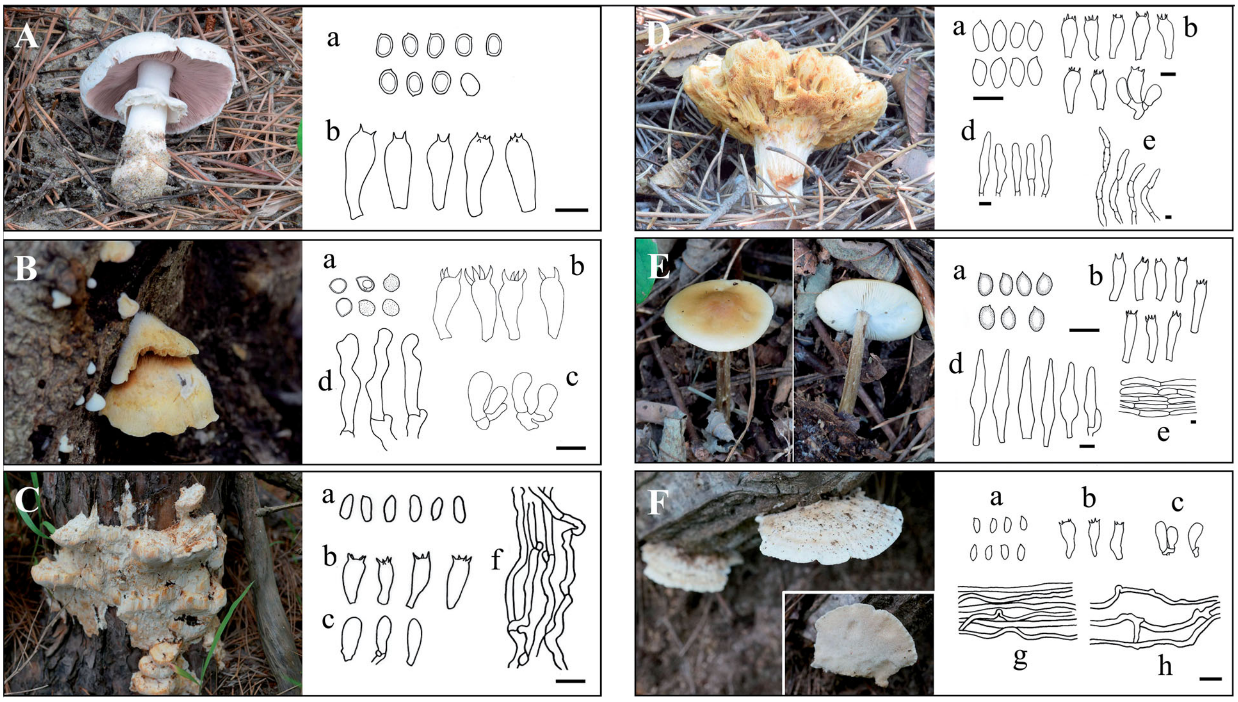

Figure 3. Fruiting bodies and microscopic features of *Agaricus menieri* (A), *Crepidotus praecipuus* (B), *Dichomitus squalens* (C), *Hortiboletus amygdalinus* (D), *Melanoleuca friesii* (E), and *Trametes lactinea* (F). (a) basidiospores; (b) basidia; (c) basidioles; (d) cystidia; (e) pileipellis; (f) generative hyphae; (g) skeletal hyphae; (h) binding hyphae. The scale bars in the microscopic images represent 10 μm.

4. Taxonomy

Basidiomycota R.T. Moore
Agaricomycetes Doweld
Agaricales Underw.
Agaricaceae Chevall

***Agaricus menieri* Bon**, Docums Mycol. 11 (no. 44): 28 (1981)

Pileus up to 12 cm in diameter, thick-fleshy, applanate or slightly umbonate, smooth, fibrillose or finely cracked, whitish, pale yellowish with a gray-pink shade, eventually pale ochraceous. Margin even, often with remnants of the veil. Lamellae free, thin, whitish-pink, pale grayish pink, with pale, sterile edges. Stipe 10 × 2.5 cm, central, equal, tightly cylindrical to slightly spindle-shaped, white but at first a pale brownish yellow, silky-fibrillose. Ring broad, apical, free-standing. Taste slightly bitter. Spore print brownish. Basidiospores 6–7.7 × 4.4–5.6 μm, broadly ellipsoid, brownish, smooth, and relatively large. Basidia 22–27.6 × 7.7–9 μm, 4-sterigmate, clavate. Sterigmata 2–2.5 μm long.

Remarks: *A. menieri* is a strictly sabulicolous species, fruiting on sandy soil and coastal dunes. Basidiomata of this species are often completely covered with sand.

Specimen examined: The specimen was collected from the pine forest, Moraeul beach, Daecheongdo Island, from the sandy soil of coastal dunes; 37°48'56.91"N 124°40'43.82"E, July 25 2018; NIBRFG0000502802 (GenBank accession no. ITS: MW578953, LSU: MW578959).

4.1. Crepidotaceae Singer

***Crepidotus praecipuus* E. Horak**, CBS Biodiversity Series 16: 44 (2018)

Pileus 10–70 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with an incurved and, later, even margin, with a surface that is minutely tomentose-scaly with yellowish-brown to brown fibrillose scales and a tough, elastic consistency. Lamellae whitish, ochre-brown to cinnamon, moderately crowded, with margins that are minutely fimbriate, remaining whitish. Stipe absent. Spore print yellowish brown. Basidiospores 6.3–7.8 × 5.1–6.6 μm, ellipsoid, smooth, thick-walled, apex obtuse, depressed, or occasionally mucronate. Basidia 26–33 × 7.6–9.3 μm, cylindrical-clavate, 4-spored. Chelliocystidia 26–65 × 4–14 μm, clavate, cylindrical, irregularly cylindrical. Pleurocystidia absent.

Remarks: *C. praecipuus* is characterized by 6.3–7.8 × 5.1–6.6 μm, ellipsoid, smooth, thick-walled basidiospores. It is distinguished from closely related species such as *C. tobolensis*, by spore quotient (*C. praecipuus: C. tobolensis* = 1.19–1.2:1.21–1.35) and basidiomata size (*C. praecipuus: C. tobolensis* = 10–70 mm: 7-43 mm) [29].

Specimen examined: The specimen was collected on Daecheongdo Island, from the branch of a dead deciduous tree; 37°49'0.97"N 124°41'40.18"E, September 4, 2018; NIBRFG0000502861 (GenBank accession no. ITS: MW578958, LSU: MW578960).

4.2. Tricholomataceae R. Heim ex Pouzar

***Melanoleuca friesii* (Bres.) Bon**, Docums Mycol. 9(no. 33): 67 (1978)

Pileus up to 5–6 cm in diameter, initially convex to plano-convex; margin not surplus, incurred, never fully extended; cuticle smooth to slightly venous; brown. Stipe 6–7 × 0.6–0.9 cm, cylindrical, generally short and wide, robust, full, progressively widening toward the base, brownish-white, pruinose. Scent mild. Flavor slightly spicy. Basidia 16–29 × 6–8.6 µm, clavate, 4-spored; Sterigmata 3.4 µm long; Basidiospore whitish, slightly cream in color when dry; 7.6–8.8 × 4.3–5.3 µm, narrowly ellipsoidal to subcylindrical, warty. Cheilocystidia 42–73 × 12–13 µm, lageniform, Pleurocystidia similar to cheilocystidia.

Remarks: *M. friesii* is distinguished by having lageniform cystidia and brownish whitish pruinose stipes.

Specimen examined: The specimen was collected on Socheongdo Island, on soil; 37°46'29.27"N 124°45'19.62"E, September 5 2018; NIBRFG0000502881 (GenBank accession no. ITS: MW578956, LSU: MW578963).

4.3. Boletales E.-J. Gilbert

Boletaceae Chevall.

***Hortiboletus amygdalinus* Xue T. Zhu & Zhu L. Yang**, in Wu, Li, Zhu, Zhao, Han, Cui, Li, Xu & Yang, Fungal Diversity 81: 98 (2016)

Pileus 6.5 cm in diameter, hemispherical, convex to applanate; surface always strong rugose, glabrous and yellow-brown, red-brown. Context cream to yellowish. Hymenophore adnate, sometimes depressed around the stipe; surface yellow to dull yellow, becoming ochraceous. Pores compound, angular, 0.5–1 per mm; tubes concolorous with hymenophoral surfaces, staining blue when injured. Stipe 5 × 1.7 cm, subcylindrical; apical part yellowish; basal part cream to dirty white; middle part cream to brownish. Basidia 30–40 × 10–11 µm, clavate, 4-spored; Basidiospores 9.3–10 × 4.9–5.6 µm subfusiform to slender, brownish yellow, smooth. Pleurocystidia scattered, 61–77 × 11–13 µm, fusoid-ventricose to clavate with wider apex, thin-walled. Pileipellis is yellowish brown, more or less broadened and often incrusted hyphal elements, with cylindrical terminal cells, 37–63 × 10–12 µm. Clamp connections absent.

Remarks: *H. amygdalinus* is characterized by its rugose pileus surface when young, eventually turning glabrous and rimose-diffract with age, unpleasant odor, and relatively wider basidiospores.

Specimen examined: The specimen was collected on Haeneomi gogae, Daecheongdo Island, from soil; 37°48'28.88"N 124°41'37.93"E, July 24, 2018; NIBRFG0000502792 (GenBank accession no. ITS: MW578955, LSU: MW578962).

4.4. Polyporales Gäum

Polyporaceae Fr. ex Corda

***Dichomitus squalens* (P. Karst.) D.A. Reid**, Revta Biol., Lisb. 5(1–2): 150 (1965)

Basidiocarps pileate, effused-reflexed, imbricate or resupinate, individual pilei, up to 3 cm wide, 1–7 cm long, tough and corky when fresh, hard when dry. Upper surface white to cream, with a very thin cuticle; margin white, narrow to glabrous. Pore surface white to wood-colored, with age more yellowish or discolored in light brown and gray shades, often unevenly. Pores circular to angular, 4–5 per mm, with thin dissepiments. Hyphal system dimitic; generative hyphae with clamps, thin-walled, hyaline, 1.5–4 µm in diameter; binding hyphae predominant, arboriform and usually dichotomously branched, hyaline, thick-walled to solid, up to 7 µm in diameter in the main stems, tapering down to thin whip-like ends. Cystidia absent, with fusoid cystidioles present, 16 × 4.5 µm. Basidia clavate, 4-sterigmate, 23–27 × 6–7 µm, with a basal clamp. Basidiospores cylindrical to oblong-ellipsoid, hyaline, thin-walled, smooth, 9.5–11.8 × 3–4.4 µm.

Remarks: *D. squalens* is characterized by circular to angular pores with 4–5 pores per mm.

Specimen examined: The specimen was collected from the pine forest, Moraeul beach, Daecheongdo Island, from the branch of a dead deciduous tree; 37°48'56.91"N 124°40'43.82"E, July 18 2018; NIBRFG0000502804 (GenBank accession no. ITS: MW578954, LSU: MW578961).

***Trametes lactinea* (Berk.) Sacc.**, Syll. fung. (Abellini) 6: 343 (1888)

Basidiocarps dimidiate to semicircular, soft and velvety to touch, mostly azonate, concentrically sulcate and zoned near the margin, white to cream, eventually turning ochraceous. Margin weakly lobed, obtuse and relatively thick, concolorous or paler than the upper surface. Pore surface cream, ochraceous to pale fulvous. Pores round to angular, mostly 1.5–2 per mm, dissepiments thin to rather thick, entire, tubes concolorous with the context, 1–10 mm long. The hyphal system, trimitic. Generative hyphae clamped, hyaline and delicately thin-walled, 1–4 µm in diameter. Skeletal hyphae abundant, hyaline to pale yellow, thin-walled, 3–8 µm wide in the tubes. Binding hyphae also abundant, hyaline to pale yellow, thick-walled, arboriform to coralloid, 1–7 µm in diameter. Basidia 14–17.3 × 5.9–6.2 µm, clavate, 4-spored. Basidiospores cylindrical-ellipsoid, 6–6.5 × 2.2–2.7 µm, smooth and hyaline.

Remarks: *T. lactinea* is recognized by soft velvety azonate pileus, thick fruitbodies, relatively large pores, and presence of interlocking hyphae.

Specimen examined: The specimen was collected at the pine forest at Moraeul beach, Daecheongdo Island, from the branch of a dead deciduous tree; 37°48'56.91"N 124°40'43.82"E, July 25 2018; NIBRFG0000502816 (GenBank accession no. ITS: MW578957, LSU: MW578964).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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