



## A new species and two new records of *Amanita* (Amanitaceae; Basidiomycota) from South Korea

JONG WON JO<sup>1,4</sup>, YOUNG-NAM KWAG<sup>1,5</sup>, HYUNG SO KIM<sup>1,6</sup>, HYUN LEE<sup>1,7</sup>, SANG-KUK HAN<sup>2,8</sup>, JAE-GU HAN<sup>3,9</sup>, SEUNG HWAN OH<sup>1,10</sup> & CHANG SUN KIM<sup>1,11\*</sup>

<sup>1</sup>Division of Forest Biodiversity, Korea National Arboretum, Pocheon, Korea.

<sup>2</sup>Research Planning and Coordination, Korea National Arboretum, Pocheon, Korea.

<sup>3</sup>Mushroom Research Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, Korea.

<sup>4</sup>✉ [jongwon82@korea.kr](mailto:jongwon82@korea.kr); <https://orcid.org/0000-0001-6415-1869>

<sup>5</sup>✉ [kyn0102@korea.kr](mailto:kyn0102@korea.kr); <https://orcid.org/0000-0001-7333-2303>

<sup>6</sup>✉ [wangsonim@naver.com](mailto:wangsonim@naver.com); <https://orcid.org/0000-0003-1635-0200>

<sup>7</sup>✉ [hodooking@korea.kr](mailto:hodooking@korea.kr); <https://orcid.org/0000-0003-2817-7812>

<sup>8</sup>✉ [hansk75@korea.kr](mailto:hansk75@korea.kr); <https://orcid.org/0000-0003-4549-8896>

<sup>9</sup>✉ [hanjaegu@korea.kr](mailto:hanjaegu@korea.kr); <https://orcid.org/0000-0002-8948-224X>

<sup>10</sup>✉ [oshwan@korea.kr](mailto:oshwan@korea.kr); <https://orcid.org/0000-0003-2190-0967>

<sup>11</sup>✉ [changsun84@korea.kr](mailto:changsun84@korea.kr); <https://orcid.org/0000-0001-6918-8863>

\*Corresponding author

### Abstract

A new species of *Amanita* sect. *Roanokenses*, *A. brunneofolia*, from South Korea, is described based on morphological and molecular evidences. The species is characterized by medium- to large-sized basidiomata, a greenish white pileus covered with brownish, floccose pyramidal volval remnants, an appendiculate margin, reddish brown lamellae, a long radicating stipe, and ellipsoid to elongate amyloid basidiospores. Based on both nrLSU and combined dataset (nrLSU, *rpb2* and *tef1- $\alpha$* ), *A. brunneofolia* formed a monophyletic clade and clearly separated from other *Amanita* species. In addition, we describe two other *Amanita* species in *A.* sect. *Roanokenses*, namely, *A. caojizong* and *A. sphaerobulbosa*. This is the first report of these species for South Korea.

**Keywords:** Amanitaceae, morphology, new taxon, phylogeny, taxonomy

### Introduction

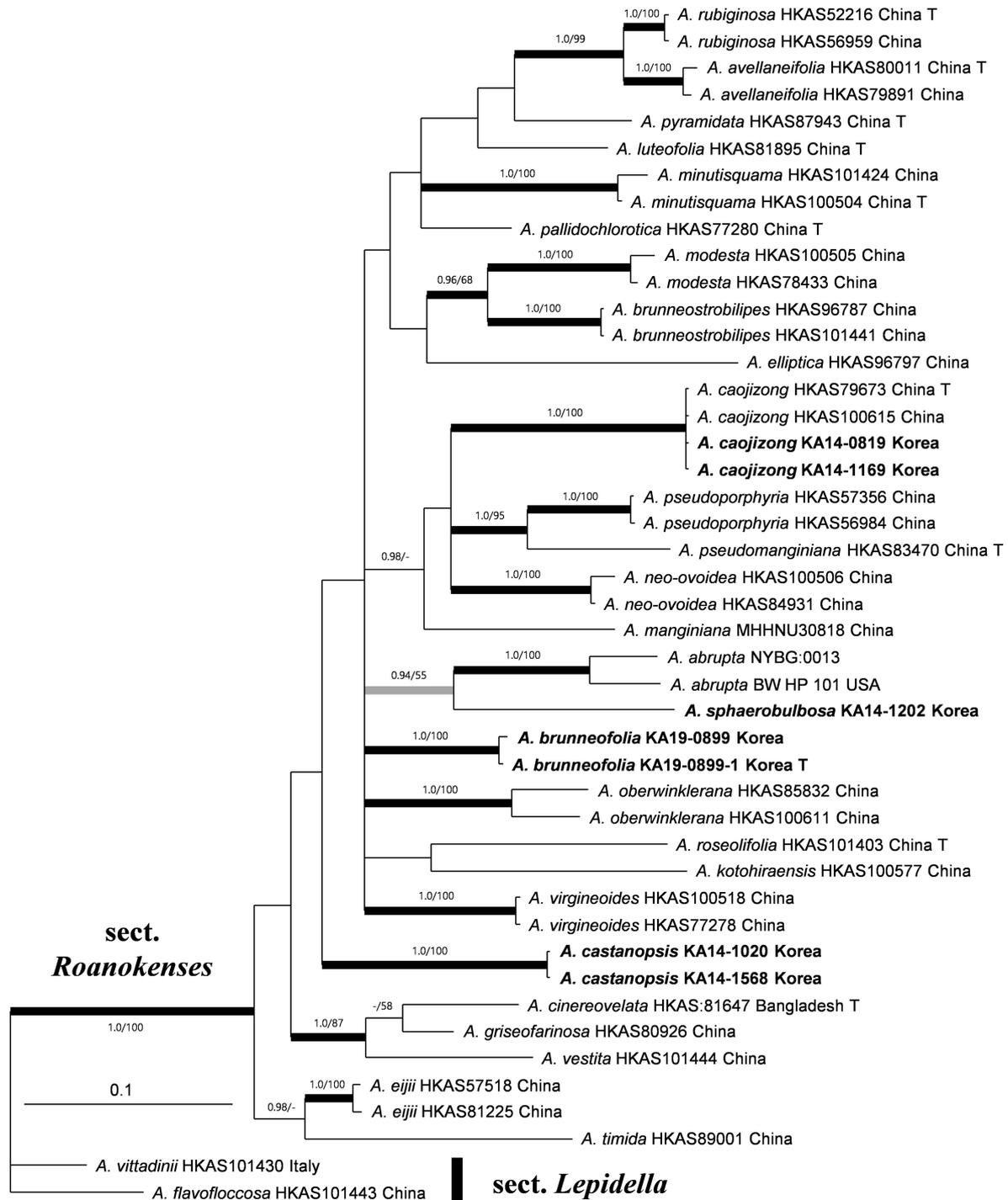
*Amanita* Pers. (1797: 65) is a cosmopolitan genus of the Amanitaceae family in Basidiomycota consisting of over 500 accepted species (Kirk *et al.* 2008, Yang 2015, Cui *et al.* 2018). Genus *Amanita* is known to comprise both edible and poisonous mushrooms (Bas 1969, Jenkins 1986). Moreover, most species of this genus play important roles in forest ecosystems by forming ectomycorrhizal associations with various tree species (Bas 1969, Jenkins 1986, Yang 2000a, Cai *et al.* 2014).

Many mycologists have proposed an infrageneric classification of the genus based on the morphological characteristics of the fruit-body (Gilbert & Kühner 1928, Gilbert 1940, 1941, Corner & Bas 1962, Bas 1969, Singer 1986). Until recently, genus *Amanita* remained divided in two subgenera comprising seven sections (Yang 1997, Weiß *et al.* 1998, Zhang *et al.* 2004). However, based on morphological and multi-locus sequence data, Cui *et al.* (2018) restructured the current classification system as three subgenera and eleven sections. As a result, one subgenus and one section, *Amanita* subgen. *Amanitina* (E.J. Gilbert) E.J. Gilbert (1941) and *Amanita* sect. *Roanokenses* Singer ex Singer (1962), were re-examined, and three sections *Amanita* sect. *Amarrendiae* (Bougher & Lebel) Zhu L. Yang, Yang-Yang Cui, Qing Cai & Li-Ping Tang (2018), *Amanita* sect. *Arenariae* Zhu L. Yang, Yang-Yang Cui & Qing Cai (2018), and *Amanita* sect. *Strobiliformes* Singer ex Qing Cai, Zhu L. Yang & Yang-Yang Cui (2018), were newly described.

Nearly 50 species of *Amanita* have been reported from Korea (The Korean Society of Mycology 2013). However, most of these reports were based on morphological data only, with short descriptions (Kim *et al.* 2013b). Henceforth,

15 species of *Amanita* have been reported as new to Korea, based on ribosomal DNA sequence data (Kim *et al.* 2013a, 2013b, Lee *et al.* 2014, Cho *et al.* 2015, Jin *et al.* 2015). Therefore, more than 60 species of *Amanita* have been recorded in Korea, but there has been no report of any new species.

During our ongoing research of ectomycorrhizal fungi in South Korea, we collected several unusual *Amanita* species belonging to sect. *Roanokenses*. Herein, we document the morphological and molecular characteristics of three *Amanita* species in sect. *Roanokenses*, including a new taxon.



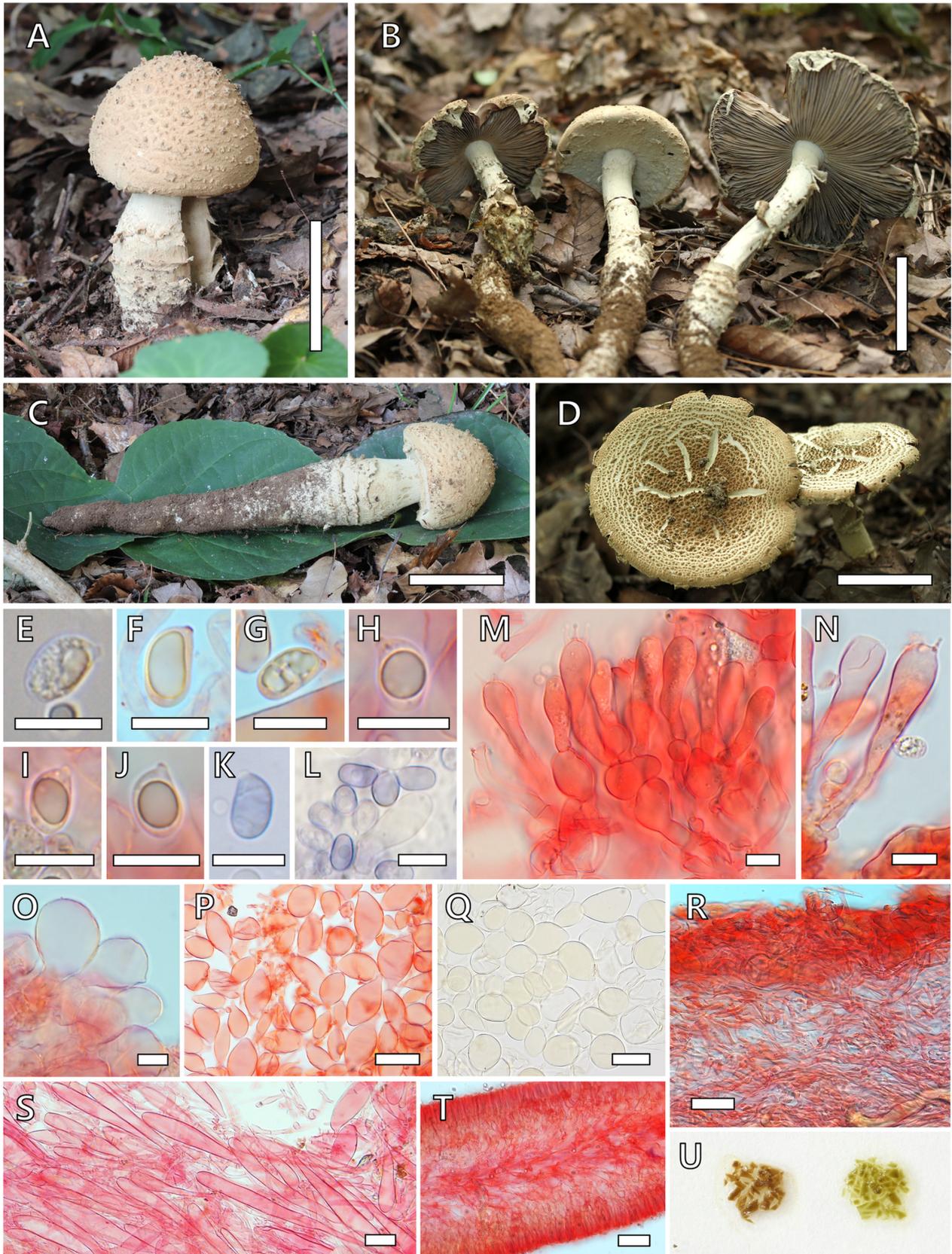
**FIGURE 1.** Bayesian 50% majority-rule consensus topology based on nrLSU sequence data. Bayesian posterior probabilities (PP) and 1000 bootstrap replicates in MP analysis (MPBS) are indicated as PP/MPBS above or below branches. Broad black branches indicate PP > 0.95 and MPBS > 60%. Broad gray branch indicates 0.90 < PP < 0.95 and MPBS > 50%. Sequences generated for this study are highlighted in bold. The symbol 'T' indicated the type materials.

## Material & methods

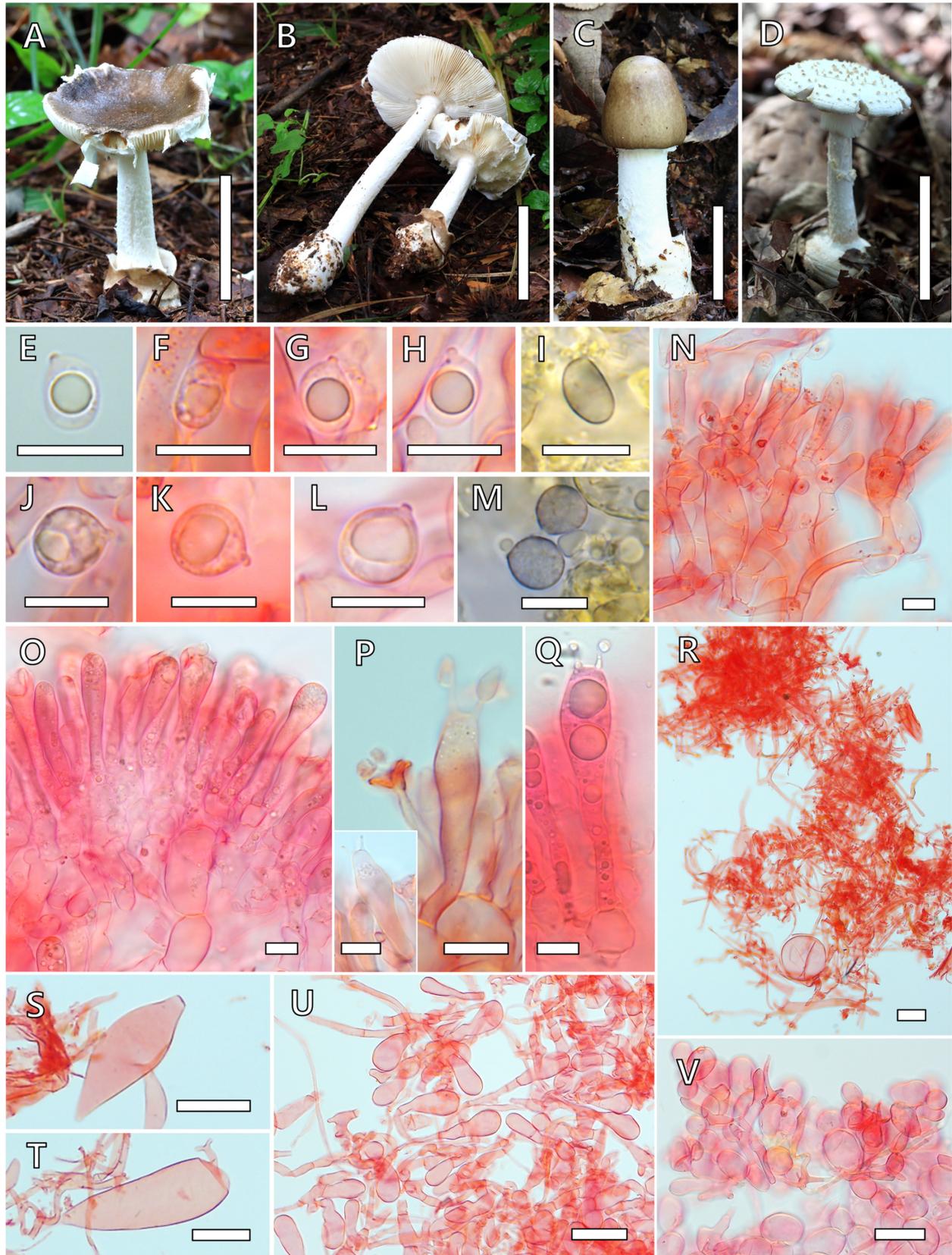
*Taxon sampling*:—As part of the mushroom surveys in the unexploited areas of Korea, we found unusual Amanitoid basidiomata near sawtooth oak (*Quercus acutissima* Carruth.) trees at Ganghwa island, South Korea, in August 2019. The specimens had brownish lamellae and a very long radicating stipe under the ground. Additionally, two other unrecorded *Amanita* species used in this study were collected in 2014 and kept at the Korea National Arboretum Herbarium (KH).



**FIGURE 2.** Bayesian 50% majority-rule consensus topology based on combined dataset (nrLSU, *rpb2* and *tef1- $\alpha$* ). Bayesian posterior probabilities (PP) and 1000 bootstrap replicates in MP analysis (MPBS) are indicated as PP/MPBS above or below branches. Broad black branches indicate PP > 0.95 and MPBS > 70%. Sequences generated for this study are highlighted in bold. The symbol ‘T’ indicated the type materials.



**FIGURE 3.** Morphological characters of *Amanita brunneofolia*. A, C: KA19-0899. B, D: KA19-0899-1. E: basidiospore in 3% KOH. F–J: basidiospores in 1% Congo Red. K, L: basidiospores in Melzer’s reagent. M, N: hymenium and subhymenium. O: inflated cells on lamellar edge. P: elements of partial veil. Q: volval remnants on pileus. R: pileipellis. S: context of stipe. T: hymenophoral trama. U: crushed lamella tissues (left: in water, right: in 3% KOH). Scales bars: A–D = 5 cm, E–O = 10  $\mu$ m, P–T = 40  $\mu$ m. Photos by: Jong Won Jo.



**FIGURE 4.** Morphological characters of *Amanita caojizong* and *A. sphaerobulbosa*. A, B: *A. caojizong* (KA14-1169). C: *A. caojizong* (KA14-0819). D: *A. sphaerobulbosa* (KA14-1202). E–I: basidiospores (KA14-1169). J–M: basidiospores (KA14-1202). N: hymenium and subhymenium (KA14-1169), O: hymenium and subhymenium (KA14-1202). P: basidia (KA14-1169). Q: basidia (KA14-1202). R–T: volval remnants on stipe base (KA14-1169). U: elements of partial veil (KA14-1202), V: volval remnants on pileus (KA14-1202). Scales bars: A–D = 5 cm, E–Q = 10  $\mu$ m, R–V = 40  $\mu$ m. Photos by: Jong Won Jo.

*Morphological studies:*—Macroscopic features were determined based on color photographs of fresh basidiomata and field notes. Color codes followed the color chart by the Royal Horticultural Society (2015). Microscopic features were observed from dried specimens after sectioning and mounting in 3% KOH solution. Melzer’s reagent was used to determine any amyloid reaction of the basidiospores, and 1% Congo Red was used for tissue staining. An Olympus BX53 (Tokyo, Japan) microscope was used for microscopic observation. Measurement of microscopic characteristics was performed with ProgRes Capture Pro v.2.8.8 (Jenoptik Co., Jena, Germany). The abbreviation [n/m/p] indicates ‘n’ basidiospores measured from ‘m’ basidiomata of ‘p’ collections. In describing basidiospore dimensions, we used the notation (a–)b–c(–d). The b–c range represents 95% of the measured values; extreme values (a, d) are given in parentheses; L’ and W’ are average basidiospore length and width; Q is the range of the length/width ratio for all measured basidiospores; Qm is the average Q value ± sample standard deviation. All voucher specimens were deposited at the herbarium of the Korea National Arboretum.

*DNA extraction, PCR and gene sequencing:*—Genomic DNA was extracted from tissues of basidiomata using a modified CTAB protocol (Doyle & Doyle 1987). Amplification of the nrLSU, *tefl-α* and *rpb2* sequence regions was performed using primer sets LR0R/LR5 (Vilgalys & Hester 1990), EF1-983F/EF1-1567R (Rehner & Buckley 2005), and Am-6F/Am-7R (Cai *et al.* 2014), respectively. The Am-b-tublin F/Am-b-tublin R (Cai *et al.* 2014) primer set was used to amplify *β-tubulin* only for KA14-1202 specimens. The protocol used for PCR amplification followed Cai *et al.* (2014). Subsequently, PCR products were sequenced by Macrogen Inc. (Seoul, Korea). New sequences generated in this study were deposited in GenBank (Table 1).

**TABLE 1.** Sequences of *Amanita* used in this study.

Species	Voucher	Locality	nrLSU	<i>rpb2</i>	<i>tefl-α</i>
<i>Amanita abrupta</i>	BW HP 101	USA	HQ539660	-	-
<i>A. abrupta</i>	NYBG:0013	N/A	KY432828	-	-
<i>A. avellaneifolia</i>	HKAS79891	China	MH486377	MH485871	MH508679
<i>A. avellaneifolia</i> (T)	HKAS80011	China	MH486378	MH485872	MH508680
<b><i>A. brunneofolia</i></b>	<b>KA19-0899</b>	<b>South Korea</b>	<b>MT385143</b>	<b>MT412396</b>	<b>MT412402</b>
<b><i>A. brunneofolia</i> (T)</b>	<b>KA19-0899-1</b>	<b>South Korea</b>	<b>MT385144</b>	<b>MT412397</b>	<b>MT412403</b>
<i>A. brunneostrobilipes</i>	HKAS96787	China	MH486416	MH485897	MH508704
<i>A. brunneostrobilipes</i>	HKAS101441	China	MH486414	MH485896	MH508702
<i>A. caojizong</i> (T)	HKAS79673	China	MH486429	MH485908	MH508714
<i>A. caojizong</i>	HKAS100615	China	MH486424	MH485904	MH508711
<b><i>A. caojizong</i></b>	<b>KA14-0819</b>	<b>South Korea</b>	<b>MT385140</b>	<b>MT412393</b>	<b>MT412400</b>
<b><i>A. caojizong</i></b>	<b>KA14-1169</b>	<b>South Korea</b>	<b>MT385141</b>	<b>MT412394</b>	<b>MT412401</b>
<b><i>A. castanopsis</i></b>	<b>KA14-1020</b>	<b>South Korea</b>	<b>MT385138</b>	<b>MT412391</b>	<b>MT412398</b>
<b><i>A. castanopsis</i></b>	<b>KA14-1568</b>	<b>South Korea</b>	<b>MT385139</b>	<b>MT412392</b>	<b>MT412399</b>
<i>A. cinereovelata</i> (T)	HKAS81647	Bangladesh	KP259291	KP259288	KP259289
<i>A. eijii</i>	HKAS57518	China	MH486483	MH485962	MH508760
<i>A. eijii</i>	HKAS81225	China	MH486483	MH485964	MH508762
<i>A. elliptica</i>	HKAS96797	China	MH486485	MH485966	MH508765
<i>A. flavofloccosa</i>	HKAS101443	China	MH486515	MH485986	MH508791
<i>A. griseofarinosa</i>	HKAS80926	China	MH486559	MH486025	MH508830
<i>A. kotohiraensis</i>	HKAS100577	China	MH486614	MH486068	MH508874
<i>A. luteofolia</i> (T)	HKAS81895	China	MH486633	MH486083	MH508890
<i>A. manginiana</i>	MHHNU30818	China	MH605436	MH614263	MH614264
<i>A. minutisquama</i> (T)	HKAS100504	China	MH486644	MH486095	MH508902
<i>A. minutisquama</i>	HKAS101424	China	MH486645	MH486096	MH508903

.....continued on the next page

**TABLE 1.** (Continued)

Species	Voucher	Locality	nrLSU	<i>rpb2</i>	<i>tefl-α</i>
<i>A. modesta</i>	HKAS78433	China	MH486650	MH486099	MH508907
<i>A. modesta</i>	HKAS100505	China	MH486647	MH486098	MH508904
<i>A. neo-ovoidea</i>	HKAS84931	China	MH486655	MH486105	MH508912
<i>A. neo-ovoidea</i>	HKAS100506	China	MH486654	MH486104	MH508911
<i>A. oberwinklerana</i>	HKAS85832	China	MH486681	MH486118	MH508928
<i>A. oberwinklerana</i>	HKAS100611	China	MH486666	MH486113	MH508923
<i>A. pallidochlorotica</i> (T)	HKAS77280	China	MH486730	MH486158	MH508964
<i>A. pseudomanginiana</i> (T)	HKAS83470	China	MH486772	MH486187	MH508999
<i>A. pseudoporphyria</i>	HKAS56984	China	KJ466450	KJ466614	KJ481953
<i>A. pseudoporphyria</i>	HKAS57356	China	KJ466451	KJ466613	KJ481952
<i>A. pyramidata</i> (T)	HKAS87943	China	MH486795	MH486209	MH509021
<i>A. roseolifolia</i> (T)	HKAS101403	China	MH486807	MH486219	MH509032
<i>A. rubiginosa</i> (T)	HKAS52216	China	MH486817	MH486229	MH509045
<i>A. rubiginosa</i>	HKAS56959	China	MH486818	MH486230	MH509046
<b><i>A. sphaerobulbosa</i></b>	<b>KA14-1202</b>	<b>South Korea</b>	<b>MT385142</b>	<b>MT412395</b>	-
<i>A. timida</i>	HKAS89001	China	MH486932	MH486325	MH509149
<i>A. vestita</i>	HKAS101444	China	MH486941	MH486335	MH509160
<i>A. virgineoides</i>	HKAS77278	China	MH486945	MH486340	MH509166
<i>A. virgineoides</i>	HKAS100518	China	MH486944	MH486339	MH509165
<i>A. vittadini</i>	HKAS101430	Italy	MH486950	MH486342	MH509169

<sup>a</sup>T: Type specimen. Newly generated sequences and new species are indicated in bold. N/A = no information/sequence available

*Phylogenetic analysis*:—We compiled two datasets based on nrLSU sequences and a concatenated dataset (nrLSU, *rpb2* and *tefl-α*). The representative species of *A. sect. Roanokenses* were selected to reconstruct phylogenetic trees. They were aligned and edited using ClustalX 1.81 (Thompson *et al.* 1997) and PHYDIT (Chun 1995).

We performed parsimony heuristic tree searches in PAUP\* v. 4.0b10 (Swofford 2002) with parameters set as 1000 random addition sequence replicates, tree bisection and reconnection (TBR) branch-swapping. One thousand bootstrap replicates were calculated to evaluate the branch support values (MPBS) of the MP tree.

Bayesian Inference (BI) was executed in MrBayes v. 3.2.2 (Ronquist & Huelsenbeck 2003). The best model for BI analysis was determined using jModelTest2 software (Darriba *et al.* 2012) based on Akaike Information Criterion (AIC). The GTR+I+G model was selected for both datasets. Posterior probabilities (PP) were calculated using the Metropolis-coupled Markov Chain Monte Carlo method. We performed two runs in parallel with one cold and three heated chains for  $3 \times 10^6$  generations starting with a random tree and sampling every 100<sup>th</sup> generation. We tested the level of convergence of two independent runs to remove all trees that did not converge as per average standard deviation of the split frequencies below 0.01 using the burn-in command. Then, all trees, which did converge, were used to calculate a 50% majority consensus-tree and to estimate posterior probability (PP).

Two species from *Amanita* sect. *Lepidella*, namely *A. vittadini* (Moretti) Vittad. (1826: 31) and *A. flavofloccosa* Nagas. & Hongo (1984: 367) were selected as outgroups. Based on previous studies by Wolfe *et al.* (2012), Cai *et al.* (2014), Hosen *et al.* (2015), and Cui *et al.* (2018), we retrieved reference sequences to construct the phylogenetic trees (Table 1).

## Results

**Molecular analysis:**—The nrLSU dataset included 45 taxa and 573 characters, of which 191 were parsimony-informative. The MP tree was 879 steps long with consistency index (CI) = 0.4266, retention index (RI) = 0.6340, and homoplasy index (HI) = 0.5734. Bayesian analyses were conducted with a GTR+I+G model and the first 9,000 trees were discarded as burn-in (burninfrac = 0.30). The combined dataset (nrLSU, *rpb2* and *tef1- $\alpha$* ) included 42 taxa and 1655 characters, of which 598 were parsimony-informative. The MP tree was 2498 steps long with a CI = 0.4291, RI = 0.6208, and HI = 0.5709. The Bayesian analyses were conducted with a GTR+I+G model and the first 7,500 trees were discarded as burn-in (burninfrac = 0.25).

The topologies from BI and MP trees of both combined and nrLSU datasets were similar. In addition, the phylogenetic trees of two datasets in this study showed a similar main branch topology as reported previously (Cui *et al.* 2018). Based on both nrLSU and the combined dataset (nrLSU, *rpb2* and *tef1- $\alpha$* ) analyses, the new species (*A. brunneofolia*) formed a monophyletic clade with high support values (MPBS/PP = 100/1.0) and was clearly separated from other taxa (Figs. 1–2).

Our two collections (KA14-0819 and KA14-1169) were clustered in high bootstrap support values (MPBS/PP = 100/1.0) together with Chinese type collection (HKAS79673), *A. caojizong* Zhu L. Yang, Yang-Yang Cui & Qing Cai (2018: 138) (Figs. 1–2). KA14-1020 and KA14-1568 specimens, belonging to *A. castanopsis* Hongo (1974: 192), were clearly separated from other taxa (Figs. 1–2).

In the nrLSU phylogenetic analyses, the KA14-1202 specimen formed a sister clade with *A. abrupta* Peck (1897: 138) (BW HP 101 and NYBG:0013) in low bootstrap support values (MPBS/PP = 55/0.94) (Fig. 1) but the  $\beta$ -tubulin sequence of the specimen was identical to that recognized as *A. sphaerobulbosa* Hongo (1969: 230) in Cui *et al.* (2018).

## Taxonomic description

*Amanita brunneofolia* J.W. Jo, H.S. Kim, Y.-N. Kwag & C.S. Kim, *sp. nov.* (Fig. 3)

Mycobank: MB 835444

**Diagnosis:**—Characterized by a yellowish white to greenish white pileus with brownish floccose pyramidal volval remnants; brownish lamellae; a long radicate stipe; a fragile and fugacious, greenish white partial veil; ellipsoid to elongate, amyloid basidiospores; clamps present in all parts of the basidioma. Associated with *Quercus acutissima*.

**Type:**—SOUTH KOREA. Incheon-si: Ganghwa-gun, Ganghwa-eup, 37° 44' 44.16" N, 126° 26' 53.90" E, elev. 163 m, under *Quercus acutissima*, 30 August 2019, JO 190315 (KA19-0899-1, holotype!), GenBank accession numbers: MT385144 (nrLSU), MT412397 (*rpb2*), MT412403 (*tef1- $\alpha$* ).

**Etymology:**—‘brunneofolia’, from brunneus = brown, and folia = leaf, referring to the characteristic brown lamellae of the species.

**Description:**—*Basidioma* medium- to large-sized. *Pileus* 8–13 cm diam, hemispherical to convex when young, then appanate when mature, sometimes slightly umbonate; yellowish white (NN155A) to greenish white (155C); volval remnants on pileus floccose to pulverulent, pyramidal, subconical at center, 1–2 mm high, 1–1.5 mm wide, and becoming smaller toward the margin, pale yellow (165D) to brownish orange (N167B–D); margin non-striate, appendiculate; context greenish white (157C–D), unchanging. *Lamellae* narrowly adnexed, close to crowded; reddish brown (174A–B), tissues changing to greenish in 3% KOH; lamellulae attenuated, of diverse lengths. *Stipe* 15–20 cm long, 2–4 cm thick; subcylindrical or slightly tapering upwards, with apex slightly expanded, yellowish white (NN155A) to greenish white (157D); context solid, greenish white (157A–C), tissues changing to greenish in 3% KOH; basal bulb fusiform to long clavate, radicate, 10–15 cm long, 2–4.5 cm thick, densely covered with appressed to recurved scales arranged in concentric rings. *Annulus* present, apical, yellowish white (NN155A) to greenish white (157D), fragile and fugacious.

*Lamella trama* bilateral. *Mediostratum* 20–45  $\mu$ m wide, composed of abundant subfusiform, ellipsoid to elongate inflated cells, 50–30  $\times$  15–35  $\mu$ m; filamentous hyphae abundant, 3–9  $\mu$ m wide; vascular hyphae scarce, 4–11  $\mu$ m wide. *Lateral stratum* composed of abundant subfusiform to ellipsoid inflated cells, 30–75  $\times$  10–20  $\mu$ m, diverging at an angle of ca. 25° to 40° to mediostratum; filamentous hyphae abundant and 3–7  $\mu$ m wide. *Subhymenium* 15–45  $\mu$ m thick, with 1–3 layers of subglobose, ovoid to ellipsoid, sometimes irregularly shaped cells, 7–25  $\times$  7–15  $\mu$ m. *Basidia* [n = 40] 35–56  $\times$  8–14  $\mu$ m, clavate, 4-spored, sterigmata 3–6  $\mu$ m long; clamps present at the base of basidia. *Basidiospores*

[120/4/2] (7.8–)8.2–9.6(–11.4) × (4.8–)5.2–5.9(–6.4) μm, [L' = 8.9 μm, W' = 5.5 μm, Q = (1.45–)1.48–1.75(–1.93), Qm = 1.61 ± 0.09, ellipsoid to elongate, amyloid, thin-walled, colorless; apiculus small. *Lamellar edge* appearing as a sterile strip, composed of subglobose to sphaeropendunculate inflated cells, 18–30 × 10–19 μm, terminal, single or 2–3 in chains, thin-walled, colorless. *Pileipellis* 150–260 μm thick, 1 layer, gelatinized, composed of radially to somewhat interwoven, thin-walled, yellowish brown, filamentous hyphae 2.5–6.5 μm wide; vascular hyphae scarce, 5–10 μm wide. *Volval remnants on pileus* composed of vertically to somewhat irregularly arranged elements: filamentous hyphae scarce, 2–8 μm wide, colorless to yellowish brown, thin-walled, branching, anastomosing; inflated cells very abundant, globose, subglobose, ellipsoid, obovoid, 35–60 × 20–45 μm, colorless to brownish, thin-walled, terminal or in chains of 2–3; vascular hyphae rare, 3–6 μm wide. *Stipe trama* composed of longitudinally arranged, long clavate, terminal cells, 70–330 × 10–40 μm; filamentous hyphae abundant, 2–7 μm wide, branching, anastomosing; vascular hyphae scarce, 3–8 μm wide. *Annulus* primarily composed of inflated cells, subglobose, subfusiform to ellipsoid, obovoid, 30–75 × 20–35 μm, colorless to brownish, thin-walled; filamentous hyphae scarce, 1.5–4.5 μm wide, colorless to yellowish brown, thin-walled, branching, anastomosing; vascular hyphae scarce, 3–9 μm wide. Clamps present in all parts of basidioma.

Habitat:—Solitary or gregarious in broad-leaved forests under *Q. acutissima*.

Distribution:—Only known from South Korea.

Edibility:—Unknown.

Additional specimens examined:—SOUTH KOREA. Incheon-si: Ganghwa-gun, Ganghwa-eup, 37° 44' 43.02" N, 126° 26' 53.75" E, elev. 161 m, under *Quercus acutissima*, 29 August 2019, JO190310 (KA19-0899).

***Amanita caojizong*** Zhu L. Yang, Y.Y. Cui & Q. Cai, in Cui, Cai, Tang, Liu & Yang, *Fungal Diversity* 91: 138, 2018 (Fig. 4)

Description:—*Basidioma*, medium- to large-sized. *Pileus* 7–13 cm diam, convex to applanate, sometimes depressed at the center, gray-brown (N199A–C) to brownish (200D), darker at the center, innately fibrillose, occasionally covered with white (NN155D) membranous volval remnants; margin non-striate, appendiculate; context white (NN155D), unchanging. *Lamellae* free to adnexed, crowded, white (NN155D) to cream-colored (NN155A–B); *lamellulae* attenuate, of diverse lengths, plentiful. *Stipe* 7–13 cm long, 1.5–2.0 cm thick; slightly tapering toward apex, white (NN155D), covered with fibrillose white (NN155D) squamules; basal bulb ellipsoid to clavate, 2–2.5 cm thick; *volval remnants on stipe base* limbate, membranous, white (NN155B–D). *Annulus* present, apical, white (NN155D), radially striate above, often torn from stipe during expansion of pileus, fragile and fugacious.

*Lamella trama* bilateral. *Subhymenium* 18–35 μm thick, with 2–3 layers of subglobose, broadly ellipsoid to ovoid, sometimes irregularly shaped cells, 7–25 × 6–12 μm. *Basidia* [n = 40] (27–)30–44 × 7–11 μm, clavate, 4-spored; sterigmata [n = 25] 2.1–3.7 μm long; clamps absent at the base of basidia. *Basidiospores* [80/2/1] (6.2–)6.8–8.5(–9.0) × (4.7–)5.0–6.0(–6.5) μm [L' = 7.6 μm, W' = 5.4 μm, Q = (1.17–)1.21–1.53(–1.60), Qm = 1.40 ± 0.09], broadly ellipsoid to ellipsoid, thin-walled, colorless, hyaline, amyloid, apiculus small. *Volval remnants on stipe base* filamentous hyphae abundant, 3–9 μm wide, branching, anastomosing, colorless, thin-walled; inflated cells scarce to scattered, subglobose, ellipsoid to obovoid, clavate, 40–80 × 15–50 μm. *Annulus* filamentous hyphae 3–7 μm wide, branching, colorless, thin-walled; inflated cells abundant, subglobose, ellipsoid to obovoid, 20–70 × 12–30 μm; vascular hyphae scarce, 8–10 μm wide. Clamps absent in all parts of basidiomata.

Habitat:—Solitary in broad-leaved forest with *Quercus* spp.

Distribution:—China, Thailand (Cui *et al.* 2018) and now South Korea.

Edibility:—Edible.

Examined specimens:—SOUTH KOREA. Gyeonggi-do, Pocheon-si, Gwangneung forest, 34° 45' 20.54" N, 127° 09' 11.41" E, elev. 340 m, 12 August 2014, JO 140205 (KA14-0819); Gyeongsangnam-do, Namhae-gun, Changsunmyeon, Mt. Daebang, 34° 52' 10.49" N, 127° 59' 07.92" E, elev. 250 m, 27 August 2014, JO 140270 (KA14-1169).

***Amanita sphaerobulbosa*** Hongo, J. Jap. Bot. 44: 230, 1969 (Fig. 4).

Description:—*Basidioma* small- to medium-sized. *Pileus* 4–7 cm diam, convex to applanate, white (NN155D) to whitish (NN155B), volval remnants on pileus pyramidal to subpyramidal, whitish (NN155B) to yellowish white (NN155A); margin non-striate, appendiculate; context white (NN155D), unchanging. *Lamellae* free, crowded, white (NN155D) to pale cream-colored (N155A); *lamellulae* attenuate, of diverse lengths. *Stipe* 5–9 cm long, 0.7–1.2 cm thick; slightly tapering toward apex, white (NN155D) to pale cream-colored (N155A); basal bulb abrupt to sub-abrupt,

2–3 cm thick, upper part covered with whitish (NN155B–C), verrucose volval remnants. *Annulus* apical, membranous, skirt-like, white (NN155C–D), radially striate above.

*Lamella trama* bilateral. *Subhymenium* 14–38  $\mu\text{m}$  thick, with 2–3 layers of globose to subglobose, broadly ellipsoid to ovoid, doliiform, sometimes irregularly shaped cells, 10–18  $\times$  8–14  $\mu\text{m}$ . *Basidia* [ $n = 40$ ] 45–59  $\times$  10–13  $\mu\text{m}$ , clavate, 4-spored; sterigmata [ $n = 20$ ] 2.1–5.0  $\mu\text{m}$  long; clamps present at the base of basidia. *Basidiospores* [75/2/1] (7.3–)7.6–9.4(–10.0)  $\times$  (6.7–)7.0–8.7(–9.5)  $\mu\text{m}$  [ $L' = 8.4 \mu\text{m}$ ,  $W' = 7.8 \mu\text{m}$ ,  $Q = (1.0\text{--})1.03\text{--}1.17(–1.31)$ ,  $Q_m = 1.08 \pm 0.06$ ], globose to subglobose, occasionally broadly ellipsoid, thin-walled, colorless, hyaline, amyloid, apiculus small. *Volval remnants on pileus* filamentous hyphae 4–8  $\mu\text{m}$ , abundant, branching, anastomosing; vascular hyphae 5–10  $\mu\text{m}$  wide; inflated cells very abundant, terminal, 2–4 in chains, subglobose, broadly ellipsoid to ellipsoid, 15–51  $\times$  11–30  $\mu\text{m}$ . *Annulus* filamentous hyphae 4–7  $\mu\text{m}$  wide, branching, anastomosing, colorless, clamps not observed; inflated cells abundant, clavate to broadly clavate, 25–47  $\times$  14–22  $\mu\text{m}$ ; vascular hyphae not observed.

Habitat:—Solitary in broad-leaved forest with *Quercus* spp.

Distribution:—China (Cui *et al.* 2018), Japan (Hongo 1969), and now South Korea

Edibility:—Unknown.

Examined specimens:—KOREA. Gyeonggi-do, Pocheon-si, Gwangneung forest, 34° 45' 04.48" N, 127° 09' 04.36" E, elev. 275 m, 27 August 2014, JO140290 (KA14-1202).

## Discussion

*Amanita brunneofolia* is treated as a new species based on morphological and molecular evidences. This new species is characterized by its greenish white pileus with floccose to pulverulent brownish volval remnants, reddish brown lamella, fragile and fugacious annulus, appressed to recurved scales on stipe base, long radicating stipe and ellipsoid to elongate amyloid basidiospores. Molecular data based on both nrLSU and combined (nrLSU, *rpb2*, and *tef1- $\alpha$* ) datasets showed that the sequences from South Korea specimens fall within *Amanita* sect. *Roanokenses* and are clearly separated from other related taxa (Figs. 1–2).

Morphologically, *A. brunneofolia* is similar to *A. sculpta* Corner & Bas (1962: 255) and *A. westii* (Murrill) Murrill (1945: 127). These two species are characterized by brownish pyramidal to conical volval remnants on pileus and brown to pinkish lamellae (Sanmee *et al.* 2008, Cui *et al.* 2018). However, *A. sculpta* differs from *A. brunneofolia* in its larger volval remnants on the pileus, white to brownish context, context changing color when injured, a shorter stipe base, white annulus, basidia without a basal clamp and globose to subglobose basidiospores: (8–)9–11(–11.5)  $\times$  (8–)9–10.5(–11)  $\mu\text{m}$ , with  $Q = 1\text{--}1.04(–1.08)$  (Yang 2005, 2015, Sanmee *et al.* 2008). Although there were no available sequences to determine the phylogenetic position of *A. sculpta*, the morphological differences between *A. brunneofolia* and *A. sculpta* were clear (Corner & Bas 1962). *Amanita westii* is distinguished from *A. brunneofolia* by having a darker pileus, a shorter stipe base, context changing colour when bruised, basidia without basal clamp, larger and longer basidiospores: (8.5–)10.2–14.0(–15.5)  $\times$  (5.8–)6.0–7.8(–8.2)  $\mu\text{m}$ , with  $Q = (1.42\text{--})1.50\text{--}1.99(–2.18)$  (Jenkins 1986, Tullos & Lewis 1994). As regards the color of lamellae, *A. brunneofolia* is comparable to *A. boudieri* var. *beillei* (Beauseign.) Neville & Poumarat (1996: 21) and *A. microlepis* Bas (1969: 424). However, *A. boudieri* var. *beillei* differs from *A. brunneofolia* by its whitish basidioma, a shorter stipe base, basidia without basal clamp, and longer basidiospores: 10–14  $\times$  5–7.5(–8)  $\mu\text{m}$ , with  $Q = (1.60\text{--})1.70\text{--}2.60$  (Neville & Poumarat 2004). *Amanita microlepis* is distinguished from *A. brunneofolia* by having a cream to brownish cream or whitish pileus, a shorter stipe base, a felted-membranous annulus, larger basidiospores: 9–11  $\times$  (6–)6.5–8  $\mu\text{m}$  (Bas 1969). Therefore, molecular and morphological data confirmed *A. brunneofolia* as a new species of sect. *Roanokenses*.

Most *Amanita* species are known to be ectomycorrhizal fungi (Wolfe *et al.* 2012, Tedersoo & Brundrett 2017). *Amanita brunneofolia* occurs in the proximity of *Quercus acutissima* trees. Therefore, the species might be ectomycorrhizal associated with *Q. acutissima*. In addition, some species of *Amanita* sect. *Roanokenses*, such as *A. gymnopus* Corner & Bas (1962: 259), *A. kotohiraensis* Nagas. & Mitani (2000: 93), *A. neoovoidea* Hongo (1976: 57), *A. oberwinklerana* Zhu L. Yang & Yoshim (1999: 120) and *A. pseudoporphyria* Hongo (1957: 141) are poisonous (Tullos *et al.* 1992, Iwafuchi *et al.* 2003, Chen *et al.* 2014, Fu *et al.* 2017), whereby this new species warrants further study to elucidate its toxicity.

*Amanita caojizong* is a recently described species from China (Cui *et al.* 2018), characterized by gray-brown pileus, and limbate volva on the stipe base. This species is closely related to *A. pseudoporphyria*. However, the latter species has relatively longer basidiospores: (6.0–)7.0–9.0(–10.0)  $\times$  4.5–6.0  $\mu\text{m}$ , with  $Q = (1.17\text{--})1.27\text{--}1.7(–1.82)$ , and

volval remnants on the stipe base consisting of abundant inflated cells (Yang 2000b, Bhatt *et al.* 2007, Cui *et al.* 2018). Two Korean *Amanita* specimens (KA14-0819 and KA14-1169) clustered with high bootstrap support values (MPBS/PP = 100/1.0) together with the Chinese type collection (HKAS79673) of *A. caojizong*, and were almost identical morphologically (Figs. 1–2). Therefore, our specimens were identified as *A. caojizong*, based on morphological and molecular data.

*Amanita sphaerobulbosa* was described by Hongo (1969) from Japan, and then reported for China (Yang 2015). This species is quite similar to *A. abrupta*. However, *A. sphaerobulbosa* can be distinguished from *A. abrupta* by its globose to subglobose basidiospores [from type material: (7.0–)8.0–10.0 × (7.0–)7.5–9.0(–9.5) μm], and volval remnants on the pileus consisting of many inflated cells and vascular hyphae (Yang 2015, Cui *et al.* 2018) (Fig. 4). The  $\beta$ -*tubulin* sequence generated from our specimen (GenBank Accession number: MT412404) was identical to the Chinese collection (MH485789). Herein, for the first time, we have also provided nrLSU and *rpb2* sequences of *A. sphaerobulbosa* (Table 1).

*Amanita castanopsis* was originally described from Japan. We have not described this species in this study because *A. castanopsis* was reported South Korea by Kim (1991). According to Cui *et al.* (2018), *A. castanopsis* also belongs to sect. *Roanokenses*, however, they did not conduct phylogenetic studies due to the lack of DNA sequences of this species. However, our nrLSU and multi-locus phylogenetic studies supported the concept of Cui *et al.* (2018) i.e., *A. castanopsis* is a member of sect. *Roanokenses* (Figs. 1–2).

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