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Lethal *Amanita* species in China

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Abstract: Lethal amanitas (*Amanita* sect. *Phalloideae*) cause many casualties worldwide. Recent molecular phylogenetic studies revealed diverse lethal *Amanita* spp. in China. Here a 5-gene phylogeny (nuc rDNA region encompassing the internal transcribed spacers 1 and 2 with the 5.8S rDNA, the D1–D3 domains of nuc 28S rDNA, and partial RNA polymerase II second largest subunit, translation elongation factor 1-α and β-tubulin genes) is used to investigate the phylogenetic lineages and species delimitation in this section. Thirteen species are recognized, including four new species, namely *A. griseorosea*, *A. molliuscula*, *A. parviexitialis*, and *A. subfuliginea*. They are documented with morphological, multigene phylogenetic, and ecological evidence, line drawings, and photographs and compared with similar species. A key to the Chinese lethal *Amanita* species is provided.

Key words: amatoxins, geographic distributions, poisonous mushrooms, species characterization

Introduction

Lethal *Amanita* species are deadly poisonous mushrooms classified in *Amanita* sect. *Phalloideae* (Fr.) Quél., characterized by a non-striate and non-appendiculate pileus, attenuate lamellulae, the persistent presence of an annulus, a bulbous stipe base with a limbate volva and amyloid basidiospores (Corner and Bas 1962; Bas 1969; Tulloss et al. 1992; Yang 1997, 2005, 2015). From 1994 to 2012, more than 90% of fatal mushroom poisonings were caused by ingestion of lethal amanitas in Australia, East Asia and North America, partly a result of the similarities between edible and poisonous amanitas, for example, *A. chepangiana* Tulloss & Bhandary (edible) vs. *A. exitialis* Zhu L. Yang & T.H. Li (poisonous) (Unluoglu and Tayfur 2003, Roberts et al. 2013, Ward et al. 2013, Chen et al. 2014). Investigations over the last 15 y in Guangdong Province, China indicated that *A. exitialis* caused at least 80 poisoning cases including 32 fatalities (Deng et al. 2011, Chen 2014, Chen et al. 2014, Li et al. 2014b). The toxins in lethal amanitas are chiefly amatoxins, phallotoxins, and virotoxins. Most of these cyclic peptide toxins are chemically stable and resistant to high temperatures, including cooking, and consequently, consumption of species containing these toxins results in serious liver and kidney damage (Wieland 1973, 1986; Chen et al. 2002; Li et al. 2014a, b).

To date, about 50 lethal *Amanita* spp. have been described worldwide (Corner and Bas 1962; Jenkins 1986; Tulloss 1989; Tulloss et al. 1992; Tulloss et al. 1995b; Wood 1997; Yang 1997, 2005, 2015; Oda et al. 2002; Simmons et al. 2002; Neville and Poumarat 2004; Zhang et al. 2010; Li et al. 2015). Recent molecular phylogenetic studies showed that the species diversity of lethal amanitas was seriously underestimated in East Asia (Zhang et al. 2010, Cai et al. 2014, Yang 2015). With multigene phylogenetic studies, Cai et al. (2014) revealed 28 phylogenetic species of lethal amanitas, including 14 putatively new species, six from East Asia, five from North America, and one each from Central America, South Asia and Oceania, of which "*Amanita* sp. 2" in Cai et al. (2014) was subsequently described as *A. subpallidorosea* Hai J. Li (Li et al. 2015).

The aims of this study are: (i) to delimitate and characterize the Chinese lethal *Amanita* spp.; (ii) to document the new Chinese species using morphological characters, molecular evidence, and ecological data; and (iii) to provide a key for identification.

Materials and methods

Morphological study.—Specimens were collected from tropical, subtropical, and temperate parts of China and deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS); the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU); the Herbarium of Microbiology Institute of Guangdong (GDGM); and the Mycological Herbarium of Microbiology Institute, Chinese Academy of Sciences (HMAS). Color codes indicated in the descriptions are from Kornerup and Wanscher (1981). Macroscopic characters are described from fresh basidiomata, detailed field notes, and photographs. Microscopic structures were observed with light microscopy using dried material revived

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in 5% KOH, dyed with Congo Red when necessary. Basidiospores were mounted in Melzer's reagent to test for amyloidy. In the descriptions of the basidiospores, the abbreviation (n/m/p) means n basidiospores measured from m basidiomata of p collections. Dimensions for basidiospores are given using a range notation of the form (a–) b–c (–d). The range b–c contains a minimum of 90% of the measured values. Extreme values (a, d) are given in parentheses. Q = length/width ratio of a basidiospore in side view. Qm = average Q of all basidiospores measured ± sample standard deviation. The statistic analysis of the measurements for the basidiospores was conducted with SPSS 16.0. The specimens examined for *A. exitialis*, *A. fuliginea* Hongo, *A. fuligineoides* P. Zhang & Zhu L. Yang, *A. pallidorosea* P. Zhang & Zhu L. Yang, *A. rimosa* P. Zhang & Zhu L. Yang, *A. subjunquillea* S. Imai, *A. subpallidorosea*, and *A. virosa* Bertill. are listed (SUPPLEMENTARY TABLE I).

Molecular phylogenetics.—Methods for genomic DNA extraction, PCR amplification, and sequencing followed those of Cai et al. (2014) and references therein. Ten sequences (KT971342–KT971347, KU168386–KU168389) were newly generated for nuc rDNA region encompassing the internal transcribed spacers 1 and 2 with the 5.8S rDNA (ITS), the D1–D3 domains of nuc 28S rDNA (28S), and partial sequences of the RNA polymerase II second largest subunit (*RPB*2), translation elongation factor 1-α (*TEF*1), and β-tubulin genes (*BenA*). The universal primer pairs ITS1/ITS4 and ITS1F/ITS4 (White et al. 1990, Gardes and Bruns 1993), LROR/LR5 (Vilgalys and Hester 1990) and 983F/1567R (Rehner and Buckley 2005) were used for the amplification and sequencing of ITS, 28S and *TEF*1, respectively. *RPB*2 and *BenA* sequences were generated from amplifications using primers Am-6 F/Am-7 R and Am-β-tubulin F/Am-β-tubulin R (Cai et al. 2014). These new sequences were combined with representatives of lethal *Amanita* spp. shown in Zhang et al. (2010), Cai et al. (2014) and Li et al. (2015) (SUPPLEMENTARY TABLE II). Multiple sequences alignments were performed with MUSCLE 3.8.31 (Edgar 2004) and manually checked with BioEdit 7.0.9 (Hall 1999). The resulting alignments can be accessed at TreeBASE with submission ID 18928.

To test for potential conflicts among the five gene fragments, maximum likelihood (ML) analyses and Bayesian Inference (BI) were performed on each individual matrix with the same setting as in the concatenated analysis. Because no conflicts were detected (e.g. well-supported differences in the topology, SUPPLEMENTARY FIGS. 1–5), the five gene fragments were combined for phylogenetic analysis. *Amanita zangii* and *A.* sp. HKAS77321 were selected as outgroups. The phylogenetic tree of the combined dataset was constructed with both ML and BI analyses conducting on RAxML 7.2.6 (Stamatakis 2006) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with the parameters employed by Cai et al. (2014).

RESULTS

Morphological data.—125 specimens were examined, including 103 collections from East and South Asia, 16 collections from North America, five collections from Europe and one collection from Australia. They represented 21 taxa of lethal *Amanita* spp. Thirteen occur in China, including eight previously known species and five novel species, four described as new taxa below, and the remaining one to be formally described later because of a paucity of material.

Phylogenetic data.—The combined dataset (ITS, 28S, *TEF*1, *RPB*2, and *BenA*), in which the aligned lengths of the 5-gene loci were 747, 881, 577, 680, and 388 bp, respectively, contained 853 parsimony informative sites. According to the genealogical concordance phylogenetic species recognition (GCPSR) criterion (Taylor et al. 2000), 16 phylogenetic lineages could be recognized (FIG. 1, see also SUPPLEMENTARY FIGS. 1–5). However, there are subtle morphological and anatomical differences between each sister lineage recognized as *A. pallidorosea* and *A. fuliginea*. The sister lineages were thus defined as the same species, respectively. In addition, the remaining seven lineages with only one specimen, for example, *A. bisporigera*, *A. ocreata*, and *A.* sp. 8, were delimitated based on the fact that they were phylogenetically and morphologically distinct from their sister groups. Therefore, 21 phylogenetic species were ultimately accepted in this study, and 13 of them from China, corresponding well to the morphological circumscriptions (FIG. 1).

TAXONOMY

Amanita exitialis Zhu L. Yang & T.H. Li, Mycotaxon 78:439. 2001. FIG. 2a

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by Fagaceae; basidiomata occurring from early spring to summer; presently known in southern and southwestern China.

Commentary: It is noteworthy that *A. exitialis* forms basidiomata in early spring to early summer in Guangdong but in middle to late summer in Yunnan. *Amanita exitialis* is phylogenetically close to *A. suballiacea* (Murrill) Murrill, but the latter differs from the former by its 4-spored basidia and broadly ellipsoidal to ellipsoidal basidiospores (Murrill 1941, Jenkins 1979). Both *A. bisporigera* G.F. Atk. and *A. parviexitialis* (see below) also have white basidiomata and 2-spored basidia. However, *A. bisporigera*, a species originally described from North America, has a superior, skirt-like, lower annulus, smaller basidiospores and more abundant inflated cells in the volval remnants on the stipe base (Lewis 1906; Jenkins 1986; Tulloss et al. 1995a; Yang and Li 2001; Yang 2005, 2015); *A. parviexitialis* has smaller basidiomata and basidiospores (see commentary under *A. parviexitialis*).

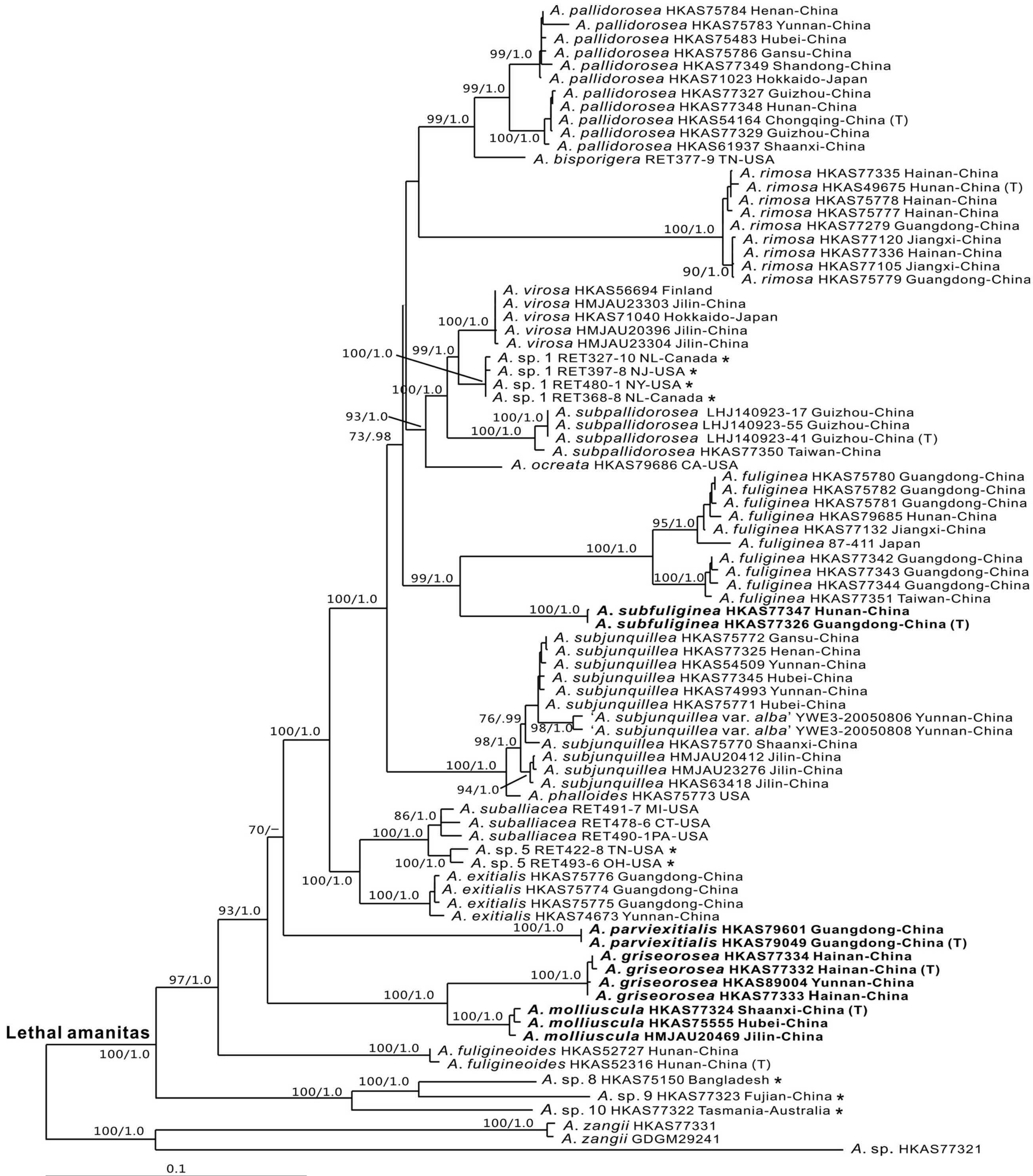

FIG. 1. Phylogenetic tree of lethal amanitas generated from the combined dataset (ITS, 28S, *TEF*1, *RPB*2, and *BenA*) by using RaxML and MrBayes. Bootstrap values over 50% and Bayesian posterior probabilities over 0.90 are reported on branches. Sequences from type collections are indicated with (T) and new species are shown in boldface. Names marked with asterisks were used in Cai et al. (2014).

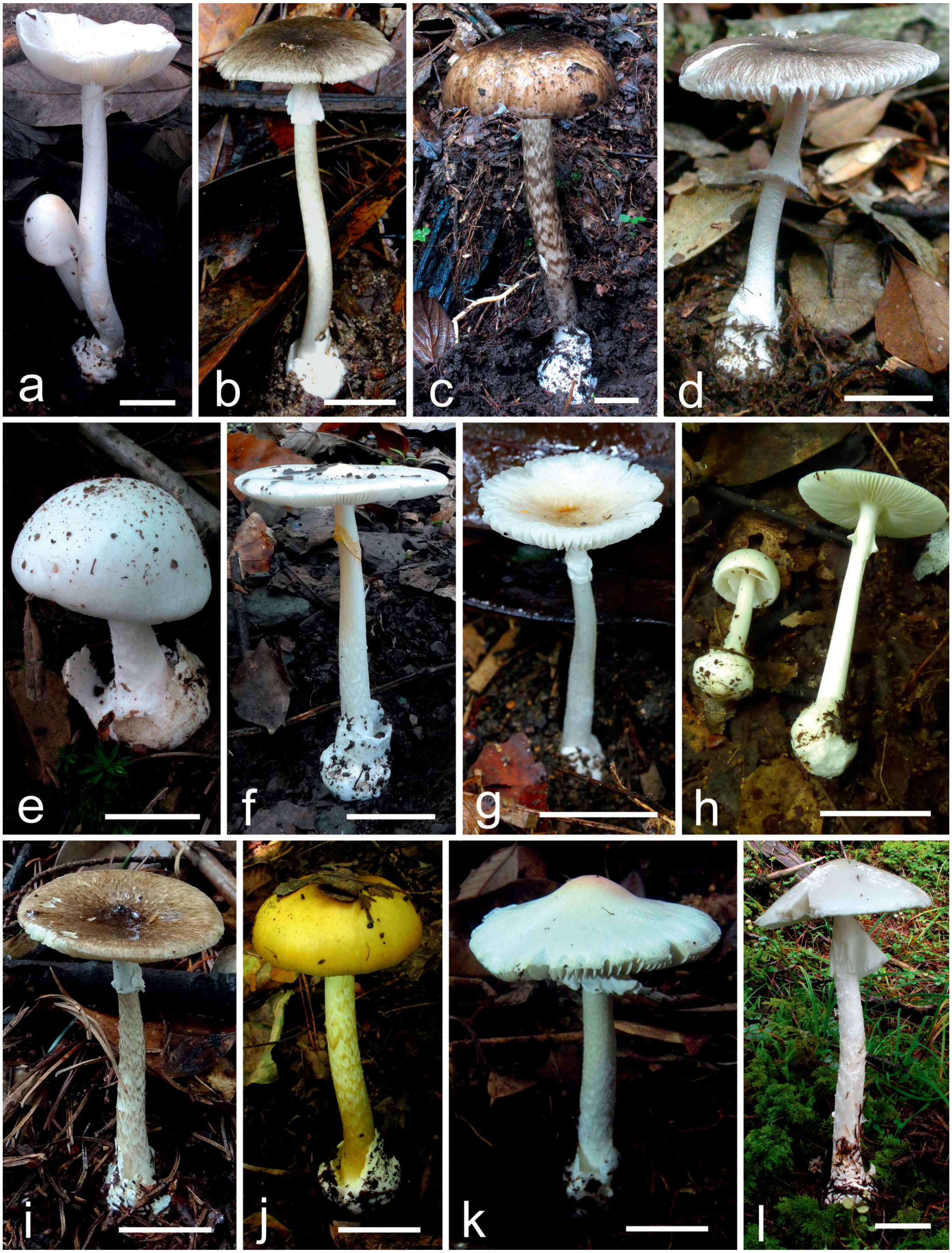

FIG. 2. Basidiomata of the 12 lethal *Amanita* species in China. a. *A. exitialis* (HKAS 90175). b. *A. fuliginea* (HKAS 78472). c. *A. fuligineoides* (HKAS 83694). d. *A. griseorosea* (HKAS 77332). e. *A. molliuscula* (HKAS 75555). f. *A. pallidorosea* (HKAS 75783). g. *A. parviexitialis* (HKAS 79049). h. *A. rimosa* (HKAS 77120). i. *A. subfuliginea* (HKAS 77326). j. *A. subjunquillea* (HKAS 90778). k. *A. subpallidorosea* (HKAS 77350). l. *A. virosa* (HKAS 90176). Scale bars = 2 cm.

Amanita fuliginea Hongo, J. Jpn. Bot. 28:69. 1953. FIG. 2b

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by Fagaceae; basidiomata occurring from late spring to summer; presently known in Japan and eastern, southern, central, and southwestern China.

Commentary: Amanita fuliginea is very similar and closely related to *A. subfuliginea*, a species described below. Diagnosis characters of these two species are discussed with the commentary on *A. subfuliginea.*

Amanita fuligineoides P. Zhang & Zhu L. Yang, Fungal Divers. 42:122. 2010. FIG. 2c

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by Fagaceae; basidiomata occurring from summer to autumn; presently known in central and southwestern China.

Commentary: Morphologically, *A. fuligineoides* is very similar to *A. fuliginea* and *A. subfuliginea.* However, the close phylogenetic relationships between *A. fuligineoides* and the latter two species are not supported in our analysis (FIG. 1). Furthermore, *A. fuliginea* has a smaller basidioma, a globose to subglobose stipe base, and the volval remnants on the stipe base composed of more inflated cells (Hongo 1953; Yang 2005, 2015; Zhang et al. 2010). The differences between *A. fuligineoides* and *A. subfuliginea* can be summarized by the size of basidioma, shape of the stipe base and form of the basidiospores (see *Commentary* below **Amanita subfuliginea**).

Amanita griseorosea Qing Cai, Zhu L. Yang & Yang-Yang Cui, sp. nov. FIGS. 2d, 3

MycoBank MB815034

Typification: HAINAN PROVINCE: Qiongzhong County, Limu Mountain, alt. 1000 m, 22 Jul 2009, *N. K. Zeng 307* (**holotype** HKAS 77332). GenBank ITS = KJ466411, 28S = KJ466474, *TEF*1 = KJ481992, *BenA* = KJ466578.

Etymology: griseorosea, from the Latin *grisea* = gray, and *rosea* = rose-colored, referring to the gray pileus and pinkish lamellae of this species.

Basidiomata (FIG. 2d) small. Pileus 3–6 cm diam, plano-convex to plane, often slightly depressed at center, glabrous, gray (4C2–4) to grayish brown (4D3–4), with innate, dark gray, radiating fibrils; margin non-striate, sometimes radially rimose, non-appendiculate; trama white, unchanging. Lamellae free, crowded, initially white (1A1), but becoming pinkish (7A2–3, 8A2) when mature; lamellulae attenuate, plentiful, evenly distributed. Stipe 5–7 cm long × 0.5–1 cm diam, subcylindrical or slightly tapering upward, with apex slightly expanded, white to dirty white, covered with minutely white to dirty white squamules; context white; basal bulb subglobose, 1–1.5 cm diam, white; volva limbate, membranous, with free limb up to 1 cm in height, both surfaces white. Annulus present, nearly median, pendant from attachment 1–3 cm below apex of stipe, white (1A1). Odor indistinct.

Lamellar trama bilateral. Mediostratum 15–40 μm wide, composed of abundant ellipsoidal inflated cells 38–80 × 15–20 μm; filamentous hyphae abundant, 2–7 μm wide; vascular hyphae rare. Lateral stratum composed of abundant ellipsoidal to subglobose inflated cells 23–65 × 13–30 μm, diverging at an angle of ca. 30–45° to the mediostratum; filamentous hyphae abundant, 3–5 μm wide; septa without clamps. Subhymenium (FIG. 3a) 15–30 μm thick, with 2–3 layers of subglobose to ellipsoidal or irregularly shaped cells, 5–25 × 8–20 μm. Basidia (FIG. 3a) 32–48 × 10–13 μm, clavate, 2-spored; sterigmata 3–5 μm long; basal septa without clamps. Basidiospores (FIG. 3b) [60/2/2] (7.5–)8.5–10.5(–11.5) × (7–) 8–10 μm, Q = 1.0–1.14(–1.40), Qm = 1.06 ± 0.05, globose to subglobose, amyloid, colorless, thin-walled, smooth; apiculus small. Lamellar edge appearing as a sterile strip, composed of ellipsoidal, subfusiform to subglobose or sphaeropedunculate inflated cells (15–43 × 10–30 μm), single and terminal or in chains of two to three, thin-walled, colorless; filamentous hyphae abundant, 2–6 μm wide, irregularly arranged or ± running parallel to lamellar edge. Pileipellis 100–120 μm; upper layer 35–60 μm thick, strongly gelatinized, composed of subradially arranged to somewhat interwoven, thin-walled, colorless to nearly colorless, filamentous hyphae 5–16 μm wide; lower layer 38–60 μm thick, composed of radially and compactly arranged, filamentous hyphae 4–20 μm wide, often with brownish to brown, vacuolar pigments; vascular hyphae rare. Interior of volval limb on the stipe base (FIG. 3c) composed of longitudinally to irregularly arranged elements; filamentous hyphae very abundant to nearly dominant, 3–15 μm wide, colorless, slightly thick-walled (≤0.5 μm), branching, anastomosing; inflated cells rare to scattered or sometimes locally abundant, ellipsoidal, subfusiform to clavate, 40–85 × 20–80 μm, colorless, thin- to slightly thick-walled, terminal, or in chains of two to three; vascular hyphae rare. Outer surface of the volval limb similar to its interior but with more abundant filamentous hyphae 7–13 μm wide; inner surface strongly gelatinized, filamentous hyphae very abundant and 3–10 (–18) μm wide, inflated cells rare. Volval remnants on the pileus not observed. Stipe trama composed of longitudinally arranged, long clavate, terminal cells 160–410 × 15–50 μm; filamentous hyphae scattered to abundant and 4–15 μm wide; vascular hyphae rare. Annulus (FIG. 3d, e) composed of subradially arranged elements: inflated cells very abundant, subglobose, subfusiform, ellipsoidal to oblong ellipsoidal, 20–110 × 20–35 μm, colorless, thin-walled, becoming

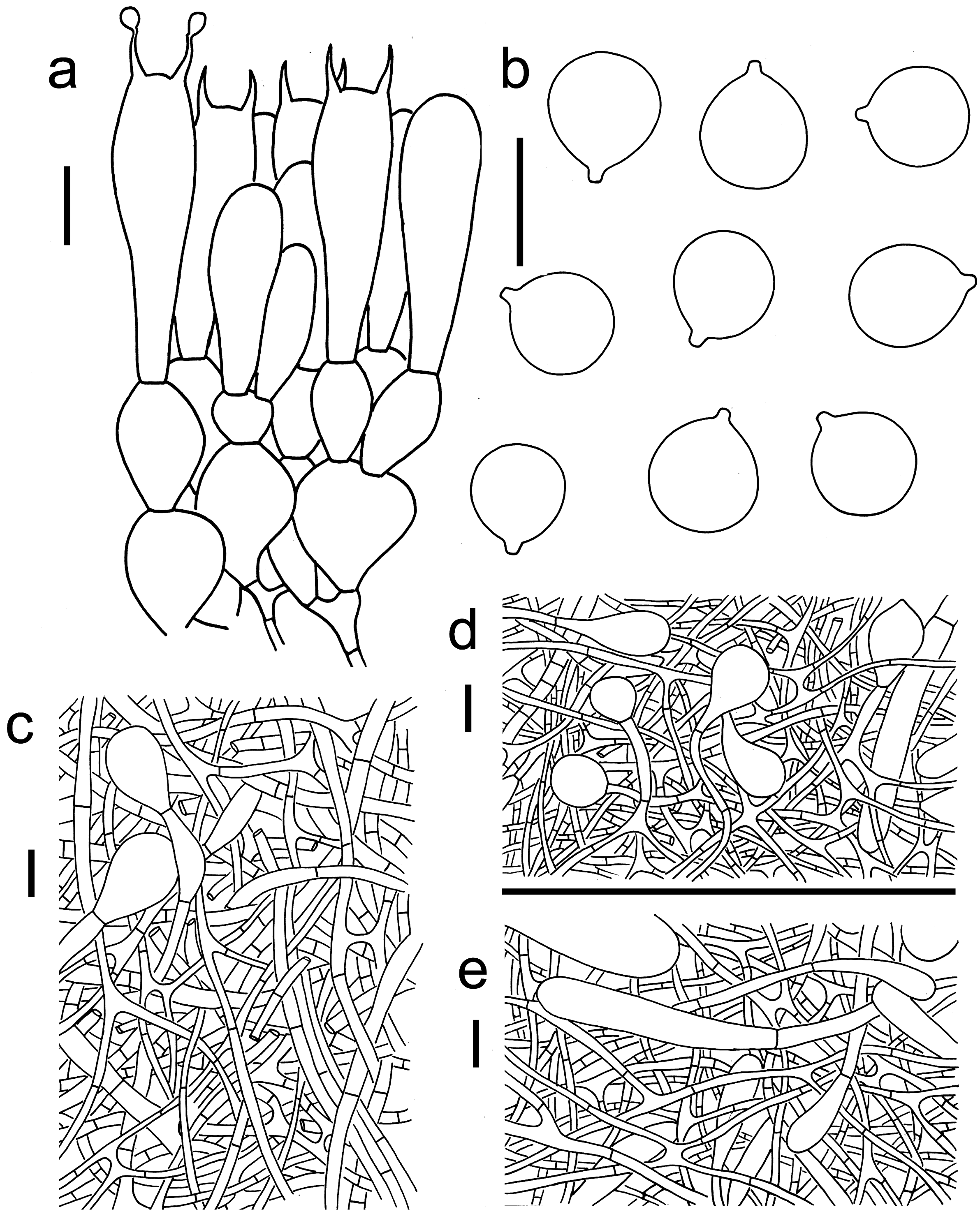

FIG. 3. Microscopic features of *Amanita griseorosea* (TYPE HKAS 77332). a. Hymenium and subhymenium. b. Basidiospores. c. Longitudinal section of trama of the volval limb on the stipe base. d. Outer part of the annulus trama. e. Inner part of the annulus trama. Scale bars: a, b = 10 μm, c–e = 20 μm.

elongated toward inner surface; filamentous hyphae abundant, 2–8(–11) μm wide, colorless, thin to slightly thick-walled; vascular hyphae rare. Clamps absent in all parts of basidioma.

Habit, habitat, and distribution: Solitary or gregarious in forests of Fagaceae; basidiomata occurring in summer; presently only known in tropical (southern and southwestern) China.

Additional specimens examined: CHINA. HAINAN PROVINCE: Baisha County, Yinggeling Nature Reserve, alt. 1300 m, 28 Jul 2009, *N.K. Zeng 385* (HKAS 77333); Location unknown, 2 Sep 1960, *R. Liu 2313* (HMAS 32819); Jianfengling, alt. 109 m, 29 Sep 1987, *T.H. Li s.n.* (GDGM 12541). YUNNAN PROVINCE: Mengla County, Xishuangbanna National Nature Reserve, alt. 1000 m, 6 Jul 2014, *G. Wu 1267* (HKAS 89004).

Commentary: Amanita griseorosea is characterized by its gray to grayish brown pileus with innate, dark gray, radiating fibrils, pinkish lamellae, a white stipe with a nearly median annulus, 2-spored basidia and globose to subglobose basidiospores.

Phylogenetically, *A. griseorosea* is closely related to *A. molliuscula* (FIG. 1, see below). However, the latter can be easily distinguished from the former by its white basidioma, 4-spored basidia, smaller basidiospores, abundant inflated cells in the pileipellis and the inner layer of the volval limb.

Morphologically, *A. griseorosea* is similar to *A. fuliginea* and *A. subfuliginea* because of its gray to grayish brown pileus with innate, dark gray, radiating fibrils (Hongo 1953; Yang 2005, 2015; Zhang et al. 2010). However, *A. fuliginea* and *A. subfuliginea* have white to cream lamellae, gray to grayish brown fibrillose or squamulose stipe with an apical to subapical annulus and 4-spored basidia, and are predominantly distributed in subtropical China (Hongo 1953; Yang 2005, 2015; Zhang et al. 2010). Furthermore, *A. subfuliginea* has smaller basidiospores.

Species in section *Phalloideae* having dark-colored pilei, globose to subglobose basidiospores and exhibiting some morphological similarities to *A. griseorosea* are *A. alauda* Corner & Bas and *A. privigna* Corner & Bas, both originally described from Singapore (Corner and Bas 1962). However, the latter two species differ from the former by their apical to subapical annulus, 4-spored basidia and smaller basidiospores (Corner and Bas 1962). Furthermore, *A. privigna* possesses a white stipe covered with pale gray, floccose-scurfy patches or fibrils (Corner and Bas 1962).

Amanita elephas Corner & Bas, originally described from Singapore, has a dark pileus and a white stipe with a median annulus that are somewhat similar to *A. griseorosea* (Corner and Bas 1962). However, *A. elephas* can be easily distinguished by its larger basidiomata with pilei ca. 9–10 cm diam, 4-spored basidia and distinctly smaller, subglobose to broadly ellipsoidal or obovoid basidiospores (Corner and Bas 1962).

Amanita molliuscula Qing Cai, Zhu L. Yang & Yang-Yang Cui, sp. nov. FIGS. 2e, 4

MycoBank MB815035

Typification: SHAANXI PROVINCE: Zhouzhi County, Taibai Mountain Nature Reserve, alt. 1400 m, 15 Jul 2012, *X.H. Wang 2916* (**type** HKAS 77324). GenBank ITS = KJ466409, 28S = KJ466472, *TEF1* = KJ481974, *RPB2* = KJ466639, *BenA* = KJ466553.

Etymology: molliuscula, from the Latin *molliusculus* = somewhat soft, referring to the somewhat soft volval limb of the species composed of abundant inflated cells.

Basidiomata (FIG. 2e) small to medium-sized. Pileus 4–6 cm diam, at first nearly hemispherical, then convex to plano-convex, glabrous, white (5A1), sometimes pale buff (5A2–3) at center; margin non-striate, non-appendiculate; trama white, unchanging. Lamellae free, crowded, white (11A1) to cream-colored (2A2); lamellulae attenuate, plentiful. Stipe 8–10 cm long × 0.6–1 cm diam, subcylindrical or slightly tapering upward, with apex slightly expanded, white, covered with fibrillose squamules; context white; basal bulb subglobose, ca. 1.5 cm diam, white; volva limbate, somewhat soft membranous, both surfaces white. Annulus present, apical to subapical, white (1A1). Odor indistinct.

Lamellar trama bilateral. Mediostratum 30–50 μm wide, composed of abundant subglobose, subfusiform to ellipsoidal inflated cells 25–100 × 20–30 μm; filamentous hyphae abundant, 2–13 μm wide; vascular hyphae rare. Lateral stratum composed of abundant subfusiform to ellipsoidal inflated cells 35–75 × 12–40 μm, diverging at an angle of ca. 30–60° to the mediostratum; filamentous hyphae abundant and 3–13 μm wide. Subhymenium (FIG. 4a) 25–45 μm thick, with 2–3 layers of subglobose to ellipsoidal or irregularly shaped cells, 10–15 × 8–15 μm. Basidia (FIG. 4a) 30–45 × 8–12 μm, clavate, 4-spored, rarely 2-spored; sterigmata 3–5 μm long; basal septa without clamps. Basidiospores (FIG. 4b) [100/3/2] (7–)7.5–9(–10) × (6.5–)7–8(–9) μm, Q = 1.0–1.14(–1.25), Qm = 1.07 ± 0.06, globose to subglobose, amyloid, colorless, thin-walled, smooth; apiculus small. Lamellar edge appearing as a sterile strip, composed of globose to ellipsoidal or sphaeropedunculate inflated cells 20–50 × 10–25 μm, single and terminal or in chains of two to three, thin-walled, colorless; filamentous hyphae abundant, 3–8 μm wide, irregularly arranged or ± running parallel to lamellar edge. Pileipellis (FIG. 4c) 80–220 μm; upper layer 40–150 μm thick, slightly or non-gelatinized, composed of subradially arranged, thin-walled, colorless to nearly colorless, ellipsoidal to clavate inflated cells 50–180 × 13–30 μm, mixed with filamentous hyphae 4–10 μm wide; lower layer 40–70 μm thick, composed of radially and compactly arranged, filamentous hyphae 4–9 μm wide, colorless; vascular hyphae rare. Interior of volval limb on the stipe base (FIG. 4d, e) composed of two layers intergrading into each other. Outer layer (FIG. 4d) dominantly composed of longitudinally arranged filamentous hyphae 5–8 μm wide, mixed with rare long clavate inflated hyphae up to 20 μm wide, lacking any distinct globose to ellipsoidal inflated

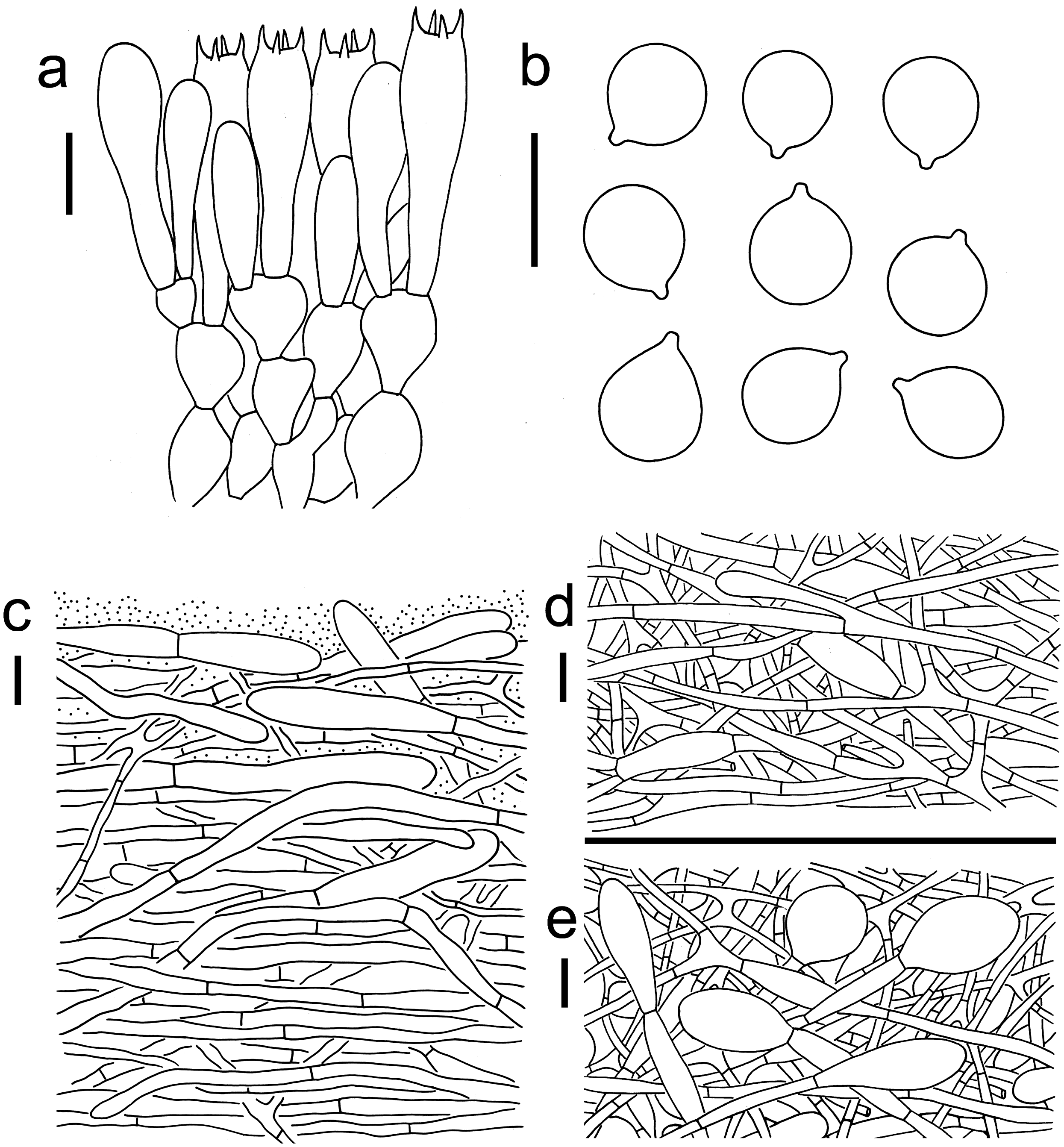

FIG. 4. Microscopic features of *Amanita molliuscula.* a. Hymenium and subhymenium. b. Basidiospores. c. Pileipellis. d. Longitudinal section of the outer layer of the volval limb. e. Longitudinal section of the inner layer of the volval limb. Scale bars: a, b = 10 μm, c–f = 20 μm. a–c from **type** HKAS 77324. d, e from HKAS 75555.

cells. Inner layer (FIG. 4e) composed of longitudinally arranged elements: filamentous hyphae abundant, 4–6 μm wide, colorless, slightly thick-walled (≤0.5 μm), branching, anastomosing; inflated cells abundant, subglobose, ellipsoidal to long clavate, 15–100 × 15–65 μm, colorless, thin- to slightly thick-walled, terminal, or in chains of two to three; vascular hyphae rare. Outer surface of the volval limb similar to the outer layer; inner surface gelatinized, composed of gelatinized filamentous hyphae. Volval remnants on the pileus not observed. Stipe trama composed of longitudinally arranged, long clavate, terminal cells, 130–450 × 15–30 μm; filamentous hyphae scattered to abundant, 3–11 μm wide; vascular hyphae rare. Annulus composed of subradially arranged elements: inflated cells very abundant, ellipsoidal to long ellipsoidal, 25–95 × 10–20 μm, colorless, thin-walled, becoming elongated toward stipitipellis; filamentous hyphae abundant, 2–11 μm wide, colorless, thin- to slightly thick-walled; vascular hyphae rare. Clamps absent in all parts of basidioma.

Habit, habitat, and distribution: Solitary to scattered on soil under trees of *Pinus* and *Quercus*; basidiomata occurring in summer; presently known from central and northwestern China.

Additional specimens examined: CHINA. HUBEI PROVINCE: Shennongjia, Hongping Town, alt. 1850 m, 15 Jul 2012, *Q. Cai 801* (HKAS 75555). JILIN PROVINCE: Antu County, Changbai Mountain, alt. 1000 m, 5 Sep 2008, collection number unknown (HMJAU 20443).

Commentary: Amanita molliuscula is characterized by its white basidioma, pileipellis composed of subradially arranged, ellipsoidal to clavate inflated cells, inner layer of the volval limb composed of abundant subglobose to ellipsoidal inflated cells, and globose to subglobose basidiospores.

Phylogenetically, *A. molliuscula* and *A. griseorosea* clustered together with strong statistic support (FIG. 1, see below). However, *A. griseorosea* has a gray to grayish brown pileus, pinkish lamellae, 2-spored basidia, larger basidiospores, a pileipellis composed of radially arranged filamentous hyphae and rare inflated cells in the volval limb.

The pileipellis composed of subradially arranged, ellipsoidal to clavate inflated cells in *A. molliuscula* is similar to that in *A. rimosa*. However, the basidiomata of *A. molliuscula* are larger than *A. rimosa* (Zhang et al. 2010, Yang 2015). Furthermore, the volval limb of *A. molliuscula* is composed of abundant inflated cells in the inner layer, while the inflated cells in the volval limb of *A. rimosa* are scattered and evenly distributed (Zhang et al. 2010, Yang 2015). Geographically, *A. molliuscula* is distributed in northeastern and central China, ranging from Hubei to Jilin provinces, while *A. rimosa* is known from southern parts of China (Hainan to Jiangxi provinces).

The white basidiomata of *A. subjunquillea* resembles *A. molliuscula* in appearance. However, the former has a pileipellis composed of filamentous hyphae, and inflated cells in the volval limb are rare (Yang 1997, 2005; Yang and Li 2001). Species in section *Phalloideae* also having white pilei, globose to subglobose basidiospores and exhibiting some morphological similarities to *A. molliuscula* are *A. virosa*, *A. pallidorosea* and *A. subpallidorosea*. However, the latter three species differ from *A. molliuscula* by their larger basidiomata, radially arranged filamentous hyphae in the pileipellis and rare to scattered inflated cells in the volval limb (Neville and Poumarat 2004, Zhang et al. 2010, Li et al. 2015, Yang 2015). Furthermore, *A. virosa* possesses a white stipe with distinctly recurved squamules below the annulus and larger basidiospores (Neville and Poumarat 2004, Yang 2015). *Amanita pallidorosea* and *A. subpallidorosea* share white pilei with a pale rose color and a conspicuous umbo over the disc, and *A. subpallidorosea* has a white stipe mostly covered with lacerate squamules (Li et al. 2015, Yang 2015).

Amanita molliuscula is somewhat similar to *A. oberwinklerana* Zhu L. Yang & Yoshim. Doi because of its volval limb composed of abundant inflated cells. However, the abundant inflated cells in the volval limb of *A. oberwinklerana* are predominantly distributed in the outer layer and become rare toward the inner surface, while the abundant inflated cells in the volval limb of *A. molliuscula* are mainly distributed in the inner layer. Furthermore, *A. oberwinklerana* has larger basidiomata and ellipsoidal to broadly ellipsoidal basidiospores (Yang and Li 2001; Yang 2005, 2015) and belongs to *A.* sect. *Lepidella* (Cai et al. 2014, Yang 2015).

Amanita pallidorosea P. Zhang & Zhu L. Yang, Fungal Divers. 42:125. 2010. FIG. 2f

Habit, habitat, and distribution: Solitary to scattered on soil in forests of Pinaceae and Fagaceae; basidiomata occurring from summer to autumn; presently known in Japan and northeastern, eastern, northwestern, central, and southwestern China.

Commentary: Phylogenetically, *A. pallidorosea* is closely related to *A. bisporigera*, but *A. pallidorosea* differs from *A. bisporigera* by its conspicuous umbo over the disc and 4-spored basidia (Lewis 1906, Jenkins 1986, Tulloss et al. 1995a, Zhang et al. 2010).

Morphologically, *A. pallidorosea* resembles *A. subpallidorosea* because of its pale rose pileus with a conspicuous umbo. However, *A. subpallidorosea* differs from *A. pallidorosea* by its larger basidia and basidiospores (Li et al. 2015, Yang 2015). The pale rose color over the disc is variable, and entirely white basidiomata of *A. pallidorosea* have been collected (Zhang et al. 2010, Yang 2015). The entirely white basidiomata of *A. pallidorosea* can be easily confused with *A. virosa* in East Asia. However, *A. virosa* has a stipe with distinctly recurved squamules and larger basidiospores (Neville and Poumarat 2004, Yang 2015).

Amanita parviexitialis Qing Cai, Zhu L. Yang & Yang-Yang Cui, sp. nov. FIGS. 2g, 5

MycoBank MB815036

Typification: GUANGDONG PROVINCE: Fengkai County, Heishiding Nature Reserve, alt. 480 m, 16 Aug 2012, *F. Li 831* (**type** HKAS 79049). GenBank 28S = KT971342, *TEF*1 = KT971343, *RPB*2 = KT971345, *BenA* = KT971346.

Etymology: parviexitialis, from the Latin *parvus* = small, named for its similarity to *A. exitialis* but with smaller basidiomata.

Basidiomata (FIG. 2g) very small. Pileus 1–3 cm diam, applanate, glabrous, center sometimes slightly

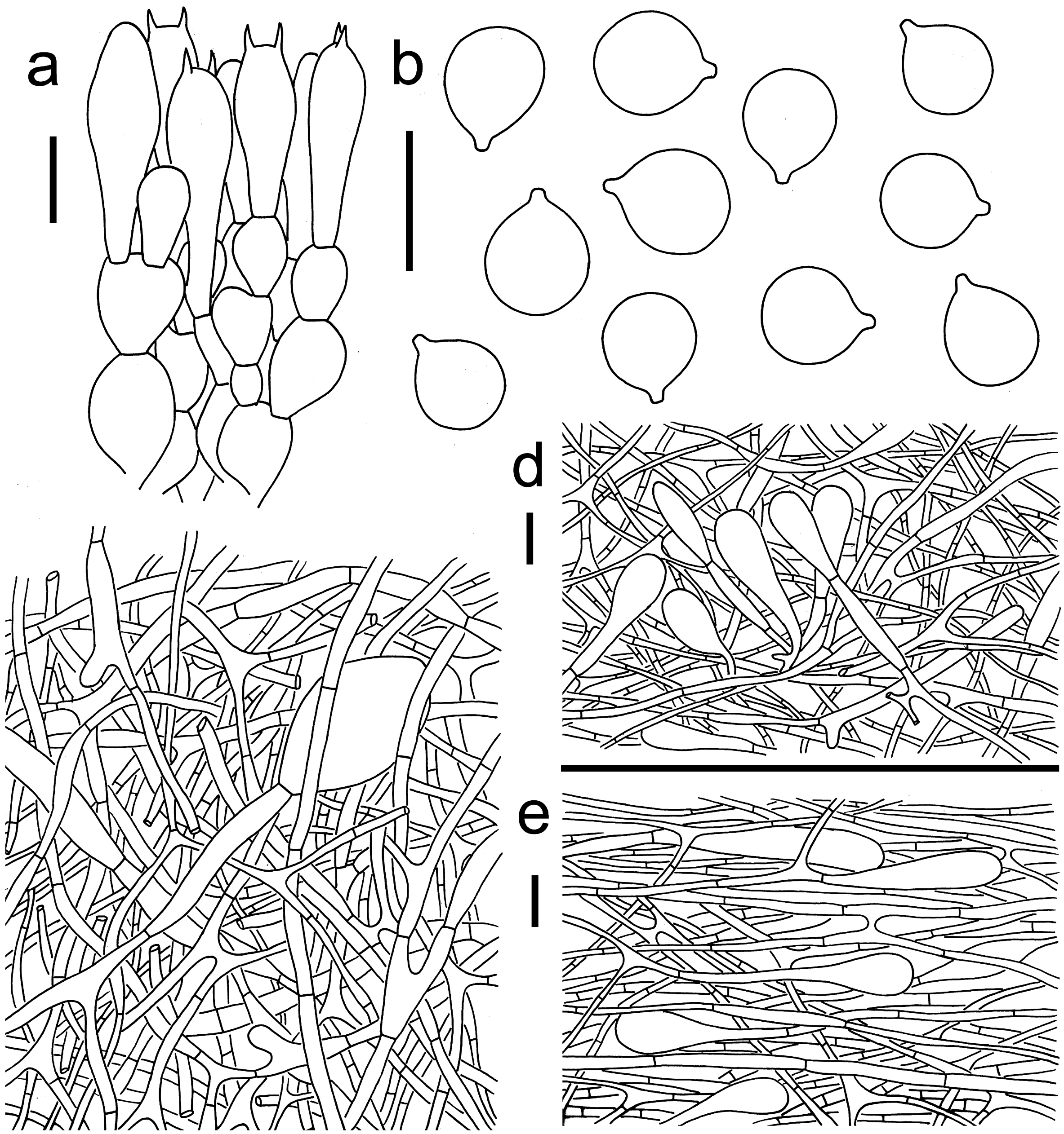

FIG. 5. Microscopic features of *Amanita parviexitialis* (**type** HKAS 79049). a. Hymenium and subhymenium. b. Basidiospores. c. Longitudinal section of trama of the volval limb on the stipe base. d. Outer part of the annulus trama. e. Inner part of the annulus trama. Scale bars: a, b = 10 μm, c–e = 20 μm.

depressed, and slightly brownish (4B3–5), becoming white (1A1) to dirty white (4A2, 4B2) toward margin; margin non-striate, non-appendiculate; trama white, unchanging. Lamellae free, crowded, white (1A1); lamellulae attenuate, plentiful, evenly distributed. Stipe 5–7 cm long × 0.2–0.5 cm diam, subcylindrical or slightly tapering upward, with apex slightly expanded, white, glabrous, or sometimes with fibrillose squamules; context white; basal bulb subglobose, ca. 1 cm diam, white; volva shortly limbate, membranous, with free limb up to 1 cm in height, both surfaces white. Annulus present, subapical, white (1A1). Odor indistinct.

Lamellar trama bilateral. Mediostratum 15–30 μm wide, composed of abundant subfusiform, ellipsoidal to long ellipsoidal inflated cells 40–110 × 20–55 μm; filamentous hyphae abundant, 2–7 μm wide; vascular hyphae rare. Lateral stratum composed of abundant subglobose, subfusiform to ellipsoidal inflated cells 25–50 × 12–27 μm, diverging at an angle of ca. 30–60° to the mediostratum; filamentous hyphae abundant, 3–5 μm wide; septa without clamps. Subhymenium (FIG. 5a) 20–50 μm thick, with 2–3 layers of subglobose to ellipsoidal or irregularly shaped cells, 5–25 × 4–25 μm. Basidia (FIG. 5a) (20–)30–40 × 8–11 μm, clavate, 2-spored; sterigmata 3–5 μm long; basal septa without clamps. Basidiospores (FIG. 5b) [100/2/2] (6.5–)7.5–9.5(–11) × (6.5–)7–9.0(–11) μm, Q = 1.0–1.17(–1.40), Qm = 1.09 ± 0.05, subglobose, rarely globose to broadly ellipsoidal, amyloid, colorless, thin-walled, smooth; apiculus small. Lamellar edge appearing as a sterile strip, composed of ellipsoidal or sphaeropedunculate inflated cells 15–25 × 7–20 μm, single and terminal or in chains of two to three, thin-walled, colorless; filamentous hyphae abundant, 4–7 μm wide, irregularly arranged or ± running parallel to lamellar edge. Pileipellis 60–100 μm; upper layer 30–50 μm thick, strongly gelatinized, composed of subradially to somewhat interwoven, thin-walled, colorless to nearly colorless, filamentous hyphae 2–5 μm wide; lower layer 30–50 μm thick, composed of radially and compactly arranged, filamentous hyphae 4–8 μm wide, colorless; vascular hyphae rare. Interior of volval limb on the stipe base (FIG. 5c) composed of longitudinally to irregularly arranged elements: filamentous hyphae very abundant to nearly dominant, 7–26 μm wide, colorless, slightly thick-walled (≤0.5 μm), branching, anastomosing; inflated cells rare, subfusiform to ellipsoidal, 50–150 × 20–90 μm, colorless, thin- to slightly thick-walled, terminal or in chains of two to three; vascular hyphae rare. Outer surface of the volval limb similar to its interior but with more abundant filamentous hyphae, 4–13 (–20) μm; inner surface strongly gelatinized with abundant filamentous hyphae, 2–8(–11) μm. Volval remnants on the pileus not observed. Stipe trama composed of longitudinally arranged, long clavate, terminal cells, 130–250 × 10–20 μm; filamentous hyphae scattered to abundant, 2–13 μm wide; vascular hyphae rare. Annulus (FIG. 5d, e) composed of subradially arranged elements: inflated cells very abundant, ellipsoidal to long ellipsoidal, 30–100 × 15–20 μm, colorless, thin-walled, becoming elongated toward stipitipellis; filamentous hyphae abundant, 2–8 μm wide, colorless, thin to slightly thick-walled; vascular hyphae rare. Clamps absent in all parts of basidioma.

Habit, habitat, and distribution: Solitary or gregarious in forests dominated by Fagaceae; basidiomata occurring in summer; presently known from southern China.

Additional specimen examined: CHINA. GUANGDONG PROVINCE: Fengkai County, Heishiding Nature Reserve, alt. 500 m, 1 Jun 2013, *Q. Cai 922* (HKAS 79601).

Commentary: Amanita parviexitialis is characterized by its very small basidiomata with white to dirty white pilei, which are sometimes brownish at center, 2-spored basidia and subglobose basidiospores. It is very similar to *A. exitialis* and *A. bisporigera* because of the white basidiomata and 2-spored basidia. However, *A. exitialis* and *A. bisporigera* have larger basidiomata. Furthermore, the basidiospores of *A. exitialis* are larger than those of *A. parviexitilais* (Yang and Li 2001; Yang 2005, 2015). *Amanita bisporigera*, originally described from North America, has a white pileus without distinct brownish tint at center and a fragile, superior, skirt-like annulus (Lewis 1906, Jenkins 1986, Tulloss et al. 1995a).

One species in section *Phalloideae* sharing small, white basidiomata similar to *A. parviexitialis* is *A. parviformis* (Murrill) Murrill, originally described from North America. However, *A. parviformis* has an annulus that is lower on the stipe and ellipsoidal to elongate basidiospores (Murrill 1945; Jenkins 1979, 1986).

Amanita rimosa P. Zhang & Zhu L. Yang, Fungal Divers. 42:124. 2010. FIG. 2h

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by Fagaceae; basidiomata occurring from late spring to autumn; presently known in eastern, central, and southern China.

Commentary: Amanita rimosa resembles *A. molliuscula*, a species described in this study, and a comparison between the two species can be found in the commentary under *A. molliuscula. Amanita rimosa* is somewhat similar to the white basidiomata of *A. subjunquillea*. However, the white basidiomata of *A. subjunquillea* have larger basidiomata, an annulus on the upper part of the stipe, and its pileipellis is composed of radially arranged, filamentous hyphae (Yang 1997, 2005, 2015; Yang and Li 2001).

Amanita subfuliginea Qing Cai, Zhu L. Yang & Yang-Yang Cui, sp. nov. FIGS. 2i, 6

MycoBank MB815037

Typification: CHINA. GUANGDONG PROVINCE: Lechang, Jiufeng Town, Yangdongshan Nature Reserve, alt. 1200 m, 15 Sep 2011, *X.H. Wang 3148* (**type** HKAS 77326). GenBank ITS = KJ466404, 28S = KJ466467, *TEF*1 = KJ481971, *RPB*2 = KJ466636, *BenA* = KJ466550.

Etymology: subfuliginea, from the Latin *sub* = near, referring to its similarity to *A. fuliginea*.

Basidiomata (FIG. 2i) small. Pileus 4–5.5 cm diam, plane, often slightly depressed at center, glabrous, brown (4D3–6) to dark brown (4E2–6), with innate, brown to dark brown, radiating fibrils; margin non-striate, non-appendiculate; trama white, unchanging. Lamellae free, crowded, white (1A1); lamellulae attenuate, plentiful, evenly distributed. Stipe 10–12 cm long × 0.5–0.7 cm diam, subcylindrical or slightly tapering upward, with apex slightly expanded, grayish (4C2–4) to brownish (4B2–4), densely covered with brown (4D4–6) to dark brown (4E5–8) fibrillose squamules;

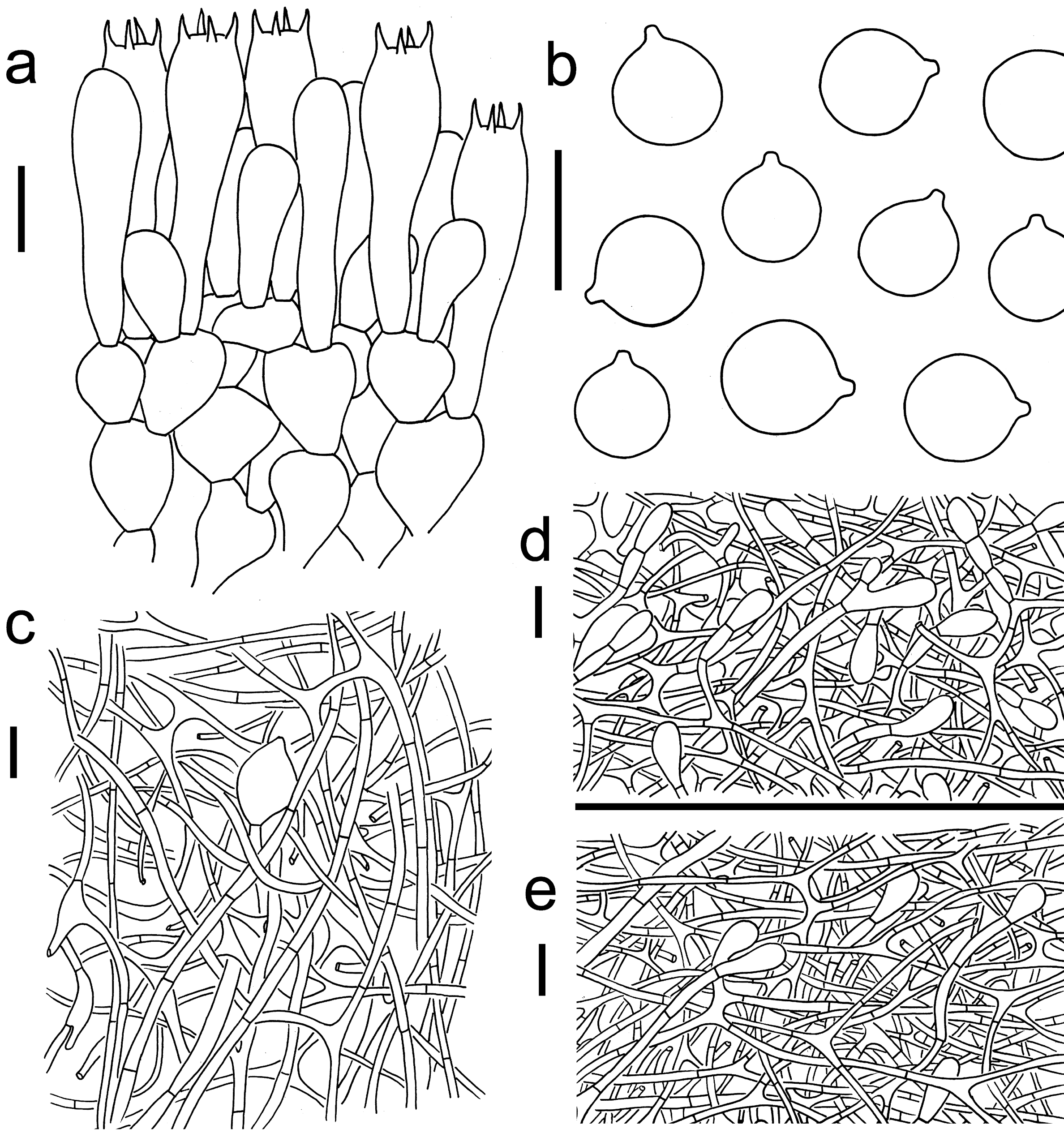

FIG. 6. Microscopic features of *Amanita subfuliginea* (**type** HKAS 77326). a. Hymenium and subhymenium. b. Basidiospores. c. Longitudinal section of trama of the volva limb on the stipe base. d. Outer part of the annulus trama. e. Inner part of the annulus trama. Scale bars: a, b = 10 μm, c–e = 20 μm.

context white; basal bulb subglobose, 1–1.3 cm diam, white to dirty white; volva limbate, membranous, with free limb up to 1 cm in height, both surfaces white. Annulus present, apical to subapical, pendant from attachment ca. 0.5 cm below apex of stipe, white (1A1). Odor indistinct.

Lamellar trama bilateral. Mediostratum 25–50 μm wide, composed of fusiform to ellipsoidal inflated cells 28–45 × 8–24 μm; filamentous hyphae very abundant, 4–9 μm wide; vascular hyphae rare. Lateral stratum composed of very abundant fusiform to ellipsoidal inflated cells 20–60 × 12–70 μm, diverging at an angle of ca. 30–45° to the mediostratum; filamentous hyphae abundant, 2–8 μm wide; septa without clamps. Subhymenium (FIG. 6a) 25–40 μm thick, with 2–3 layers of subglobose or irregularly shaped cells, 6–15 × 7–15 μm. *Basidia* (FIG. 6a) 30–45 × 8–11 μm, clavate, 4-spored; sterigmata 3–5 μm long; basal septa without clamps. Basidiospores (FIG. 6b) [90/2/2] (6–)7–9 (–11) × (6–)7–9(–10.5) µm, Q = 1–1.03(–1.06), Qm = 1.01 ± 0.02, globose, rarely subglobose, amyloid, colorless, thin-walled, smooth; apiculus small. Lamellar edge appearing as a sterile strip, composed of ellipsoidal, subfusiform to subglobose inflated cells 15–20 × 8–12 µm, single and terminal or in chains of 2–3, thin-walled, colorless; filamentous hyphae abundant, 2–6 µm wide, irregularly arranged or ± running parallel to lamellar edge. Pileipellis 50–125 µm; upper layer 25–60 µm thick, strongly gelatinized, composed of subradially to somewhat interwoven, thin-walled, colorless to nearly colorless, filamentous hyphae 3–6(–10) µm wide; lower layer 25–65 µm thick composed of radially and compactly arranged, filamentous hyphae 5–10 µm wide, often with brownish to brown, vacuolar pigments; vascular hyphae rare. Interior of volval limb on the stipe base (FIG. 6c) composed of longitudinally to irregularly arranged elements: filamentous hyphae very abundant to nearly dominant, 3–11 µm wide, colorless, slightly thick-walled (≤0.5 µm), branching, anastomosing; inflated cells rare to scattered, subfusiform to ellipsoidal, 18–90 × 13–65 µm, colorless, thin- to slightly thick-walled, terminal or in chains of two to three; vascular hyphae rare. Outer surface of the volval limb similar to its interior, filamentous hyphae very abundant, 3–5 µm wide; inner surface somewhat slightly gelatinized, filamentous hyphae very abundant, 2–7 µm wide, inflated cells rare. Volval remnants on the pileus not observed. Stipe trama composed of longitudinally arranged, long clavate, terminal cells, 240–425 × 20–40 µm; filamentous hyphae scattered to abundant, 3–6 µm wide; vascular hyphae rare. Annulus (FIGS. 6d, e) composed of subradially arranged elements: inflated cells abundant, ellipsoidal to long ellipsoidal, 15–30 × 7–10 µm, colorless, thin-walled, becoming elongated toward stipitipellis; filamentous hyphae very abundant, 3–7 µm wide, colorless, thin- to slightly thick-walled; vascular hyphae rare. Clamps absent in all parts of basidioma.

Habit, habitat, and *distribution:* Solitary to scattered on soil in forests of Fagaceae and *Pinus*; basidiomata occurring in autumn; presently known from central and southern China.

Additional specimens examined: CHINA. HUNAN PROVINCE: Yizhang County, Mangshan National Nature Reserve, alt. 1300 m, 9 Sep 2005, *P. Zhang 460* (HKAS 77347); same location, alt. 1300 m, 29 Sep 1981, *Y.C. Song* and *X.L. Mao 63* (HMAS 42198).

Commentary: Amanita subfuliginea is characterized by its brown to dark brown pileus with innate, dark brown, radiating fibrils, a brownish stipe covered with fibrillose squamules and globose basidiospores.

Phylogenetically, *A. subfuliginea* is closely related to *A. fuliginea* (FIG. 1). Indeed, *A. subfuliginea* is very similar to *A. fuliginea* because of its small, brown to dark brown basidiomata (Hongo 1953; Yang 2005, 2015). However, *A. fuliginea* has a paler stipe (white, gray to brownish), and globose to subglobose basidiospores (Hongo 1953; Yang 2005, 2015).

Amanita fuligineoides, a species originally described from central China, also has a grayish brown to fuliginous umber pileus, however, *A. fuligineoides* differs from *A. subfuliginea* by its significantly larger basidioma, a subclavate to napiform stipe base and globose to subglobose basidiospores (Zhang et al. 2010, Yang 2015).

Species in section *Phalloideae* with small basidioma and a dark-colored pileus comparable to *A. subfuliginea* are *A. privigna* and *A. alauda*, both originally described from Singapore. However, *A. privigna* differs from *A. subfuliginea* by its fuliginous umber pileus with a paler margin, a shorter stipe, a lower annulus and globose to subglobose, sometimes broadly ellipsoidal basidiospores (Corner and Bas 1962). *Amanita alauda* possesses a smooth white stipe, and globose to subglobose basidiospores (Corner and Bas 1962).

Amanita subjunquillea S. Imai, Bot. Mag. (Tokyo) 47:424. 1933. FIG. 2j

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by Fagaceae; basidiomata occurring from summer to autumn; presently known in Japan, Korea and China.

Commentary: Amanita subjunquillea is closely related and morphologically similar to *A. phalloides* (Vaill. ex Fr.) Link (FIG. 1). However, *A. phalloides*, originally described from Europe, has robust basidiomata and subglobose to broadly ellipsoidal basidiospores (Yang 1997, Galli 2001, Neville and Poumarat 2004). The entirely white basidiomata of *A. subjunquillea* were treated as *A. subjunquillea* var. *alba* Zhu L. Yang by Yang (1997, 2005, 2015). Because few genetic differences between the white and yellow basidiomata are present, it is reasonable to treat *A. subjunquillea* var. *alba* as a synonym of *A. subjunquillea*.

Amanita subpallidorosea Hai J. Li, Mycol. Prog. 14 (43):5. 2015. FIG. 2k

Habit, habitat, and distribution: Solitary to scattered on soil in forests dominated by *Quercus* and *Cyclobalanopsis*; basidiomata occurring in autumn; presently known in eastern and southwestern China.

Commentary: Amanita subpallidorosea and *A. virosa* are grouped in the same subclade (FIG. 1). However, *A. virosa* has a stipe covered with recurved squamules, which are evenly distributed below the annulus, and larger basidiospores (Neville and Poumarat 2004, Zhang et al. 2010, Li et al. 2015, Yang 2015). Furthermore, *A. subpallidorosea* is mainly distributed in subtropical regions, while *A. virosa* is dominantly distributed in temperate

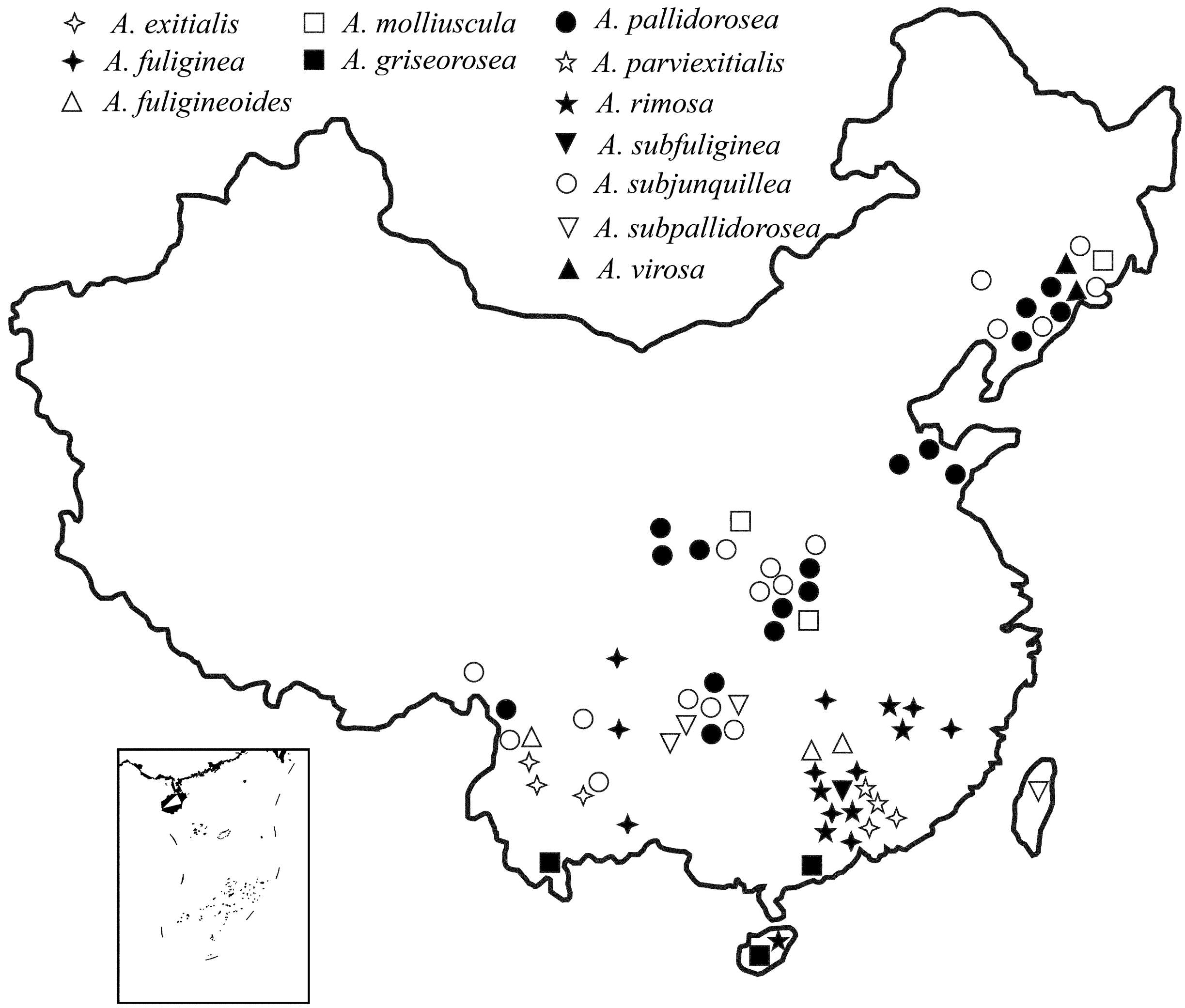

FIG. 7. Primary known locations of lethal *Amanita* spp. in China.

Eurasia (Neville and Poumarat 2004, Zhang et al. 2010, Li et al. 2015, Yang 2015).

Amanita subpallidorosea is very similar to *A. pallidorosea* because of its white pileus with a pallid rose color over the disc. A comparison between the two species can be found in *Commentary* below **Amanita pallidorosea**.

Amanita virosa (Fr.) Bertillon, Dict. Encycl. Sci. Médic.:497. 1866. FIG. 21

Habit, habitat, and distribution: Solitary to scattered on soil in forests of Pinaceae and Fagaceae; basidiomata occurring from summer to autumn; presently known in Europe and East Asia (northeastern and central China and Japan).

Commentary: Amanita virosa is very similar to *A. exitialis* in appearance. However, *A. exitialis* has a pure white pileus without a distinct umbo over the disc, a white stipe without distinctly recurved squamules, an apical annulus, 2-spored basidia and larger basidiospores (Yang and Li 2001; Yang 2005, 2015).

DISCUSSION

Phylogenetic relationships among the lethal Amanita *spp.*—21 species were delimitated among samples collected from East Asia, South Asia, North America, Europe, and Australia. Among them, a high frequency of intercontinental sister relationships was observed between East Asian and North American or between Eurasian and North American species, for example, *A. pallidorosea* vs. *A. bisporigera*, *A. exitialis* vs. *A. suballiacea*, *A. virosa* vs. *A.* sp. 1., etc. (FIG. 1). A close phylogenetic relationship between East Asian *A. subjunquillea* and European *A. phalloides* was uncovered. Furthermore, there were close relationships among the taxa in the basal group (*A.* spp. 8–10), which were collected from Bangladesh, southern China, and Australia, respectively. For the remaining seven species from East Asia, *A. fuliginea* is closely related to *A. subfuliginea*, which share a small, brown to dark brown basidiomata, and *A. molliuscula* is sister to *A. griseorosea*, which differs in the color of the basidiomata and the number of the sterigmata on each basidium. Because *A. fuligineoides*, *A. parviexitialis*, and *A. rimosa* show great divergence from other species and occupy isolated positions in the phylogenetic tree, they might be relics of lethal *Amanita* spp. in the Palaeotropics.

The taxonomic significance of macro- and microscopic characters for the delimitation of Chinese lethal Amanita *spp.*—Our data revealed that several macro- and microscopic characters could be useful for the delimitation of lethal *Amanita* species. Three macroscopic characters are most informative, namely the size of the basidiomata, the color of the basidiomata, and the location of the annulus. Bas (1969) divided the size of basidiomata into five types by the diameter of the pileus. Our research revealed that the majority of the lethal amanitas from China have small (3–5 cm) to medium-sized (5–9 cm) basidiomata. However, *A. parviexitialis* and *A. fuligineoides* have very small (1–3 cm diam) or large (7–14 cm diam) basidiomata, respectively. The color of the basidiomata, including the color of the pileal surface, the lamellae and the squamules on the stipe surface, is also important for the recognition of species (Corner and Bas 1962, Bas 1969). In our study, *A. subjunquillea* is the only species with a pileus having a yellow tinge. *Amanita fuliginea*, *A. fuligineoides*, *A. griseorosea*, and *A. subfuliginea* share brown, gray-brown to dark gray pilei. The remaining six lethal amanitas have white basidiomata, including *A. exitialis*, *A. molliuscula*, *A. pallidorosea*, *A. parviexitialis*, *A. rimosa*, *A. subpallidorosea*, and *A. virosa*. Among these white lethal amanitas, the pilei of *A. pallidorosea* and *A. subpallidorosea* often feature a pale rose-colored umbo. However, the pale rose color is inconsistent, and entirely white basidiomata were collected. It should be noticed that there are also white basidiomata in *A. subjunquillea* (Yang 1997, Cai et al. 2014). *Amanita griseorosea* has pink lamellae, while other lethal amanitas from China share white to cream-colored lamellae. Based on field observations, the colors of the squamules on the stipe surface are chiefly yellow to yellowish (*A. subjunquillea*), brown to grayish (*A. fuliginea*, *A. fuligineoides*, and *A. subfuliginea*) or white (*A. exitialis*, *A. molliuscula*, *A. griseorosea*, *A. pallidorosea*, *A. parviexitialis*, *A. rimosa*, and *A. virosa*). Although the location of the annulus may change during the development of the basidioma, this character is useful to distinguish similar lethal amanitas. For example, *A. griseorosea* is very similar to *A. fuliginea* and *A. subfuliginea*. However, *A. griseorosea* has a nearly median annulus while the latter two species share apical to subapical annuli.

In microscopic aspects, four characters are informative, namely the number of sterigmata on each basidium, the structure of volval remnants on the stipe base, the structure of pileipellis, and the size of basidiospores. *Amanita exitialis*, *A. griseorosea*, and *A. parviexitialis* have 2-spored basidia, while others have mainly 4-spored basidia. Volval remnants on the stipe base are made of filamentous hyphae and inflated cells. The ratio of hyphae and inflated cells can be used to delimitate species (Bas 1969, Yang 1997). The inner layer of *A. molliuscula* has abundant inflated cells, which separates this species from other members of this group with rare to scattered inflated cells. Most lethal *Amanita* spp. have a pileipellis with radially arranged filamentous hyphae, but *A. rimosa* and *A. molliuscula* share a pileipellis composed of abundant somewhat inflated cells. Although all lethal Chinese *Amanita* spp. have mainly globose to subglobose basidiospores, the size of the basidiospores can be used to distinguish species. For example, *A. pallidorosea* and *A. subpallidorosea* have smaller (6–8 × 6–7.5 μm) and larger [(7.5–) 8–10 (–11.5) × (7–) 7.5–9 (–10) μm] basidiospores, respectively.

Geographic distributions of the lethal amanitas in China.—Based on collections of the 12 species available (FIG. 7), ten lethal amanitas were collected predominantly in the subtropical regions of China, including *A. exitialis*, *A. fuliginea*, *A. fuligineoides*, *A. molliuscula*, *A. pallidorosea*, *A. parviexitialis*, *A. rimosa*, *A. subfuliginea*, *A. subjunquillea*, and *A. subpallidorosea*. Among them, *A. rimosa* extends into tropical China, while *A. pallidorosea*, *A. subjunquillea*, and *A. molliuscula* extend into temperate China. *Amanita griseorosea* is the only species restricted to tropical China, while *A. virosa* can only be found in the temperate regions of China (FIG. 7). It is necessary to mention that our major target areas of collection were in eastern and central parts of China. Information on lethal amanitas in the vast western areas with both semiarid and arid vegetation, and temperate or subalpine forests is still lacking. More attention to western China should be paid in the near future.

KEY TO THE SPECIES OF *AMANITA* SECT. *PHALLOIDEAE* IN CHINA

1. Basidia 2-spored 2
1. Basidia 4-spored 4
2. Pileus white, center occasionally cream, yellow, or pale brown; lamellae white to cream; annulus apical to subapical 3

2. Pileus brown, gray brown to dark gray, center dark brown; lamellae pinkish; annulus nearly median . . . . *A. griseorosea*
3. Pileus larger, 4–8 cm diam; basidiospores 9.5–12 × 9–11.5 µm. . . . . *A. exitialis*
3. Pileus smaller, ca. 3 cm diam; basidiospores 7.5–9.5 × 7–9 µm . . . . *A. parviexitialis*
4. Basidiomata white 5
4. Basidiomata differently colored (white basidiomata occasionally found in *A. subjunquillea*) 9
5. Basidiospores average less than 9 µm diam; basidiomata occurring dominantly in the subtropical regions of China, occasionally extending to tropical or temperate regions of China 6
5. Basidiospores average more than 9 µm diam; basidiomata only occurring in the northeast temperate regions of China . . . . *A. virosa*
6. Pileus often with pale rose-colored umbo; pileipellis composed of radially arranged filamentous hyphae . . .7
6. Pileus with neither rose-color tinge nor umbo; pileipellis composed of abundant long ellipsoidal to clavate inflated cells 8
7. Stipe covered with finely fibrillose, evenly distributed squamules below the annulus; basidia 30–45 × 9–11 µm; basidiospores 6–8 × 6–7.5 µm . . . . . . *A. pallidorosea*
7. Stipe covered with distinctly lacerate, locally distributed squamules below the annulus; basidia 35–50 × 11–13 µm; basidiospores 8–10 × 7.5–9 µm . . . . *A. subpallidorosea*
8. Basidiomata smaller; interior of volval limb composed of dominantly filamentous hyphae and evenly distributed scattered inflated cells; mainly distributed in tropical to subtropical China . . . . *A. rimosa*
8. Basidiomata larger; interior of volval limb composed of two layers intergrading into each other, the inner layer composed of abundant filamentous hyphae and abundant inflated cells, the outer layer composed of dominantly filamentous hyphae; mainly distributed in subtropical to temperate China. . . . . *A. molliuscula*
9. Pileus brown, gray brown to dark brown; stipe covered with dirty white, grayish or grayish brown squamules. . . 10
9. Pileus brownish yellow, dirty citrine-yellow to mustard yellow, occasionally white; stipe white to yellowish covered with yellowish fibrillose squamules, occasionally entirely white . . . .*A. subjunquillea*
10. Basidiomata smaller; pileus 3–6 cm diam; basal bulb on the stipe globose to subglobose 11
10. Basidiomata larger; pileus 7–12 cm diam; basal bulb on the stipe subclavate to napiform . . . . *A. fuligineoides*
11. Stipe darker, covered with brown to grayish brown squamules; basidiospores globose, rarely subglobose, 7–9 × 7–9 µm . . . .*A. subfuliginea*
11. Stipe lighter, covered with white, gray to brownish squamules; basidiospores globose to subglobose, 7–9 × 6.5–8.5 µm . . . . *A. fuliginea*

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