Taxonomic Study of Amanita Subgenus Lepidella and Three Unrecorded Amanita Species in Korea

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Abstract  Amanita Pers. is a well-known monophyletic mushroom genus with a broad distribution. However, the diversity of Korean Amanita species has been underestimated, and most taxonomic studies conducted in Korea have only investigated their morphological characteristics. This approach is frequently insufficient for correct identification in fungal classification; therefore, we constructed a phylogeny of Amanita subgen. Lepidella in order to understand the phylogenetic placements of 16 Amanita specimens collected in Korea in 2012. The phylogeny constructed using the sequence data of the internal transcribed spacers and the partial large subunit of ribosomal RNA identified nine Amanita species (A. citrina, A. excelsa var. spissa, A. flavipes, A. fritillaria, A. oberwinklerana, A. pallidorosea, A. rubescens, A. subjunquillea, and A. volvata); of these, A. fritillaria, A. oberwinklerana, and A. pallidorosea are new to Korea.

Keywords  Amanita subgen. Lepidella, A. fritillaria, A. oberwinklerana, A. pallidorosea, Morphology, Phylogeny

Amanita Pers. is a well-known monophyletic mushroom genus with a broad distribution [1-4]. Using the traditional taxonomy, Amanita was divided into two subgenera: 1) subgen. Amanita Pers., which produces inamyloid spores, and 2) subgen. Lepidella (E. J. Gilbert) Veselý emend. Corner & Bas, whose spores are amyloid [5]. Although most recent phylogenetic studies have weakly supported the separation of these two subgenera, each section of genus Amanita was supported as monophyletic [2, 3, 6, 7]. Based on their phylogenetic placement, Justo et al. [7] recently transferred the Torrendia and Amarrendia genera—which are characterized by their scerotoid and gastroid forms, respectively—into genus Amanita. Amanita subgen.

Lepidella consists of the following four sections based on its morphology of the remnant of the universal veil on the margin of the pileus: 1) sect. Amidella (E. J. Gilbert) Konrad & Maubl., characterized by volval remnants on a pileus, and often with appendiculates at the margin of the pileus, and marginal remnants; 2) sect. Lepidella (E. J. Gilbert) Corner & Bas; characterized by a pileus that is usually white to cream colored, with a margin that is frequently appendiculate and exceeding the gill margin, a basal bulb that is usually small, and a stipe that is not encased in a saccate volva; 3) sect. Phalloideae (Fr.) Qué., characterized by a pileus margin that is not appendiculate, a stipe with a bulbous base, a persistent partial veil, a universally membranous veil, and a limbate or saccate volva; and 4) sect. Validae (Fr.) Qué., characterized by a pileus that is usually distinctly colored, a margin that is not appendiculate and does not exceed the gill margin, and a basal bulb that is usually small (studies in the Amanitaceae: http://www.amanitaceae.org/) [8, 9].

Introduction of molecular phylogeny into fungal taxonomy has resulted in re-description and characterization of an increasing number of species based on molecular markers [4, 10, 11]. With the advent of molecular phylogeny, it has also been essential for correct identification of fungi in addition to investigation of phylogenetic relationships [4, 6, 10, 12, 13]. Although approximately 500 Amanita species have been reported [14], most Amanita specimens in Korea have been identified based on the morphology of only around 70 species [15]. Therefore, the application of
molecular phylogeny to evaluation of Korean *Amanita* species is necessary. During a survey of fungal diversity of Korean mushrooms conducted in 2012, we collected 16 specimens of *Amanita* species that were morphologically classified as *Amanita* subgen. *Lepidella*. The goal of the present study was to understand the phylogenetic positions of these 16 Korean *Amanita* specimens based on the molecular phylogeny constructed using the sequence data of the internal transcribed spacer (ITS) and partial large subunit (nLSU) of ribosomal RNA. This study identified three *Amanita* species—*A. fritillaria*, *A. oberwinklerana*, and *A. pallidorosea*—which have not been previously reported in Korea.

**MATERIALS AND METHODS**

**Specimens and morphological observations.** The dried specimens of 16 *Amanita* species used in the present study were deposited in the herbarium of the Korea National Arboretum (KA); their detailed information, including the accession numbers of sequences deposited in GenBank, is shown in Table 1. Reliable DNA sequence data of other reported *Amanita* species for use in phylogenetic analyses were obtained from GenBank. Macro-morphological descriptions were based on the field notes and color photographs of basidiomata. Micro-morphological data were obtained from dried specimens after sectioning and rehydration in 3% KOH solution under a light microscope. Descriptions of spore shapes are based on the study reported by Bas [1].

**PCR amplification and sequencing.** Genomic DNA was isolated from fresh fruiting bodies (approximately 0.1 g) using a modified version of the CTAB procedure reported by Doyle and Doyle [16]. The ITS region and partial nLSU of ribosomal RNA were amplified using two different primer sets: ITS5 and ITS4 for the ITS region [17], and LR0R and LR5 for nLSU [18]. PCR mixtures contained 0.5 pmol of each primer, dNTPs at 0.25 mM, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 2.5 U of Taq DNA polymerase, and 15 ng of template DNA. PCR conditions for ITS and nLSU were as follows: an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 40 sec, 55°C (ITS) or 52°C (nLSU) for 40 sec, and 72°C for 1 min, and a final elongation step at 72°C for 8 min. PCR products were purified using an ExoSAP kit (USB, Cleveland, OH, USA). The purified double-stranded PCR fragments were directly sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer’s instructions. The same primer sets used for amplification of ITS and nLSU were used for sequencing. Capillary electrophoresis and data collection were performed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

**Phylogenetic analyses.** In order to understand the phylogenetic placement of Korean *Amanita* subgen. *Lepidella*, reference sequences of *Amanita* species retrieved from GenBank and our 16 specimens of *Amanita* species were used in construction of a phylogeny in the phylogenetic framework of maximum parsimony (MP) and Bayesian analysis. The sequences were edited using the PHYDIT program (version 3.2) [19]. DNA sequences were aligned manually with the aid of Clustal X 1.81 [20]. Ambiguously aligned regions of ITS and nLSU sequences were excluded from phylogenetic analyses. Three species of *Amanita* sect.

Table 1. Information on *Amanita* specimens investigated in this study

| Species           | Specimen | Locality                      | Collection date | GenBank accession No. |
|-------------------|----------|-------------------------------|-----------------|-----------------------|
|                   |          |                               |                 | ITS                   | nLSU                  |
| *A. citrina*      | KA12-1226| Mt. Seodae, Geumsan-gun, Chungnam | 29 Aug 2012     | KF245892              | KF245908              |
|                   | KA12-1612| Gwangneung Forest, Pocheon-si, Gyeonggi | 4 Oct 2012     | KF245893              | KF245909              |
|                   | KA12-0884| Gwangneung Forest, Pocheon-si, Gyeonggi | 16 Jul 2012    | KF245894              | KF245910              |
| *A. excelsa var. spissa* (= *A. spissa*) | KA12-0685| Mt. Minjuji, Yeongdong-gun, Chungbuk | 16 Jul 2012     | KF245895              | KF245911              |
|                   | KA12-1517| Saneum Natural Recreation Forest, Yangpyeong-gun, Gyeonggi | 20 Sep 2012 | KF245896              | KF245912              |
| *A. flavipes*     | KA12-0685| Mt. Seodae, Geumsan-gun, Chungnam | 29 Aug 2012     | KF245897              | KF245913              |
|                   | KA12-1517| Gwangneung Forest, Pocheon-si, Gyeonggi | 4 Oct 2012     | KF245898              | KF245914              |
| *A. fritillaria*  | KA12-1231| Mt. Gumi, Boeun-gun, Chungbuk | 30 Aug 2012     | KF245899              | KF245915              |
| *A. oberwinklerana* | KA12-0898| Gwangneung Forest, Pocheon-si, Gyeonggi | 27 Sep 2012    | KF245900              | KF245916              |
| *A. palidorosea*  | KA12-1231| Mt. Gumi, Boeun-gun, Chungbuk | 30 Aug 2012     | KF245897              | KF245913              |
| *A. rubescens*    | KA12-0641| Mt. Minjuji, Yeongdong-gun, Chungbuk | 16 Jul 2012     | KF245899              | KF245915              |
|                   | KA12-1144| Mt. Minjuji, Yeongdong-gun, Chungbuk | 27 Aug 2012    | KF245900              | KF245916              |
|                   | KA12-1579| Mt. Seodae, Geumsan-gun, Chungnam | 26 Sep 2012     | KF245901              | KF245917              |
| *A. subjunquillea*| KA12-0936| Gwangneung Forest, Pocheon-si, Gyeonggi | 8 Aug 2012     | KF245902              | KF245918              |
|                   | KA12-1221| Mt. Seodae, Geumsan-gun, Chungnam | 29 Aug 2012     | KF245903              | KF245919              |
| *A. volvata I*    | KA12-1550| Mt. Jinak, Geumsan-gun, Chungnam | 25 Sep 2012     | KF245904              | KF245920              |
| *A. volvata II*   | KA12-1221| Gwangneung Forest, Pocheon-si, Gyeonggi | 9 Aug 2012     | KF245905              | KF245921              |
|                   | KA12-1194| Mt. Seodae, Geumsan-gun, Chungnam | 29 Aug 2012     | KF245906              | KF245922              |
|                   | KA12-1367| Naeseujeon-Seokpo Sunrise Sunset Observatory, Ulleung-gun, Gyeongbuk | 6 Sep 2012 | KF245907              | KF245923              |

Boldface indicates the *Amanita* species that were previously unrecorded in Korea.
Fig. 1. One of the 54 most-parsimonious trees from maximum parsimony (MP) analysis of internal transcribed spacer (ITS) sequences. Nodes supported with MP bootstrap support (MPBS) > 50% and posterior probability (PP) > 0.95 are indicated by thicker lines. Only MPBS values > 50% are shown above or below branches. Species of sect. Amanita (i.e., A. ibotengutake, A. muscaria, and A. regalis) were used as outgroups. Accession numbers of sequences obtained from GenBank are shown within parentheses. Amanita species identified in Korean specimens appear within gray boxes, and specimen voucher Nos. for Korean specimens are colored either red (for unrecorded species) or blue (for previously recorded species). CI, consistency index; HI, homoplasy index; RI, retention index.
Fig. 2. One of the 75 most-parsimonious trees from maximum parsimony (MP) analysis of partial large subunit (nLSU) sequences. Nodes supported with MP bootstrap support (MPBS) > 50% and posterior probability (PP) > 0.95 are indicated by thicker lines. Species of sect. Amanita (i.e., A. ibotengutake, A. muscaria, and A. pantherina) were used as outgroups. Accession numbers of sequences obtained from GenBank are shown within parentheses. Amanita species identified in Korean specimens appear within gray boxes, and specimen voucher nos. for Korean specimens are colored either red (for unrecorded species) or blue (for previously recorded species). CI, consistency index; HI, homoplasy index; RI, retention index.
Amanita (A. ibotengutake, A. muscaria, and A. regalis) were used as outgroups in phylogenetic analyses. MP analyses were performed using PAUP version 4.0b10 [21] with the option of heuristic searching with the random addition of sequences and subsequent tree bisection-reconnection branch swapping. For nodal support, MP bootstrap support (MPBS) values were calculated using 1,000 replicates. Bayesian analyses were performed using MrBayes 3.1.2 [22]. The nodal support was estimated in Bayesian analyses with posterior probability (PP) values using the Metropolis-coupled Markov-chain Monte Carlo method. To determine the convergence of likelihood, two parallel runs were performed using one cold and three heated chains for 10 million generations, starting with a random tree, while sampling every 100th generation. The three chains were heated at 0.2 for the analyses (the heated for cold chain 1 and heated chains 2, 3, and 4 were 1.00, 0.83, 0.71, and 0.63, respectively). We assumed that the two runs had reached convergence when the average standard deviation of the split frequencies dropped below 0.01. Use of this approach resulted in a set of four chains reaching convergence after approximately 4.5 and 4.0 million generations for the ITS and nLSU data sets, respectively. PP values below 0.95 were not considered significant; values below 0.9 are shown on the resulting phylograms in Figs. 1 and 2.

RESULTS AND DISCUSSION

Phylogenetic analyses of ITS and nLSU sequences. As shown in Fig. 1, MP analysis of the ITS sequence data resulted in 54 most-parsimonious trees comprising 1,329 steps (consistency index [CI], 0.4989; retention index [RI], 0.7511; homoplasy index [HI], 0.5011; and 290 were parsimony-informative). Bayesian analysis using a GTR + I + G model of evolution for 10 million generations was performed on the ITS sequence data set (with 76 taxa, 614 characters, and burninfrac [0.45]). As shown in Fig. 2, MP analysis of the nLSU sequence data resulted in 75 most-parsimonious trees comprising 756 steps (CI, 0.4167; RI, 0.7687; HI, 0.5833; and 169 were parsimony-informative). Bayesian analysis using a GTR + I + G model of evolution for 10 million generations was performed on the nLSU sequence data set (with 74 taxa, 534 characters, and burninfrac [0.40]). The phylogenetic trees of ITS and nLSU were very similar to the results of previously published studies in terms of phylogenetic placement of species of Amanita subgen. Lepidella [2-7].

The phylogeny for placement of 16 Korean specimens used in the present study and understanding their phylogenetic relationships within Amanita subgen. Lepidella identified nine phylogenetic species: A. citrina (Schaeff.) Pers., A. excelsa var. spissa (Fr.) Neville & Poumarat, A. flavipes S. Imai, A. fritillaria Sacc., A. oberwinklerana Zhu L. Yang & Yoshim. Doi, A. pallidorosea P. Zhang & Zhu L. Yang, A. rubescens Pers., A. subjunquillea S. Imai, and A. volvata (Peck) Lloyd. Most of the identified Amanita species formed a group with strong support (MPBS > 70% and PP > 0.95) in the ITS and nLSU trees (Figs. 1 and 2). However, A. rubescens KA12-0936 and KA12-1221 formed a group with moderate support in the ITS tree (MPBS/PP = 60/0.95). In addition, A. subjunquillea KA12-1550 formed a group with A. subjunquillea HKAS:75770 and HKAS:75772 (HKAS: deposited in the Cryptogamic Herbarium of Kunming Institute of Botany of the Chinese Academy of Sciences) with moderate support (MPBS/PP = 55/0.95) in the nLSU tree, and KA12-1550 formed a group with A. subjunquillea HKAS:52315 and A. phalloidesKF02-19 (KF: Botanical Institute, University of Copenhagen, Copenhagen, Denmark; referred from Zhang et al. [23]) without nodal support from MPBS/PP in the ITS tree (Figs. 1 and 2). Amanita citrina formed a species complex in the ITS and nLSU trees (Figs. 1 and 2). KA12-0685 was closely grouped with reference specimens of A. flavipes HKAS:36582 and A. cf. franchetii KGP82 (deposited by Peay et al. [24] as A. franchetii in GenBank, however, this specimen was only approximately identified), and KA12-1517 was grouped with A. flavipes LEM960088a (LEM: deposited in the Herbarium, Laboratory of Environmental Mycoscience, Faculty of Agriculture, Kyoto University) in ITS (Fig. 1). However, the sequences of our two A. flavipes specimens (KA12-0685 and KA12-1517) in the nLSU tree were identical to that of A. flavipes HKAS:32505. A. flavipes KA12-0985, KA12-1194, and KA12-1367 were divided into two groups (A. volvata I: KA12-0985; A. volvata II: KA12-1194 and KA12-1367) in the ITS and nLSU trees (Figs. 1 and 2).

Previously reported species of Amanita subgen. Lepidella in Korea. Amanita citrina possesses a fleshy pale yellow to white basidiocarp, a 3~12 cm pileus, a large volva at the base of the 6~8 cm tall stem, and a smell of rapeseed or potato (studies in the Amanitaceae: http://www.amanitaceae.org/) [25]. This species consists of several varieties and formae (three varieties and five formae are recorded in Index Fungorum: http://www.indexfungorum.org/ Names/Names.asp). KA12-1226 and KA12-1612 were not placed in the A. citrina complex with only weak support (Figs. 1 and 2). The lack of reliable sequence data of several varieties and formae of A. citrina in GenBank indicated that our two specimens could not be confidently assigned and classified at the levels of varieties and formae. Based on morphological characteristics and sequences data of ITS and nLSU, our two specimens are members of the A. citrina complex (Figs. 1~3). In addition, in our phylogenetic analyses, some A. porphyria specimens (LEM960303 in the ITS tree and HKAS:31531 in the nLSU tree) were included in the A. citrina complex (Figs. 1 and 2). In future studies, extensive phylogenetic and morphological investigations of this species complex should be conducted in order to clarify the relationships between the varieties and formae. Amanita excelsa, which was originally described from Sweden, is characterized by a gray brown to olive brown
pileus, a nonstriate and nonappendiculate margin, which is covered with a volva at the pileus, which is white to pale gray and striate above the annulus, with broadly ellipsoid to ellipsoid and amyloid spores, and no clamps at the bases of basidia (studies in the Amanitaceae: http://www.amanitaceae.org/) [9]. According to Jenkins [9], A. excelsa should be synonymized with A. spissa because both species grow in the same fairy ring. A. spissa was transferred to A. excelsa var. spissa based on an intensive morphological investigation by Neville and Poumarat in 2004 [26], who also accepted several varieties and designated a neotype of A. excelsa. Our KA12-0884 specimen was grouped with A. spissa KF02-47 (sequences submitted to GenBank but not published) with strong support in the ITS tree and was closely related to A. spissa NYBG47779 (Organismic and Evolutionary Biology, Harvard University, USA; sequences submitted to GenBank but not published) in the nLSU tree. The KA12-0884 specimen was closely related to A. excelsa HKAS:31510 and A. excelsa Ge 816 (Organismic and Evolutionary Biology, Harvard University, USA; sequences submitted to GenBank but not published) in the nLSU tree. In contrast, our specimen was distantly related to A. flavoconia (deposited by Zhang et al. [5]) in the ITS tree.

Amanita flavipes is characterized by a olive to yellow pileus and is paler toward the margin, decorated with yellow floccose to patch-like volval remnants, and has basidiospores measuring 7–8 × 5.5–8 µm in size, with Q = 1.06–1.47 (studies in the Amanitaceae: http://www.amanitaceae.org/) [25]. This species, which was originally described from Japan in 1933, is widely distributed in China, India, Japan, Pakistan, and Korea [5, 25]. Amanita flavipes is morphologically similar to A. flavoconia G. F. Atk., however, they have different distributions: A. flavipes is distributed in Asia, while A. flavoconia is one of the most common and widespread species found in eastern North America (studies in the Amanitaceae: http://www.amanitaceae.org/). Our two specimens (KA12-0685 and KA12-1517) were grouped with Chinese A. flavipes HKAS:32505, A. flavoconia RV5Aug96, and A. flavorubescens RV96/102 (RV: collections of Moncalvo et al. [4]; Duke University Herbarium and Culture Collection) with weak support in the nLSU tree (Fig. 2). Therefore, conduct of further study will be necessary before these two specimens can be placed with confidence, since this requires an understanding of their phylogenetic positions obtained from more taxon sampling and intensive morphological observations.

Our two A. rubescens specimens (KA12-0936 and KA12-1221) were clearly identified based on their morphological taxonomic description. They are characterized by a brownish pileus, flesh that is slowly stained a dingy reddish color when bruised or cut, adnate to narrowly adnate gills, usually absent volval remnants, and smooth, elliptical, and amyloid spores, and were phylogenetically matched with the sequences deposited in GenBank (studies in the Amanitaceae: http://www.amanitaceae.org/) (Figs. 1 and 2) [8]. Amanita subjunquillea KA12-1550 was closely related to European A. phalloides KF02-19 (referred from Zhang et al. [23]) in the ITS tree and A. phalloides UPS2701 (Uppsala Herbarium Sweden; referred from Moncalvo et al. [27]) in the nLSU tree (Figs. 1 and 2). However, they can be distinguished based on the size of spores, the morphologies of the basidia and basidiomata, and the fact that A. subjunquillea are normally smaller than A. phalloides (studies in the Amanitaceae: http://www.amanitaceae.org/) [8, 9].

Amanita volvata is characterized by a white to bruised-brown or reddish basidiocarp, tough volva, no annulus, a nonstriate to faintly striate margin at the pileus, and oblong or elliptical and amyloid spores, and is the type species of Amanita sect. Amidella and has worldwide distribution (studies in the Amanitaceae: http://www.amanitaceae.org/) [8, 9]. Morphologically this species is similar to species such as A. avellaneosquamosa (S. Imai) S. Imai, A. clarisquamosa (S. Imai) S. Imai, and A. duplex Corner & Bas (studies in the Amanitaceae: http://www.amanitaceae.org/). Our three specimens (A. volvata I: KA12-0985; A. volvata II: KA12-1194 and KA12-1367) were grouped with A. volvata sensu

![Fig. 3. Three unrecorded Amanita species in Korea. A–F, Fruiting bodies (A, B, A. fritillaria [KA12-1231]; C, A. oberwinklerana [KA12-0898]; D–F, A. pallidorosea [D, KA12-0641; E, F, KA12-1579]); G–M, Basidia and basidiospores (G, H, A. fritillaria; I, J, A. oberwinklerana; K–M, A. pallidorosea). Scale bars: A–C = 5 cm, D–F = 3 cm, G–M = 10 µm.](image-url)
Yagame et al. [28] and A. volvata LEM960165 in the ITS tree, respectively (Fig. 1). In the nLSU tree, A. volvata I and A. volvata II were related to A. aff. volvata BW_STF 090506-8 (Organismic and Evolutionary Biology, Harvard University, USA; sequences submitted to GenBank but not published) and A. volvata RV97/24, respectively. They were well separated in the ITS and nLSU trees, which is consistent with the results of the recent phylogenetic analyses reported by Zhang et al. [5] and Moreno et al. [15]. Because our specimens (KA12-0985, KA12-1194, and KA12-1367) were phylogenetically placed as A. volvata, which is not a clearly monophyletic group in future molecular studies and intensive morphological surveys that include the type or equivalent materials for a taxonomic revision should be conducted.

Three unrecorded species of Amanita subgen. Lepidella in Korea. Amanita fritillaria is characterized by a grayish, brownish gray to brownish basidiocarp, verrucose to feltly volval remnants, a smooth and nonappendiculate pile margin, broadly ellipsoid to ellipsoid and amyloid spores, and no clamps at the bases of basidia, and was originally described from northern India [25]. It is widely distributed in China, Singapore, and Thailand [25]. According to Sanmee et al. [25], some Korean mycologists reported this species as A. spissacea in Korea in 1993; however, they were not formally reported and were actually misidentified. Herein, we formally report on A. fritillaria in Korea based on morphology and phylogenetic analyses using ITS and nLSU sequence data of KA12-1231 (Table 1, Figs. 1~3).

Amanita oberwinklerana was originally described from Japan [29]. Based on its morphological characteristics, including a white basidiocarp, a nonappendiculate pile margin, a membranous limbate volva on the base of the stipe, and amyloid spores, this species was placed in sect. Phalloideae [30]. However, molecular analyses from both ITS and nLSU resulted in categorization of this species into sect. Lepidella (Figs. 1 and 2), although it is not clear whether this section is monophyletic [5, 7, 23].

Amanita pallidorosea was recently reported from China by Zhang et al. [23]. This species is characterized by its pale rose pileus with a conspicuous umbo over the disc and small globose to subglobose basidiospores. Although the pallid rose pileus is an important characteristic of A. pallidorosea, a purely white form may also exist in nature [23]. Based on their fruiting-body morphology, we initially identified the specimens KA12-0641 and KA12-1144 as A. virosa (Fr.) Bertill. However, in our molecular analyses, these specimens, as well as the KA12-1579 specimen (initially regarded as an Amanita species), were clearly identified as A. pallidorosea based on strong nodal support (Figs. 1~3).

Taxonomical descriptions of three newly recorded Amanita species in Korea.

Amanita fritillaria (Berk.) Sacc., Sylloge Fungorum 9: 2 (1891), Fig. 3A, 3B, 3G, and 3H.

Pileus ca. 4~5 cm diam., gray to brownish, covered with verrucose to feltly volval remnants; a smooth and nonappendiculate pile margin. Stipe ca. 10 × 0.5~1.0 cm, slightly tapering upward, bright-brown with floccose volval remnants; annulus present. Basidia 32~35 × 6~10 µm, 4-spored; basal septa without clamps; sterigmata 3.2~4.7 µm long. Basidiospores 7.2~9 × 6~7.5 µm, broadly ellipsoid to ellipsoid and amyloid spores.

Specimen examined: Mt. Geumjeok, Boeun-gun, Chungbuk Province, Korea, 30 Aug 2012, coll. Han et al. (specimen: KA12-1231).

Amanita oberwinklerana Zhu L. Yang & Yoshim. Doi, Bull. Natl. Sci. Mus., Tokyo, Ser. B. 25: 120 (1999), Fig. 3C, 3I and 3J.

Pileus ca. 3.5~4 cm diam., white, non-appendiculate pileal margin. Stipe ca. 5.5~8 × 0.5~1.2 cm, attenuate upward, membranous limbate volva on the base of the stipe; annulus present below the apex of the stipe. Basidia 34~54 × 8.6~13 µm, clavate, usually 4-spored; basal septa without clamps; sterigmata 2.6~4.9 µm long. Basidiospores 7.5~9.7 × 5.3~7.5 µm, ellipsoid to elongate and amyloid spores.

Specimen examined: Gwangneung Forest, Pocheon-si, Gyeonggi Province, Korea, 27 Sep 2012, coll. Han et al. (specimen: KA12-0898).

Amanita pallidorosea P. Zhang & Zhu L. Yang, Fungal Divers. 42: 125 (2010), Fig. 3D, 3E, 3F, 3K, 3L, and 3M.

Pileus ca. 8~10 cm diam., white to pallid rose in center; margin non-striate, non-appendiculate. Stipe ca. 8~11 × 0.5~1.2 cm, slightly tapering upward; annulus present. Basidia 29.5~37 × 4~10 µm, clavate, usually 4-spored, rarely 2-spored. Basidiospores 6.2~8.5 × 5.8~8 µm, globose to subglobose and amyloid spores; sterigmata 3.8~8 µm long. Absence of clamp-connection in all tissues.

Specimen examined: Mt. Minjuji, Yeongdong-gun, Chungbu Province, Korea, 16 Jul 2012, coll. Han et al. (specimen: KA12-0641); 27 Aug 2012, coll. Han et al. (specimen: KA12-1144). Mt. Seodaeg, Geumsan-gun, Chungnam Province, 26 Sep 2012, coll. Han et al. (specimen: KA12-1579).

Conclusion. The phylogenetic positions of three of our Korean Amanita specimens in Amanita subgen. Lepidella (complex and/or doubtful species: A. citrina, A. excelsa var. spissa, and A. volvata) could not be determined with confidence, however, based on the sequence data of ITS and nLSU and morphological characteristics, six other species (A. flavipes, A. fritillaria, A. oberwinklerana, A. pallidorosea, A. rubescens, and A. subjunquillea) were clearly identified as previously reported Amanita species. Three of these—A. fritillaria, A. oberwinklerana, and A. pallidorosea—are newly recorded Amanita species in Korea.

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