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RESEARCH ARTICLE

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Macrolepiota in Korea: New Records and a New Species

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ABSTRACT

The genus *Macrolepiota* (Agaricales, Basidiomycota) is easy to recognize at the genus level because of big, fleshy basidiocarps with squamules covering the pileus; a single or double annulus; and big, thick-walled basidiospores with a germ pore. However, morphological identification is often unreliable in *Macrolepiota* due to similar morphological features among species. Due to the uncertainty of previous morphological identification in the genus *Macrolepiota,* it is necessary to re-examine Korean *Macrolepiota* using molecular data. We re-examined 34 *Macrolepiota* specimens collected from 2012 to 2018 in Korea using a reverse taxonomic approach, whereby species identification was first done based on the internal transcribed spacer (ITS) region analysis, followed by morphological confirmation. We identified the presence of four species: *M. detersa*, *M. mastoidea*, *M. procera*, and *M. umbonata* sp. nov. Two species (*M. detersa* and *M. mastoidea*) were previously unrecorded from Korea and *M. umbonata* is a new species. Detailed descriptions of all four species and taxonomic key are provided in this study. *Macrolepiota procera* and *M. umbonata* are distributed through the country, but *M. detersa* and *M. mastoidea* are distributed only in limited areas. According to our results, the combination of ITS locus and morphology proved to be a robust approach to evaluate the taxonomic status of *Macrolepiota* species in Korea. Additional surveys are needed to verify the species diversity and clarify their geographic distribution.

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Agaricaceae; reverse taxonomy; ITS; new taxa; *Macrolepiota umbonata*

1. Introduction

The genus *Macrolepiota*, with a global distribution, is characterized by big, fleshy basidiocarps with squamules covering the pileus [1]. Because of these noticeable morphological features, *Macrolepiota* species are easily detected in natural (grasslands, forest clearings) and man-made (gardens, lawns) open spaces. Globally, about 39 species of *Macrolepiota* have been recognized [2–7]. The type species, *M. procera* (Scop.) Singer, is an edible mushroom cultivated and sold in some Asian countries [8–10]. Unfortunately, there have been mushroom poisoning cases of mistakenly consuming toxic *Chlorophyllum* species which are visually similar to edible *Macrolepiota* species in Asia and North America [11–15].

The taxonomy of *Macrolepiota* has been studied extensively in Europe and North America based on morphological features [1,16]. Singer [1] characterized *Macrolepiota* to have a white-to-pink spore print, thick-walled big spores (above 10 µm in length) with a broad germ pore, absence of pleurocystidia, pileus scaly, and movable annulus. *Macrolepiota* was divided into two sections based on the presence or absence of clamp connections [1]. Later the section *Laevistipedes* (Pázmány) Bon. was added [17]. However, traditional classification and identification based only on morphology are unreliable because of the morphological disparities according to environmental conditions and developmental stage [18,19] or the morphological similarities of different species that originate from different geographical origin [6]. Applying molecular data to study fungal taxonomy has been crucial in shedding light on the species diversity and phylogenetic relationships among *Macrolepiota* species.

Johnson [20] suggested that *Macrolepiota* is not monophyletic based on DNA data from the internal transcribed spacer (ITS) region, the nuclear large subunit rDNA (nLSU), and the mitochondrial small subunit rDNA (mtSSU) region. Vellinga and colleagues established *Macrolepiota* sensu stricto after the section *Laevistipedes* was moved to the genus *Chlorophyllum* based on morphology (hymenidermal pileus covering, smooth stipe, basidiospore with

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Table 1. Korean *Macrolepiota* species, collection information, and GenBank accession numbers used in this study.

Species	Specimen code	Locality	GenBank accession number
Macrolepiota detersa	SFC20160712-42	Sindo island, Ongjin-gun, Incheon-si	MK453219
	SFC20160726-06	Jangbongdo island, Ongjin-gun, Incheon-si	MK453220
	SFC20160816-20	Jangbongdo island, Ongjin-gun, Incheon-si	MK453221
	SFC20160906-01	Jangbongdo island, Ongjin-gun, Incheon-si	MK453222
	SFC20160906-29	Jangbongdo island, Ongjin-gun, Incheon-si	MK453223
	SFC20160907-22	Taehwasan University Forest, Gwangju-si, Gyeonggi-do	MK453224
	NIBRFG0000122520	Mt. Simhak, Gyeonggi-do, Paju-si	MK453225
	NIBRFG0000131193	Jeju island, Jeju-si, Jeju-do	MK453226
M. mastoidea	SFC20150718-01	Woljeongsa temple, Pyeongchang-gun, Gangwon-do	MK453227
	SFC20160927-52	Nature Environment Research Park, Hongcheon-gun, Gangwon-do	MK453228
	SFC20171012-09	Dadohaehaesang Marine & Coastal National Parks, Wando-gun, Jeollanam-do	MK453229
M. procera	SFC20120821-38	Seongjusan Natural Recreation Forest, Boryeong-si, Chungcheongnam-do	MK453230
	SFC20120919-46	Mt. Museong, Gongju-si, Chungcheongnam-do	MK453231
	SFC20150902-98	Wondae-ri Birch forest, Inje-gun, Gangwon-do	MK453232
	SFC20160908-03	Kangwon National University Forest, Hongcheon-gun, Gangwon-do	MK453233
	SFC20160920-02	Sido island, Ongjin-gun, Incheon-si	MK453234
	SFC20160920-15	Sido island, Ongjin-gun, Incheon-si	MK453235
	SFC20160920-32	Sindo island, Ongjin-gun, Incheon-si	MK453236
	SFC20171001-07	Dolsando island, Yeosu-si, Jeollanam-do	MK453237
	SFC20181013-03	Yongso Falls, Inje-gun, Gangwon-do	MK453238
M. umbonata sp. nov.	SFC20120716-10	Seoul National University, Gwanak-gu, Seoul-si	MK453239
	SFC20120903-06	Seoul National University, Gwanak-gu, Seoul-si	MK453240
	SFC20130917-H01	Hakgasan Natural Recreation Forest, Yecheon-gun, Gyeongsangbuk-do	MK453241
	SFC20150819-27	Taehwasan University Forest, Gwangju-si, Gyeonggi-do	MK453242
	SFC20160906-05	Jangbongdo island, Ongjin-gun, Incheon-si	MK453243
	SFC20160909-01	Yeongheungdo island, Ongjin-gun, Incheon-si	MK453244
	SFC20160909-16	Yeongheungdo island, Ongjin-gun, Incheon-si	MK453245
	SFC20170919-18	Soando island, Wando-gun, Jeollanam-do	MK453246
	SFC20180704-82	Wando-gun, Jeollanam-do	MK453247
	SFC20180905-78	Socheong island, Ongjin-gun, Incheon-si	MK453248
	SFC20180924-08	Mt. Bongcheon, Ganghwa-gun, Incheon-si	MK453249
	NIBRFG0000125792	Wonju-si, Gangwon-do	MK453250
	NIBRFG0000133860	Gokseong-gun, Jeollanam-do	MK453251
	NIBRFG0000142479	Asan-si, Chungcheongnam-do	MK453252

lacking germ pore), ecology (wide distribution in the tropics or a preference for compost heaps [thermophilic or thermotolerant]), phylogeny based on ITS and nLSU [21,22]. Ge et al. [3] proposed a new section, *Volvatae* Z. W. Ge, Zhu L. Yang & Vellinga, to accommodate the species with a volva within the genus *Macrolepiota*. Therefore, to date the genus *Macrolepiota* is partitioned into three sections: *Macrolepiota*, *Macrosporae*, and *Volvatae*. As a result, the genus *Macrolepiota* in the present sense is characterized by the combination of the following characters: pileal squamules of a trichodermal layer made up of long subcylindric elements, basidiospores with a germ pore, the presence of stipe squamules, and noticeable color bands in mature specimens [2,6,21,22].

Since *M. procera* was first reported to Korea in 1940 (as *Lepiota procera*) [23], four other species have been reported in Korea: *M. alborubescens*, *M. molybdites, M. neomastoidea*, and *M. rhacodes* [24]. According to the most recent classification system [22], *M. procera* is the only Korean species that should remain in the genus *Macrolepiota*, with the other four species being transferred to the genus *Chlorophyllum*. In previous Korean studies involving *M. procera*, identification was based solely on basidiocarp morphology. Molecular approaches have been useful in distinguishing morphologically similar species, discovering new species, and clarifying classification [3–6]. For *Macrolepiota* several new species have been reported based on molecular data in North America [21], China [3], and Brazil [6]. Due to the uncertainty of previous morphological identification in the genus *Macrolepiota*, it is necessary to re-examine Korean *Macrolepiota* using molecular data. In this study, we investigate the species diversity of *Macrolepiota* in Korea based on molecular data from the ITS locus. Additionally, we provide detailed morphological description of each species.

2. Materials and methods

2.1. Taxon sampling and molecular analysis

A total of 34 *Macrolepiota* specimens were used in this study. Specimens were collected throughout South Korea from 2012 to 2018 and kept in the Seoul National University Fungus Collection (SFC) and National Institute of Biological Resources (NIBR) (Table 1 and Figure 1). Due to limited distinguishing morphological features, we applied a reverse taxonomic approach, whereby species identification was first done based on DNA data, followed by morphological confirmation [25].

Genomic DNA was extracted from basidiocarp tissue using a modified CTAB extraction protocol

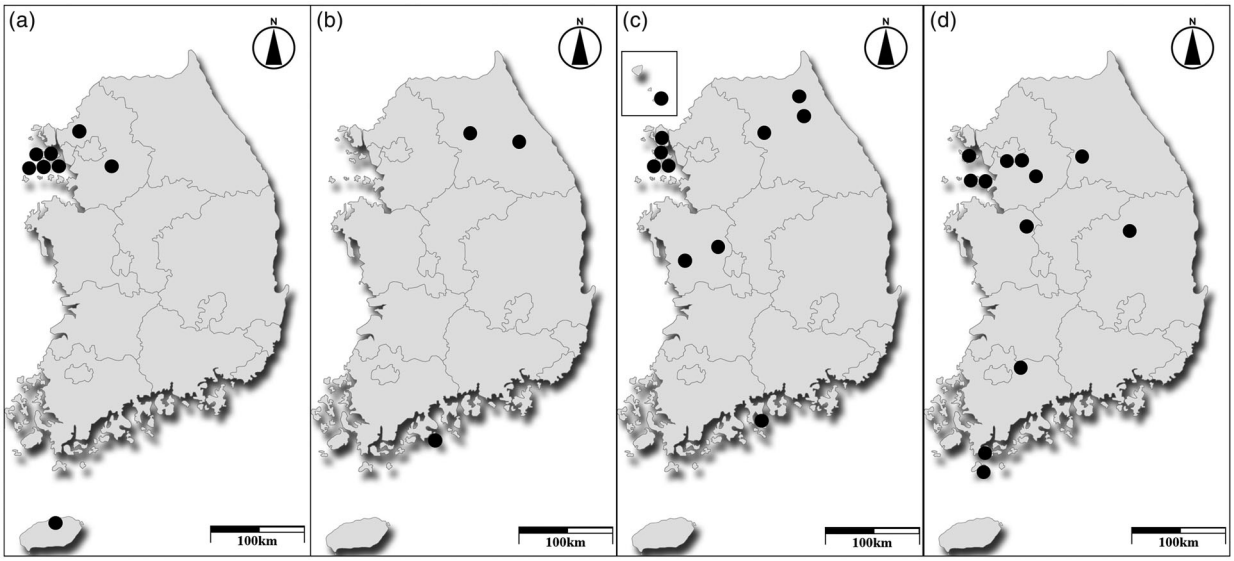

Figure 1. Distribution maps of *Macrolepiota* species in Korea. (a); *Macrolepiota detersa*, (b); *M. mastoidea*, (c); *M. procera*, and (d); *M. umbonata*.

[26]. The ITS region was amplified using the primers ITS1F and ITS4B [27]. PCR amplifications were performed on a thermal cycler (C1000TM; Bio-Rad, Richmond, CA, USA) using the AccuPower PCR premix (Bioneer Co., Daejeon, Korea) following the protocol outlined in Park et al. [28]. PCR products were visualized on a 1% agarose gel and purified using the Expin^{TM} PCR Purification Kit (GeneAll Biotechnology, Seoul, Korea). Sanger sequencing was performed at Macrogen (Seoul, Korea) on an automated DNA sequencer (ABI PRISM 3730XL Analyzer; Applied Biosystems, Foster City, CA, USA) using the aforementioned PCR primers.

ITS sequences for each individual were assembled and proofread using MEGA version 5 [29] and deposited in GenBank (accession numbers in Table 1). All new sequences were aligned using Multiple Alignment Fast Fourier Transform (MAFFT ver. 7) [30] with closely related ITS sequences of *Macrolepiota* obtained from GenBank. Alignments were checked, and ambiguous positions were adjusted manually. A maximum likelihood (ML) phylogenic analysis was performed in RAxML 8.0.2 [31] implemented on the CIPRES Web portal [32] using a GTRCAT model of sequence evolution and 1000 bootstrap replicates [33]. *Leucoagaricus barssii* and *L. meleagris* were selected as outgroup based on a previous study [3].

2.2. Morphological observations

Macromorphological features of studied materials were described based on fresh and dried specimens, comparing with published *Macrolepiota* data [1,3,6,21,22,34,35]. All color names and alphanumeric codes followed the Methuen Handbook of Colour [36]. Microscopic features were observed under a Nikon Eclipse 80i optical microscope (Nikon, Tokyo, Japan) at either 400× or 1000×. The amyloidity of basidiospores was observed using Melzer's reagent [37]. Other structures were observed in 3% aqueous KOH, Congo red solution, and cresyl blue.

At least 20 basidiospores, 10 basidia, and 10 cheilocystidia were measured per specimen. In the descriptions of each species presented in this paper, the abbreviation [a/b/c] behind basidiospores and hymenial elements is as follows: a—number observed, b—number of basidiocarps, c—number of collection site. Dimensions for basidiospores and hymenial elements and are presented as minimum–maximum.

3. Results

In this study, 34 new ITS sequences of *Macrolepiota* from Korea were generated and submitted to GenBank (Table 1). An additional 68 ITS sequences were retrieved from GenBank and used in constructing the alignment. The final ITS alignment was 696 bp long. The phylogenetic tree based on this ITS dataset separates Korean *Macrolepiota* specimens into four distinct taxa (Figure 2). Three taxa were identified based on the clustering with previously reported species (*M. detersa*, *M. mastoidea*, and *M. procera*), while one was distinct from all recognized species and considered a new species. *Macrolepiota detersa*, *M. procera*, and the undescribed species (*Macrolepiota* sp.) belong to the section *Macrolepiota*, while *M. mastoidea* belongs to the section *Macrosporae* (Figure 2). This is the first report of *M. detersa* and *M. mastoidea* in Korea. *Macrolepiota* spp. forms a distinct lineage with strong support (89%), as sister species to a clade

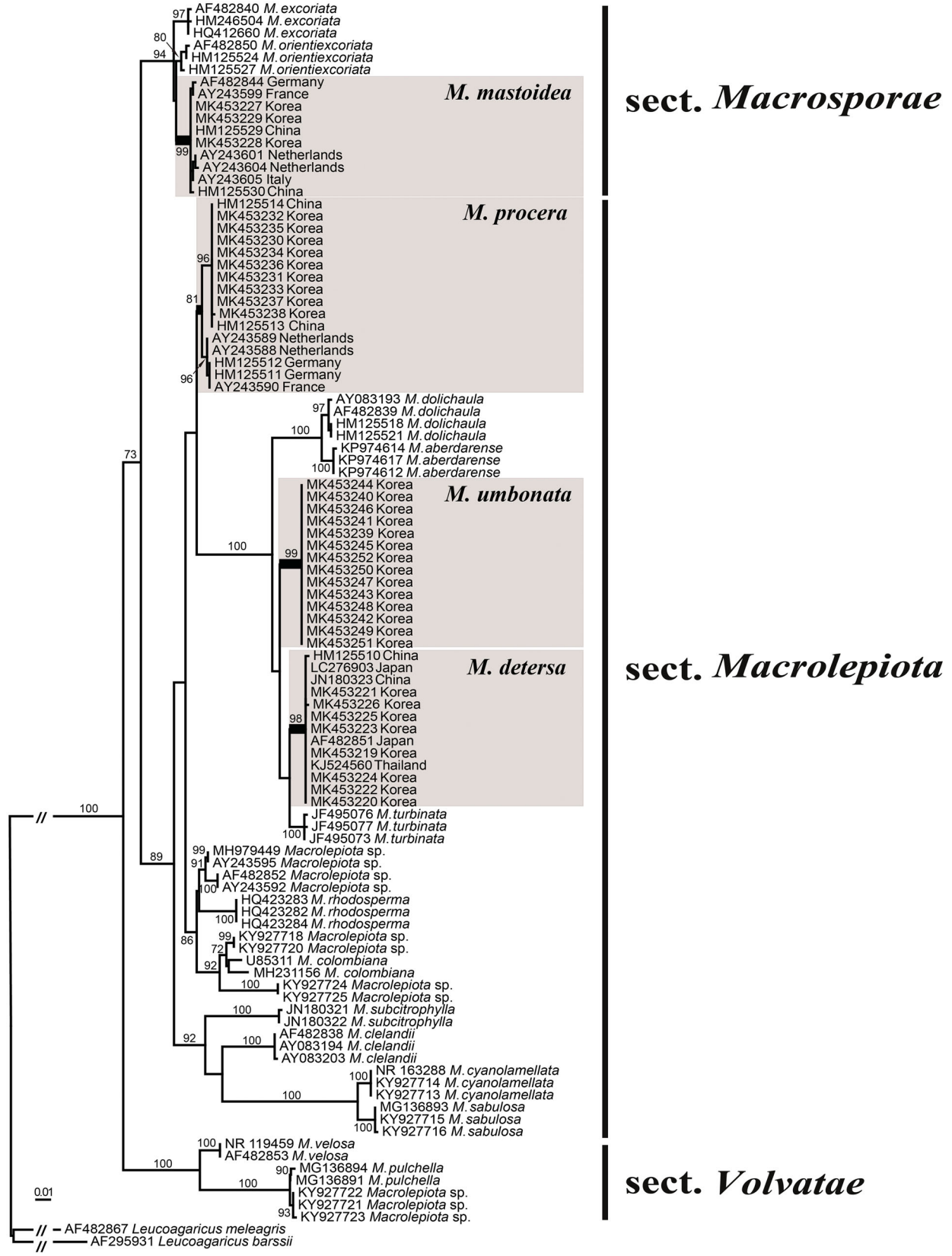

Figure 2. Phylogeny of *Macrolepiota* species based on a maximum-likelihood analysis of the ITS region. Bootstrap values >70% are indicated. The scale bar indicates the number of expected nucleotide substitutions per site. *Macrolepiota* species found in Korea are denoted by the gray boxes.

including *M. aberdarense*, *M. detersa*, *M. dolichaula*, and *M. turbinata* (Figure 2). *Macrolepiota* sp. showed sequence similarity of 93.9–94.1% to *M. aberdarense*, 96.2–96.5% to *M. detersa*, 94.7–94.9% to *M. dolichaula*, and 97.1–97.4% to *M. turbinata*.

The 34 specimens from Korea previously identified morphologically as *M. procera* have been confirmed as four different species using ITS data, and their detailed morphological characters are provided below.

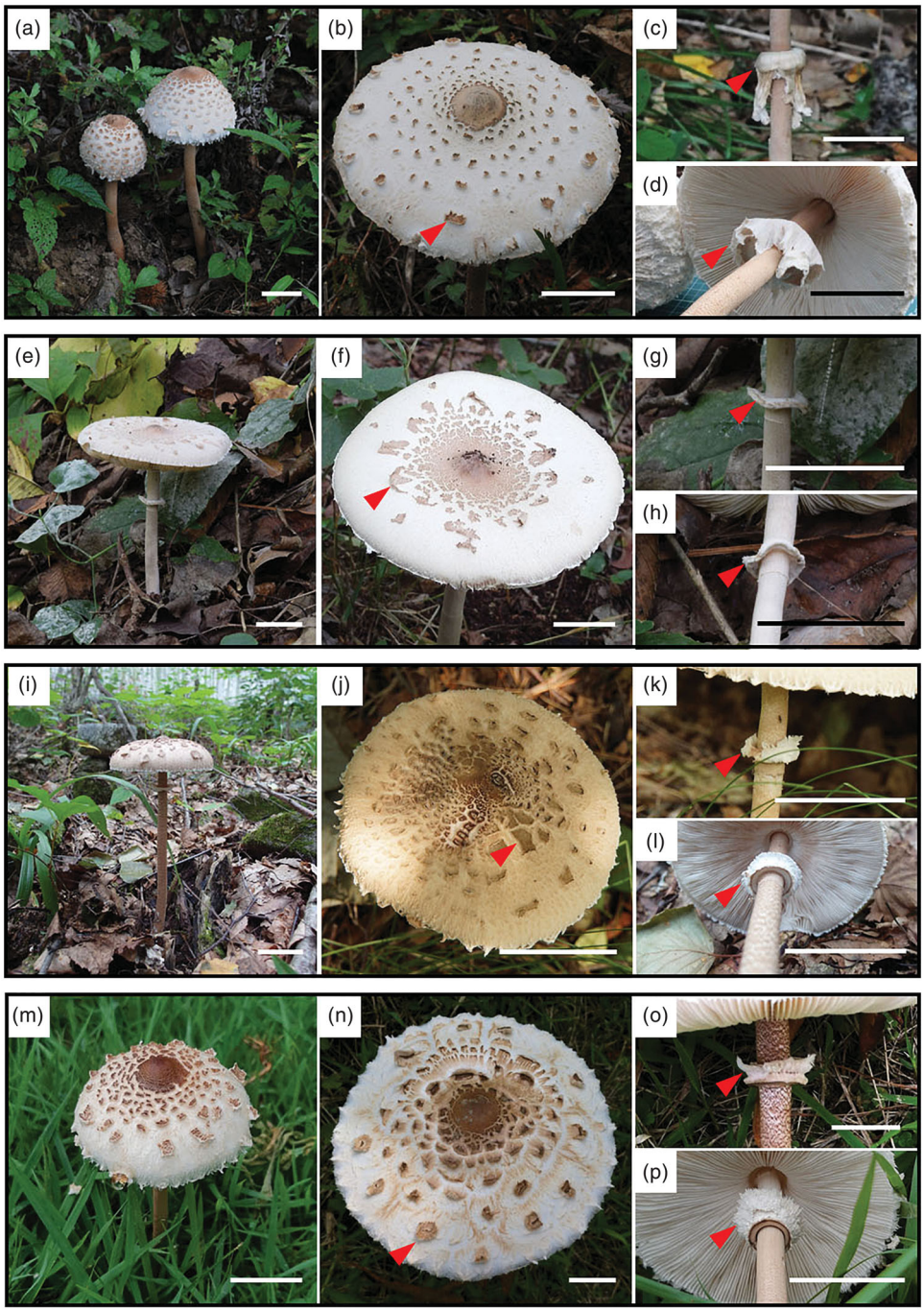

Figure 3. Basidiocarp (left column), pileal squamules (middle column), and annulus (right column) of *Macrolepiota* species. (a–d); *M. detersa* (bar = 5 cm), (e–h); *M. mastoidea* (bar = 2.5 cm), (i–l); *M. procera* (bar = 5 cm), and (m–p); *M. umbonata* (bar = 2.5 cm). Red arrow on the middle and right columns points to the pileal suamules and annulus, respectively.

4. Taxonomy

Macrolepiota detersa Z. W. Ge, Zhu. L. Yang & Vellinga in Fungal Diversity 45: 83 (2010) (Figures 3(a–d) and 4).

Basidiocarp medium-sized to large. *Pileus* 9–15 cm in diam., ovoid to campanulate when young, becoming convex to applanate with age, whitish, covered with scattered, greyish orange (5B4) to brownish orange (6C6) floccus- or crust-like squamules which are easily detachable from the pileus; disc smooth, light brown (6D7). *Lamellae* free, crowded, white when young, white to pale cream colored when mature, thin, with lamellulae, sometimes with greyish brown spots on the lamellae. *Stipe* whitish, cylindrical to subcylindrical, 15.0–38.0 × 1.8–3.2 cm, slightly tapering upwards, with tiny brownish scales, hollow; base slightly bulbous. *Annulus* ascending, superior, about 4–6 cm below stipe apex, whitish, membranous, complex, with brownish squamules on the underside; movable when mature. *Context* white to whitish, spongy, unchanging when cut.

Basidiospores [40/2/2] 15.0–18.4 × 10.2–12.4 µm, $Q = 1.32–1.66$, ellipsoid to ovoid, thick-walled,

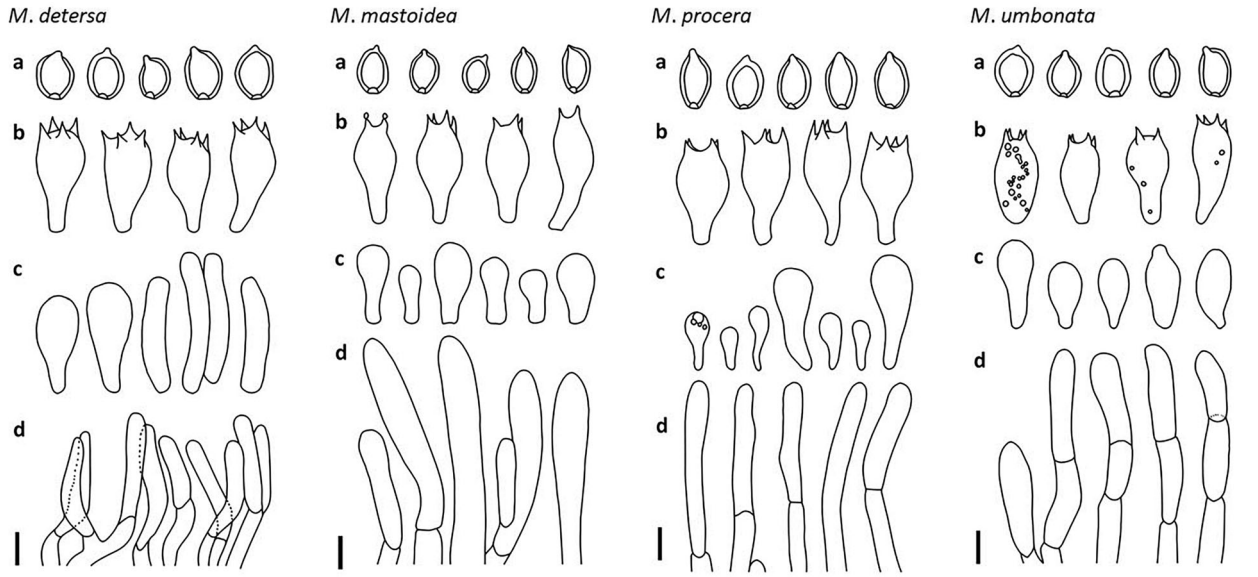

Figure 4. Microscopic features of the *Macrolepiota detersa*: *M. mastoidea*, *M. procera*, and *M. umbonata*. (a); basidiospores, (b); basidia, (c); cheilocystidia, and (d); hyphae of squamules on pileus. Scale bar = 10 mm.

smooth, hyaline, dextrinoid, congophilous, metachromatic in cresyl blue, a disconnection in the episporium on the rounded apex with germ pore, covered with a hyalinous cap in KOH; apiculus about 0.8–1.1 µm long. *Basidia* [20/2/2] 29.6–37.3 × 12.5–15.0 µm, clavate, thin-walled, hyaline, 4-spored, sometimes 2-spored. *Cheilocystidia* [20/2/2] 21.2–41.6 × 6.6–13.1 µm, cylindrical to pyriform, sometimes broadly clavate, hyaline, thin-walled. *Pleurocystidia* absent. *Squamules on pileus* a palisade of vertically arranged subcylindric, clampless hyphae (20.2–42.2 µm in length, 5.8–13.1 µm in diam.), sometimes shortly septate, rarely branched, with terminal elements slightly tapering toward the tip, with yellowish to brownish vacuolar pigment, slightly thick-walled. *Clamp connections* very common at the base of basidia and cheilocystidia.

Habitat: Solitary to scattered on ground beside roads or edge of deciduous forest.

Comment: *Macrolepiota detersa*, compared to other species in the section *Macrolepiota*, has the most easily detachable pileus squamules. The presence of cylindrical cheilocystidia are distinguishable character of this species.

Macrolepiota mastoidea (Fr.: Fr.) Singer in Lilloa 22: 417. 1951 ("1949") (Figures 3(e–h) and 4).

Basidiocarp medium-sized to large. *Pileus* 7–15 cm in diam., ovoid to campanulate when young, becoming convex to applanate when mature, with a papilla at disc, white to greyish white, covered with reddish grey (7B3) to light brown (7D4) scaly squamules, which are at first smooth and entire, then gradually break up into irregular patches, and become minute and sparse toward margin; margin appendiculate with the fibrillose veil when young; disc smooth, reddish brown (8E4). *Lamellae* free, crowded, white to greyish white, with lamellulae. *Stipe* cylindrical to subcylindrical, 9–25 × 0.8–2.0 cm, tapering upwards, whitish, covered with tiny furfuraceous cream to pale brown squamules; base slightly enlarged or bulbous. *Annulus* ascending, superior, about 3–5 cm below stipe apex, simple, whitish, membranous. *Context* whitish, not changing color when cut.

Basidiospores [40/2/2] 11.2–14.4 × 8.0–10.4 µm, Q = 1.31–1.67, ellipsoid to ovoid, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic in cresyl blue, a disconnection in the episporium on the rounded apex with germ pore, covered with a hyalinous cap in KOH; apiculus 1.2–1.4 µm long. *Basidia* [28/2/2] 33.0–39.7 × 11.6–13.8 µm, clavate, thin-walled, hyaline, rarely with small granules and bubble-like contents, 4-spored. *Cheilocystidia* [20/2/2] 15.6–25.0 × 6.1–11.3 µm, clavate, hyaline, thin-walled, in bunches forming a sterile edge. *Pleurocystidia* absent. *Squamules on pileus* a palisade of cylindric to subcylindric, clampless hyphae (36.9–71.9 µm in length, 8.2–10.9 µm in diam.), with terminal elements slightly tapering toward the tip, with yellowish to brownish vacuolar pigment, slightly thick-walled. *Clamp connections* occasionally observed at the base of basidia, not observed at the base of cheilocystidia.

Habitat: Solitary or scattered on ground in open meadows or edge of forest.

Comment: *Macrolepiota mastoidea* is only a species belonging to the section *Macrosporae* in Korea. This species is easily distinguished from other species in Korea by simple annulus and absence of clamp connection at the base of cheilocystidia.

Macrolepiota procera (Scop.: Fr.) Singer in Papers Mich. Acad. Sci., Arts Letters 32: 141. 1948 ("1946") (Figures 3(i–l) and 4).

Basidiocarp medium-sized to large. *Pileus* 10–21 cm in diam., ovoid when young, becoming convex to plano-convex with age, with an umbo at disc, white to greyish white, covered with brownish grey (5C2), light brown (6D4) to dark brown (7F5) thin squamules; disc smooth, dark brown (6F5 to 7F5); covering easily peelable, thin squamules which are irregularly arranged toward margin on the greyish brown speckled with whitish background. *Lamellae* free, crowded, thin, white when young, pale cream colored when mature, with lamellulae. *Stipe* whitish, subcylindrical, 16.5–28.0 × 1.2–2.0 cm, tapering upwards, at base enlarged or bulbous, covered with brown to dark brown speckled squamules, sometimes hollow. *Annulus* superior, about 4–5 cm below stipe apex, upper side white, underside grayish brown, membranous, complex, moveable when mature. *Context* spongy, whitish at the pileus, grayish red to grayish brown at the stipe.

Basidiospores [40/2/2] 12.6–16.8 × 7.8–10.2 µm, Q = 1.35–1.70, ellipsoid to ovoid, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic in cresyl blue, a disconnection in the episporium on the rounded apex with germ pore, covered with a hyalinous cap in KOH; apiculus not distinctive. *Basidia* [33/2/2] 34.5–40.3 × 14.7–17.2 µm, clavate, thin-walled, hyaline, 4-spored. *Cheilocystidia* [25/2/2] 11.5–35.5 × 5.0–14.5 µm, clavate to subclavate, hyaline, thin-walled, massed forming a sterile edge. *Pleurocystidia* absent. *Squamules on pileus* a palisade of subcylindric to cylindric, slightly thick-walled, clampless hyphae which are 8–13.5 µm in diam., seldom branched, with terminal elements slightly attenuate toward the tip, with yellowish vacuolar pigment, slightly thick-walled. *Clamp connections* common both of basidia base and cheilocystidia base.

Habitat: Solitary or scattered on ground in open meadows or edge of forest.

Comments: *Macrolepiota procera* can be distinguished from species in the same section by thin squamules on pileus. In addition, the background of the pileus surface is darker greyish color than other *Macrolepiota* species distributed in Korea.

Macrolepiota umbonata H. J. Cho, H. Lee & Y.W. Lim, *sp. nov* (Figures 3(m–p) and 4).

MycoBank: MB829835

Diagnosis: ***Macrolepiota umbonata*** is characterized by a large basidiocarp, big and knob-like umbo at disc, long and brownish fragmented stipe, superior and movable annulus, and squamules on pileus composed of subcylindrical, clampless yellowish brown hyphae.

Type:—SOUTH KOREA. Incheon-si, Ongjin-gun, Yeongheungdo island, 72 m elev., N37°16'12″ E126°27'35″, Nam Kyu Kim, Jae Young Park, 9 September 2016, SFC20160909-16 (Holotype, SFC!)

Basidiocarp large-sized. *Pileus* 12– 25 cm in diam., white to whitish, convex to plano-convex, sometimes applanate with slightly reflexed margin, umbonate to knobbed at disc, covered with brownish orange (5C3) to light brown (6D5) floccus-like squamules, which become sparse toward margin, revealing a white flesh between them; margin occasionally crenulate, the fibrillose veil when young; disc smooth, dark brown (6F8). *Lamellae* free, crowded, white when young, cream to greyish cream colored when mature, sometimes orange brown to greyish brown tinge, thin, with lamellulae. *Stipe* whitish, subcylindrical, 15–40 × 1–3 cm, slightly tapering upwards, orange brown to greyish brown fragmented bands, hollow; base club shaped to bulbous, 3.5–4.5 cm wide. *Annulus* ascending, superior, about 3–4 cm below stipe apex, whitish, membranous, slightly complex, with brownish patchy squamules on the underside; movable when mature. *Context* white to whitish, spongy, unchanging color when cut, but sometimes at edge of stipe with pinkish tinge.

Basidiospores [60/3/3] 11.8–16.6 × 9.2–12.0 µm, *Q* = 1.17–1.50, broadly ellipsoid to ellipsoid, thick-walled, smooth, hyaline, dextrinoid, congophilous, metachromatic in cresyl blue, a disconnection in the episporium on the rounded apex with germ pore, covered with a hyalinous cap in KOH; apiculus not distinctive, about 0.8–1 µm long. *Basidia* [42/3/3] 26.0–35.1 × 12.3–15.6 µm, clavate, thin-walled, hyaline, sometimes with small granules and bubble-like contents, 4-spored. *Cheilocystidia* [30/3/3] 12.6–35.2 × 5.3–13.8 µm, mostly clavate to subclavate, occasionally obtusely fusiform, hyaline, thin walled, in bunches forming a sterile edge. *Pleurocystidia* absent. *Squamules on pileus* a palisade of cylindric to subcylindric, clampless hyphae (7–31 µm in length, 6–11 µm in diam.), rarely branched, terminal elements slightly tapering toward the tip, yellowish to brownish vacuolar pigment, slightly thick-walled. *Clamp connections* occasionally observed at the base of basidia, common at the base of cheilocystidia.

Habitat: Solitary or scattered on ground in open meadows or edge of forest.

Etymology: "umbonata" means umbonate pileus which refers to the big and knob-like umbo of this species.

Additional studied material: SOUTH KOREA. Seoul, Gwanak-gu, Seoul National University, 101 m elev., N37°27′27″ E126°56′58″, Hyun Lee, Young Woon Lim, 3 September 2012, SFC20120903-06 (Paratype SFC!); SOUTH KOREA. Gyeongsangbuk-do, Yecheon-gun, Hakgasan Natural Recreation Forest, 367 m elev., N36°40′31″ E128°35′31″, Hyun Lee, Won Ju Kim, 17 September 2013, SFC20130917-H01

(Paratype SFC!); SOUTH KOREA. Gyeonggi-do, Gwangju-si, Taehwasan University Forest, 159 m elev., N37°18′42″ E127°18′41″, Hyun Lee, Hae Jin Cho, 19 August 2015, SFC20150819-27 (Paratype SFC!); SOUTH KOREA. Incheon-si, Ongjin-gun, Jangbongdo island, 105 m elev., N37°31′57″ E126°21′05″, Nam Kyu Kim, Jae Young Park, 6 September 2016, SFC20160906-05 (Paratype, SFC!); Ibid., Yeongheungdo island, 81 m elev., N37°15′26″ E126°28′09″, Nam Kyu Kim, Jae Young Park, 9 September 2016, SFC20160909-01 (Paratype, SFC!).

Comment: *Macrolepiota umbonata* belongs to the *Macrolepiota* section *Macrolepiota* and is morphologically similar to *M. detersa*, *M. dolichaula*, and *M. procera*. *Macrolepiota detersa* is distinguished from *M. umbonata* by its small basidiocarp (9–15 cm) and cylindrical cheilocystidia. *Macrolepiota umbonata* differs from *M. dolichaula* by having a simple annulus [3]. Pileus squamule is a character that distinguishes *M. umbonata* from *M. procera*: the former has floccus-like squamules while the latter has thin pileus squamules.

Key to the recognized species of *Macrolepiota* from Korea

Annulus simple; clamp connections lack at the base of cheilocystidia . . . . . *M. mastoidea*
Annulus complex; Clamp connections present at the base of basidia and cheilocystidia 2
Squamules on the pileus thin and greyish brown . . . . . *M. procera*
Squamules on the pileus floccus and light brown to dark brown 3
Cheilocystidia subclavate to clavate . . . . . *M. umbonata* sp. nov.
Cheilocystidia manily cyilindrical to pyriform . . . . . *M. detersa*

5. Discussion

Macrolepiota procera is easily recognized in the field due to its large basidiocarp and conspicuous pileal squamules. For this reason, there have been little attempts to clarify identity in Korea using microscopic features or sequence analysis. Surprisingly, results of our ITS analysis of 34 specimens previously identified morphologically as *M. procera* revealed that there are three additional species in Korea besides *M. procera*. Two species, *M. detersa* and *M. mastoidea*, are new records to Korea. *Macrolepiota* sp. is morphologically similar to other species in the section *Macrolepiota*, but clearly differentiated from them based on phylogenetic analysis of the ITS locus. We named this species as *M. umbonata* because it is characterized by umbonate to knobbed pileus center.

The four Korean *Macrolepiota* species are members of two sections: *Macrolepiota* and *Macrosporae*. The section *Macrolepiota* is characterized by the pileus that forms big plate-like squamules, a complex annulus, stipe usually two to three times the pileus diameter with fine brown squamules, relatively big ovoid-ellipsoid basidiospores (usually 14– 20 µm), common presence of clamp connections at the base of the cheilocystidia and basidia, and mainly broadly clavate cheilocystidia [1,3,17]. *Macrolepiota detersa*, *M. procera*, and *M. umbonata* are included in the section *Macrolepiota* and share common characters of this section. However, *M. detersa* primarily has cylindrical cheilosystidia, with clavate cheilocystidia occurring occasionally. The section *Macrosporae* is characterized by a smooth stipe, simple annulus, rare clamp connections, furfuraceous fine squamules composed of a single layer with rarely branched, pale brownish, and thin-walled cylindrical hyphae [1,3]. *Macrolepiota mastoidea*, in the section *Macrosporae*, has a simple annulus, tiny furfuraceous cream to pale brown squamules, smaller basidiospore 11.2–14.4 × 8.0–10.4, and no clamp connection at the base of cheilocystidia. However, clamp connections at the base of basidia are occasionally observed.

Macrolepiota procera and *M. mastoidea* are known as European species [38,39] and distributed worldwide. European and Asian populations are similar based on basidiocarp morphology, but differ slightly genetically. For both species, Korean samples show a closer relationship to other Asian samples compared to European samples (Figure 2). Similarly, Ge et al. [3] found that Chinese *M. procera* differ European samples in the phylogeny, but exhibit no distinct morphological characters. Since *M. detersa* was reported as a new species from China [3], it has also been discovered to occur in Korea, Japan, and Thailand. Therefore, *M. detersa* should be regarded as a species restricted to Asia.

In conclusion, we confirmed the presence of four *Macrolepiota* species in Korea using a reverse taxonomic approach. *Macrolepiota procera* and *M. umbonata* are distributed widely through the country, but *M. detersa* and *M. mastoidea* are distributed only in limited areas (Figure 1). These results were obtained from a limited number of specimens collected in a short period of time. Additional surveys are needed to verify the species diversity and clarify their geographic distribution.

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