Fungal Diversity (2010) 42:119–133
DOI 10.1007/s13225-010-0018-4

Lethal amanitas of East Asia characterized by morphological and molecular data

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Received: 10 March 2009 / Accepted: 23 December 2009 / Published online: 2 February 2010

Abstract This study investigated the morphological characteristics and molecular phylogenetic relationships among lethal *Amanita* species occurring in East Asia. The results revealed that at least nine phylogenetic taxa exist in the region. Among them, five were identical to previously known morphological taxa including *A. exitialis*, *A. fuliginea*, *A. subjunquillea*, *A. subjunquillea* var. *alba* and *A. virosa*; three new taxa, *A. fuligineoides*, *A. rimosa* and *A. pallidorosea* were described and illustrated here. The remaining one was labeled "*A.* sp. 1" because no robust morphological evidence was found to distinguish it from *A. subjunquillea* var. *alba*. The occurrence of *A. virosa* in East Asia, a well-known lethally poisonous mushroom originally described from Europe, was confirmed by both ITS sequences and morphology. Another lethal species native to Europe, *A. phalloides*, was determined as closely related to *A. subjunquillea* from East Asia. *Amanita oberwinklerana* was treated in section *Phalloideae* from a morphological point of view, but appeared to be a member of section *Lepidella* by the analyses of sequences from both the ITS regions and the large subunit of nuclear ribosomal RNA gene. Distribution features of East Asian *Phalloideae* and the phylogenetic relationships of these species with their counterparts from Europe and North America were also discussed. A key to species of section *Phalloideae* in East Asia is furnished.

Keywords Distribution · Morphological species · Phylogenetic species · Species diversity

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Introduction

The genus *Amanita* (Agaricales, Basidiomycota) is a cosmopolitan genus, consisting of nearly 500 described and accepted species (Yang 2000). However, some estimated that an additional 500 taxa needing to be described (Bas 2000; Tulloss 2000, 2005). This genus is important to humans because it contains both famous deadly poisonous species and valued edible species. Moreover, most species of this genus form ectomycorrhizal relationships with vascular plants and play important roles in ecosystems (Yang et al. 1999). In recent decades, mushroom poisoning cases caused by amanitas have been frequently reported in East Asia (Kawase et al. 1992; Li 1996; Yang and Li 2001; Zhang et al. 2002). Extensive investigation and collecting showed that lethal amanitas in this region are abundant and diverse (Yang 2005).

In Europe, lethal *Amanita* species, including *A. phalloides* (Fr.: Fr.) Link (Death Cap), *A. verna* (Bull.: Fr.) Lam. (Spring Poisonous Mushroom), and *A. virosa* (Fr.) Bertill. (Destroying Angel) caused dramatic poisonings or even deaths in the last century (Wieland 1973, 1986; Bresinsky and Besl 1985). These European poisonous mushrooms were also reported to occur in East Asia in the early

mycological literature (e.g. Imazeki and Hongo 1987; Mao 1991; Teng 1996). Recent intensive collecting and taxonomic studies demonstrated that in many cases, these earlier identifications were incorrect (Yang 1997; Weiß et al. 1998; Yang 2005). On the other hand, several new, endemic taxa, such as *A. subjunquillea* S. Imai, *A. fuliginea* Hongo, *A. subjunquillea* var. *alba* Zhu L. Yang, and *A. exitialis* Zhu L. Yang & T. H. Li were described from Japan and China (Imai 1933; Hongo 1953; Yang 1997; Yang and Li 2001). Although quite similar to their relatives from Europe or North America, these lethal amanitas are distinct taxa, and most of the lethal cases of human mushroom poisoning in this region were associated with one of these poisonous mushrooms endemic to East Asia.

In the traditional classifications based on morphological and anatomical characters, the genus *Amanita* was divided into two subgenera [*Amanita* and *Lepidella* (E.-J. Gilbert) Veselý], comprising seven sections [*Amanita, Caesareae* Singer, *Vaginatae* (Fr.) Quél., *Amidella* (E.-J. Gilbert) Konrad & Maubl., *Lepidella, Phalloideae* (Fr.) Quél., *Validae* (Fr.) Quél.] (Yang 1997, 2005). The most familiar lethal amanitas those capable of manufacturing amatoxins are included in section *Phalloideae*. Molecular phylogentic studies on *Amanita* have been carried out based on nucleotide sequences of the ITS (Oda et al. 1999) or nLSU (Weiß et al. 1998; Drehmel et al. 1999) or both (Zhang et al. 2004). These studies generally supported the separation of the genus into two subgenera, as well as the monophyly of the sections except section *Lepidella*. Although the molecular phylogeny of eastern Asian *Amanita* species has been reported (Oda et al. 1999; Zhang et al. 2004), a comprehensive molecular phylogenetic investigation focusing on lethal *Amanita* species of this region remains unaddressed.

The objectives of this study are: (i) to understand the species diversity and distribution feature of lethal amanitas in East Asia, (ii) to clarify phylogenetic relationships among these taxa, and (iii) to shed new light on biogeographic relationships of East Asian *Amanita* species with their counterparts from Europe and North America.

Materials and methods

Sample source

The internal transcribed spacer (ITS) regions of 23 specimens morphologically assigned to section *Phalloideae* were sequenced and analyzed. Eight ITS sequences of related taxa were retrieved from the GenBank. Three *Lepidella* species, *A. japonica*, *A. kotohiraensis* and *A. virgineoides* were also included in the ITS analysis due to their close relationships with *A. oberwinklerana* based on preliminary morphological works and DNA sequence blast.

In order to determine the phylogenetic position of *A. oberwinklerana*, the nuclear large subunit of ribosomal RNA gene (nLSU) sequences of two species of each section within *Amanita* were analyzed with that of *A. oberwinklerana*.

Samples sequenced in this study were deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Science (HKAS) or Mycological Herbarium of Hunan Normal University (MHHNU). Scientific names, GenBank accession numbers, and other relevant information for the specimens included in this study were listed in Table 1.

Morphological descriptions

Macro-morphological descriptions are based on the field notes and color photos of basidiomata. Color codes of the form "5F2" that indicate the plate, row, and color block are from Kornerup and Wanscher (1981); colour names with the first letters capitalized (e.g. Light Buff) are from Ridgway (1912). Micro-morphological data were obtained from the dried specimens after sectioning and rehydrating in 5% KOH solution under a light microscope. In the descriptions of basidiospores, the abbreviation [*n/m/p*] shall mean *n* basidiospores measured from *m* basidiomata of *p* collections; Dimensions for basidiospores are given using the following notation form *(a-) b-c (-d)*. The range *b-c* contains a minimum of 90% of the measured values. Extreme values, i.e. *a* or *d*, are given in parentheses. *Q* is used to mean "length/width ratio" of a spore in side view; **Q** means the average *Q* of all basidiospores measured ± ample standard deviation.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was obtained from dried specimens using a modified CTAB procedure of Doyle and Doyle (1987). The primers ITS 1 and ITS 4 (White et al. 1990) were used for amplification of the ITS region. Primers for nLSU included LROR and LR5. PCR reactions were performed with an Eppendorf Mastercycler thermal cycler (Eppendorf Inc., Germany) in 50 μL reaction mixtures containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.5 mM dNTP mix, 0.5 μM of each primer, 5 U of Taq polymerase, and 1 μL DNA template. Cycling conditions were set as follows: initial denaturation at 94°C for 4 min, followed by 34 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 40 s, extension at 72°C for 50 s (1 min for amplifying nLSU), and a final extension at 72°C for 8 min. Amplified PCR products were quantified by gel electrophoresis on a 1% agarose gel, then purified using Sangong's purification kit (Sangong, China). Purified PCR products were sequenced on an ABI 3730 automated DNA

Table 1 Taxa included in DNA analyses

Taxa	Specimen no.	Geographic origin	GenBank accession	
			ITS	nLSU
A. avellaneosquamosa (S. Imai) S. Imai	HKAS 29500	Lijiang, Yunnan, China		[a]AF024441
A. bisporigera G. F. Atk.	MSC 380551	USA	[e]AY325827	
A. clarisquamosa (S. Imai) S. Imai	HKAS 29514	Lijiang, Yunnan, China		[a]AF024448
A. citrina Pers.	HKAS 31449	Tübingen, Germany		[a]AF024446
A. exitialis Zhu L. Yang & T. H. Li	MHHNU 6778	Guangzhou, Guangdong, China	[c]AY855212	
A. flavipes S. Imai	LEM 960088a	Kutsuki-mura, Shiga Pref. Japan	[b]AB015696	
A. flavipes	HKAS 32505	Kunming, Yunan, China		[a]AF024451
A. fuliginea Hongo	MHHNU 6650	Changsha, Hunan, China	[c]DQ072730	
A. fuliginea	MHHNU 6853	Mangshan, Hunan, China	FJ176716	
A. fuliginea	MHHNU 6903	Chengbu, Hunan, China	FJ176717	
A. fuliginea	MHHNU 6960	Mangshan, Hunan, China	FJ176718	
A. fuliginea	MHHNU 7135	Sanming, Fujian, China	FJ176719	
A. fuligineoides P. Zhang & Zhu L. Yang	HKAS 49666	Mangshan, Hunan, China	FJ176720	
A. fuligineoides	HKAS 52316	Mangshan, Hunan, China	FJ176721	
A. hemibapha var. *ochracea* Zhu L. Yang	HKAS 29522	Lijiang, Yunnan, China		[a]AF024458
A. japonica Bas	LEM 960167	Tatsuno-shi, Hyogo Pref., Japan	[b]AB015684	
A. japonica	HMAS 59778	Bingchuan, Yunnan, China		[a]AF024460
A. kotohiraensis Nagas. & Mitani	MHHNU 6998	Mangshan, Hunan, China	FJ176722	
A. kotohiraensis	MHHNU 7112	Baiyunshan, Guangdong, China	FJ176723	FJ011682
A. muscaria (L.: Fr.) Lam.	HKAS 31495	Tübingen, Germany		[a]AF024465
A. oberwinklerana Zhu L. Yang & Yoshim. Doi	MHHNU 6977	Mangshan, Hunan, China	FJ176724	
A. oberwinklerana	MHHNU 7113	Baiyunshan, Guangdong, China	FJ176725	FJ011683
A. oberwinklerana	MHHNU 7114	Baiyunshan, Guangdong, China	FJ176726	
A. oberwinklerana	MHHNU 6826	Zhangjiajie, Hunan, China	FJ176727	
A. phalloides (Fr.: Fr.) Link	FVORO-0023	Voronej region, Russia	[e]AJ308097	
A. phalloides	KF 02-19	NZ, Gribskov, Demark	[e]AJ889921	
A. phalloides	HKAS 31457	Tübingen, Germany		[a]AF024469
A. pseudovaginata Hongo	HKAS 29524	Qujing, Yunnan, China		[a]AF024472
A. rimosa P. Zhang & Zhu L. Yang	HKAS 49675	Mangshan, Hunan, China	FJ176728	
A. rubrovolvata S. Imai	HKAS 32511	Pingbian, Yunnan, China		[a]AF024473
A. sp. 1	MHHNU 6714	Changbaishan, Jilin, China	[c]DQ072729	
A. pallidorosea P. Zhang & Zhu L. Yang	MHHNU 6838	Zhangjiajie, Hunan, China	FJ176734	
A. pallidorosea	HKAS 52314	Huinan, Jilin, China	FJ176735	
A. pallidorosea	HKAS 54164	Nanchuan, Chongqing, China	FJ176736	
A. subjunquillea S. Imai	MHHNU 6827	Zhangjiajie, Hunan, China	FJ176729	
A. subjunquillea	MHHNU 7041	Shennongjia, Hubei, China	FJ176730	
A. subjunquillea	MHHNU 7049	Shennongjia, Hubei, China	FJ176731	
A. subjunquillea	HKAS 50910	Changbaishan, Jilin, China	FJ176732	
A. subjunquillea	HKAS 52315	Huinan, Jilin, China	FJ176733	
A. subjunquillea var. *alba* Zhu L. Yang	YWE3-20050806	Wuding, Yunnan, China	[d]EF442102	
A. umbrinolutea (Secr. ex Gillet) Bataille	HKAS 31451	Tübingen, Germany		[a]AF024481
A. virgineoides Bas	LEM 960205	Matsugasaki, Kyoto-shi, Japan	[b]AB015686	
A. virosa (Fr.) Bertillon	LEM 960310	Takane-mura, Gifu Pref. Japan	[b]AB015676	
A. virosa	F. Massart 98025	France	[e]AY325829	
A. virosa	HKAS 50912	Changbaishan, Jilin, China	FJ176737	
A. virosa	HKAS 55298	Czech Republic	FJ755188	FJ755189
A. cf. *virosa*	HKAS 27133	Wuyishan, Jiangxi, China		[a]AF024486

Table 1 (continued)

Taxa	Specimen no.	Geographic origin	GenBank accession	
			ITS	nLSU
A. yuaniana Zhu L. Yang	HKAS 29516	Lijiang, Yunnan, China		[a]AF024488
Limacella glioderma (Fr.) Maire	HKAS 31561	Tübingen, Germany		[a]AF024489

[a] Sequences from Weiß et al. (1998)

[b] Sequences from Oda et al. (1999)

[c] Sequences from Zhang et al. (2005)

[d] Sequence from Li et al. (2007)

[e] Sequences submitted to GenBank, but unpublished. The others were obtained in this study

sequencer (Perkin-Elmer Inc., USA). The same primers described above for PCR were used for the sequencing reactions. Sequences generated in this study have been deposited in the Genbank.

Alignment and phylogenetic analyses

Two data sets were analyzed: one was composed of 34 ITS sequences and the other was composed of 15 nLSU sequences. DNA sequences were assembled using SeqMan™ II (DNASTAR Inc., WI, USA), aligned in ClustalX (Thompson et al. 1997) and manually modified where necessary. Both data sets were analyzed in PAUP version 4.0b10 (Swofford 2003), with gaps treated as missing and all characters and substitutions weighted equally. Maximum-parsimony (MP) trees were constructed by running the heuristic search with tree-bisection-reconnection (TBR) branch swapping and up to 1,000 random-addition sequence replications. To assess the relative support for each clade, bootstrap values were calculated from 1,000 replicates. The ITS tree was rooted with *A. flavipes* S. Imai. *Limacella glioderma* (Fr.) Maire was designated as the outgroup in the nLSU tree.

Bayesian analyses were also performed on ITS and nLSU data sets respectively using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). A preliminary run of 200,000 generations using four Metropolis-Coupled Monte Carlo Markov chains was done to estimate how many generations were required for likelihood scores to reach stationarity. This result then dictated our burn-in value for a second run of 2,000,000 generations, also using four chains. A total of 19,000 trees, among 20,000 sampled, were used to calculate posterior probabilities.

Results

Taxonomy

Amanita fuligineoides P. Zhang & Zhu L. Yang, **sp. nov.** (Fig. 1, 6, 7)

MycoBank: MB 515097

Etymology: the epithet refers to the similarity to *A. fuliginea*.

Pileus (7-) 10–12 cm latus, convexus vel applanatus, fuligineo-umbrinus vel griseolo-brunneus, nudus, innato-fibrillosus, margine non striata, non appendiculata. Lamellae liberae, 8 mm latae, albae, confertae, lamellulis attenuatis. Stipes 10–14× 0.8–1.5 cm, solidus, fibrilloso-squamulosus, griseolo-brunneus, annulatus, volvatus; bulbus sublavatus vel elongatus, 1.2–2.5 cm latus. Volva limbata, alba, tenuissima, ex hyphis filamentosis praecipue composita. Annulus membranaceus, minute striatus, albus vel griseus, apicalis, ex hyphis filamentosis et, cellulis clavatas, lato-clavatis vel elliposoideis in permixtione partium subaequalium compositus. Caro alba. Basidia 4-sporigera. Sporae (7.0-) 7.5–9.0 (−10.0)× (6.5-) 7.0–8.5 (−9.0) µm, globosae vel subglobosae, amyloideae. Fibulae absentes. ***Holotypus:*** *P. Zhang 0664 (HKAS 52316) 6. IX. 2007, Mangshan, Yizhang County, Hunan Province, China.*

Basidioma (Fig. 1a, 6, 7) large, seldom medium-sized. Pileus (7) 10–12 cm in diameter, convex to applanate,

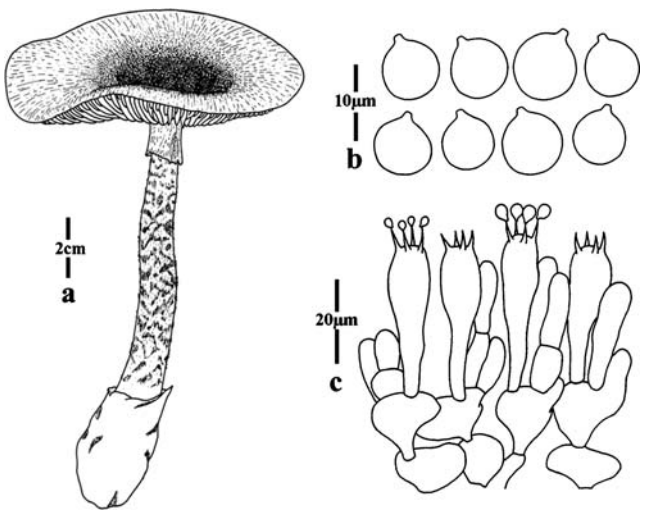

Fig. 1 *Amanita fuligineoides* (HKAS 52316, holotypus). **a** Basidioma. **b** Basidiospores. **c** Basidia and subhymenium

glabrous, fuliginous umber [5F2-4] in center, paler and becoming grayish brown [5C2-4, 5E2-4; Raw Umber, Mouse Gray] toward margin, with innate dark radiating fibrils, subviscid when wet, margin non-striate, non-appendiculate; trama white. Lamellae free, white [1A1], crowded, up to 8 mm high; lamellulae attenuate, plentiful, in two to three ranks. Stipe 10–14×0.8–1.5 cm, subcylindric or slightly tapering upward, solid, with grayish brown [5E2-4; Raw Umber] fibrils or squamules; context white. Basal bulb slightly elongated downward, subclavate to napiform, 1.2–2.5 cm wide. Volva limbate, membranous, firm, with free limb up to 1.5 cm high, both surfaces white. Annulus present, apical to subapical, thin, membranous, persistent; upper surface whitish, somewhat striate; lower surface whitish, then slightly grayish.

Lamellar trama bilateral: mediostratum 20–30 µm wide, made up of fairly abundant to abundant, ellipsoid to fusiform cells (30–60×10–15 µm), mixed with 4–8 µm wide, branching, interwoven, sometimes anastomosing, filamentous hyphae; lateral stratum made up of fairly abundant to abundant, clavate to fusiform cells (50–150×15–20 µm), mixed with abundant, filamentous hyphae, 3–6 µm wide. Subhymenium (Fig. 1c) 20–30 µm thick, with two to three layers of subglobose, ovoid to irregularly shaped cells, 10–20×8–18 µm. Basidia (Fig. 1c) 30–48×9–13 µm, clavate, 4-spored, rarely 2-spored; sterigmata 5–7 µm long. Basidiospores (Fig. 1b) [105/5/4] (7.0-) 7.5–9.0 (−10.0)×(6.5-) 7.0–8.5 (−9.0) µm [*Q*=1.0–1.13 (−1.29), **Q**=1.08±0.05], globose to subglobose, amyloid, colorless, hyaline, thin-walled, smooth; apiculus small. Lamellar edge consisting of numerous globose to broadly clavate, thin-walled, colorless, hyaline cells (10–20×8–10 µm), single or 2–3 in chain. Pileipellis 40–80 µm thick: upper layer (20–40 µm thick) gelatinized, made up of radially arranged, 4–6 µm wide, filamentous hyphae colorless or with brown intracellular pigment; the lower layer (20–40 µm thick) made up of radially and compactly arranged non-gelatinized, filamentous hyphae 4–6 (10) µm wide, with brown intracellularly pigmented. Volval limb on the stipe base dominantly made up of interwoven, somewhat longitudinal arranged, 5–10 (15) µm wide, thin-walled to slightly thick-walled (≤0.5 µm thick), colorless, hyaline, sometimes anastomosing, filamentous hyphae; inflated cells rare, ellipsoid (30–60×10–20 µm), terminal; vascular hyphae rare. Inner surface of the limb made up of gelatinized, filamentous hyphae 3–5 µm wide; outer surface of the limb made up of filamentous hyphae 3–6 µm wide, colorless, hyaline. Volval remnants on pileus not seen. Stipe trama dominantly consisting of longitudinally arranged, long-clavate, terminal cells (100–300×15–40 µm), mixed with scattered (in interior) to fairly abundant (on stipe surface) filamentous hyphae 3–5 (8) µm wide. Annulus consisting of 3–6 µm wide, thin to slightly thick-walled, branching, sometimes anastomosing filamentous hyphae, mixed with abundant to locally very abundant, clavate to broadly clavate to ellipsoid, occasionally subglobose to ovoid 10–30 (40)×10–15 (20) µm, colorless, hyaline, thin-walled inflated cells, which are terminal, single or 2–3 in chain; vascular hyphae rare. Clamp-connection absents in all tissues.

Habitat and Distribution: Solitary on ground in broad-leaved forests dominated by Fagaceae. Only known from central-southern China at present.

Specimens examined: China, Hunan Province, Yizhang County, Mangshan, alt. 1,100 m, 6. IX. 2007, P. Zhang 0664 (HKAS 52316, **holotype!**); same location, alt. 1,200 m, 5. IX. 2005, P. Zhang 0459 (HKAS 49666); same location, alt. 900 m, 3. IX. 2007, P. Zhang 0647 (HKAS 52717); same location, alt. 900 m, 5. IX. 2007, P. Zhang 0657 (HKAS 52727).

Notes: Amanita fuligineoides is characterized by its large-sized basidioma with a grayish brown to fuliginous umber pileus, an apical to subapical annulus, a firm limbate volva and amyloid, globose to subglobose spores. It is similar to *A. fuliginea*, originally described from Japan and widely distributed in China (Yang 1997, 2005). *Amanita fuligineoides* differs, however, from *A. fuliginea* in its significantly larger basidioma, umber tinge on pileus, an elongated stipe bulb, relatively fewer inflated cells in the trama of the volval limb. According to the original description (Hongo 1953), *A. fuliginea* is a small-sized species with a fuliginous to almost blackish coloured pileus 3–5 cm in diameter. Collections of *A. fuliginea* from China usually produce basidiomata with a pileus less than 6 cm in diameter, and a subglobose bulb at the stipe base. The volval limb of *A. fuliginea* made up of filamentous hyphae 3–10 µm wide, intermixed with scattered inflated cells, 60–90×15–25 µm (based on our examinations of MHHNU 6650, 6853, 6903, 6960 and 7135).

Three members of sect. *Phalloideae* with dark-coloured basidiomata reported from Singapore seem to be comparable to the present species: *A. privigna* Corner & Bas and *A. alauda* Corner & Bas also possess globose spores (Corner and Bas 1962), but the former differs on account of its lower annulus (10 mm below apex of stipe), grey volval limb and smaller basidioma, and the later differs by its white stipe and much smaller size of the basidioma (pileus 3 cm wide). *Amanita elephas* Corner & Bas has basidiomata similar in size and with an elongated bulb, but differs in the higher "**Q**" of the spores.

Amanita arocheae Tulloss, Overbo & Halling from Central and South America has spores similar to those of *A. fuligineoides* in size and shape. However, it differs from *A. fuligineoides* by having a gray-brown pileus and a distinctly expanded, globose to subglobose bulb at the stipe base (Tulloss et al. 1992).

Amanita rimosa P. Zhang & Zhu L. Yang, **sp. nov.** (Fig. 2, 8, 9)

MycoBank: MB 515098

Etymology: the epithet refers to the finely rimose pileus.

Pileus 4.8 cm latus, convexus vel applanatus, cremeus vel pallide bubalinus, nudus, siccus, rimosus, margine non striata, non appendiculata. Lamellae liberae, 5 mm latae, albae, subconfertae, lamellulis attenuatis. Stipes 7 cm longus, apice 0.7 cm crassa, basi 1 cm crassa, albus, solidus, annulatus, volvatus; bulbus subbulbosus, 1.6 cm latus. Volva limbata, alba, tenuissima, ex hyphis filamentosis praecipue, composita. Annulus membranaceus, minute striatus, albus, subapicalis, ex hyphis filamentosis, et cellulis clavatis, lato-clavatis vel elliposiodeis, in permixtione partium subaequalium compositus. Caro alba. Basidia 4-sporigera. Sporae 7.0–8.5 (−10.0)× 6.5–8.0 (−9.0) µm, globosae vel subglobosae, amyloideae. Cellulae pileipellis inflatae, 50–280× 15–40 µm. Fibulae absentes. ***Holotypus:*** *P. Zhang 0487 (HKAS 49675) 10. IX. 2005, Mangshan, Yizhang County, Hunan Province, China.*

Basidioma (Fig. 2a, 8, 9) small to medium-sized. Pileus 4.8 cm in diameter, convex to applanate, glabrous, pale buff [5A2-3; Light Buff] at disc, dirty white [4A2-3; Cartridge Buff] toward margin, minutely rimose especially on the outer half of the pileus, dry, margin non-striate, non-appendiculate; trama white. Lamellae free, white to whitish [1A1, 1A2], subcrowded, up to 5 mm high; lamellulae attenuate, plentiful, in two to three ranks. Stipe 7 cm long, subcylindric, slightly tapering upward, with apex slightly expanded, 0.7 cm wide at apex, 1 cm near base, white to whitish [1A1, 1A2], solid, with finely fibrillose squamules; context white. Basal bulb subglobose, 1.6 cm wide. Volva limbate, membranous, rather firm, with free limb up to 8 mm high, both surfaces white. Annulus present, subapical, thin, membranous, white, persistent. Macrochemical test: quick yellow reaction to 5% KOH solution.

Lamellar trama bilateral: mediostratum 25–35 µm wide, made up of fairly abundant to abundant, ellipsoid to fusiform cells (30–45×10–15 µm), mixed with branching, interwoven, sometimes anastomosing, 4–7 µm wide hyphae; lateral stratum made up of fairly abundant to abundant, clavate to fusiform cells (30–80×10–30 µm), mixed with abundant, filamentous hyphae 3–8 µm wide. Subhymenium (Fig. 2c) 20–30 µm thick, with two to three layers of subglobose, ovoid to irregularly shaped cells 10–20×8–15 µm. Basidia (Fig. 2c) 36–48×10–13 µm, clavate, 4-spored; sterigmata 4–6 µm long. Basidiospores (Fig. 2b) [40/1/1] 7.0–8.5 (−10.0)×6.5–8.0 (−9.0) µm [*Q*=(1.0-)1.05–1.15 (−1.23), **Q**=1.08±0.05], globose to subglobose, amyloid, colorless, hyaline, thin-walled, smooth; apiculus small. Lamellar edge consisting of numerous, thin-walled, colorless, hyaline, subglobose to sphaeropedunculate cells (15–30×10–18 µm), which are single or 2–3 in chain. Pileipellis 150–200 µm thick: the upper layer (80–100 µm thick) weakly gelatinized, made up of radially arranged, inflated, ellipsoid to clavate to fusiform, almost colorless, hyaline terminal cells (50–280×15–40 µm), constricted at septa, mixed with filamentous hyphae 4–10 µm wide; the lower layer (50–100 µm thick) non-gelatinized, made up of radially and compactly arranged 4–8 µm wide filamentous hyphae, mixed with abundant, cream-colored intracellularly pigmented, inflated cells (20–50×10–20 µm). Volval limb on the stipe base dominantly made up of interwoven, somewhat longitudinal, 5–10 (15) µm wide, thin-walled to slightly thick-walled (≤0.5 µm thick), colorless, hyaline, sometimes anastomosing, filamentous hyphae; inflated cells

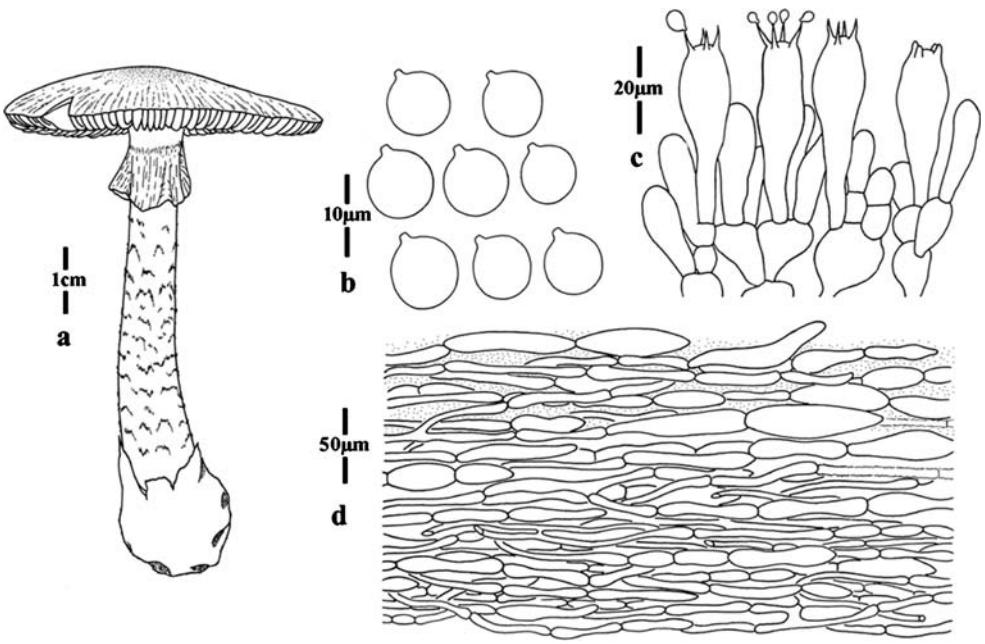

Fig. 2 *Amanita rimosa* (HKAS 49675, holotypus). **a** Basidioma. **b** Basidiospores. **c** Basidia and subhymenium. **d** Radial section of pileipellis

(60–100×20–35 μm) terminal, scattered, subfusiform to clavate; inner surface of the limb strongly gelatinized, made up of filamentous hyphae 4–10 μm wide; outer surface of the limb made up of colorless, hyaline, 4–10 μm wide filamentous hyphae. Annulus consisting of thin to slightly thick-walled, branching and anastomosing, 3–6 μm wide, filamentous hyphae, mixed with abundant to very abundant (at upper surface and at edge of annulus), clavate (50–100× 15–30 μm) to ellipsoid (20–30×10–20 μm) to subglobose (10–20×8–15 μm), colorless, hyaline, thin-walled inflated cells, terminal, single or 2–3 in chain; vascular hyphae rare. Clamp-connection absents in all tissues.

Habitat and Distribution: Solitary on ground in broad-leaved forest dominated by Fagaceae. Only known from the type locality at present.

Specimen examined: China, Hunan Province, Yizhang County, Mangshan, alt. 1,300 m, 10. IX. 2005, P. Zhang 0487 (HKAS 49675, **holotype!**).

Notes: A remarkable feature of *A. rimosa* is its rimose pileal surface, which is caused by the slightly gelatinized upper layer of the pileipellis with abundant inflated cells. This type of pileipellis structure is rare in section *Phalloideae*, or even in genus *Amanita*. *Amanita rimosa* was treated as *A. subjunquillea* var. *alba* on account of the whitish basidioma, globulose to subglobose small spores, yellow reaction to KOH solution. However, discovery of the pileipellis structure revealed the striking difference between the two taxa. The upper layer of the pileipellis of *A. subjunquillea* var. *alba* is gelatinized and made up of filamentous hyphae 2–4 μm wide.

Amanita pilosella Corner & Bas (Corner and Bas, 1962) from Singapore and *A. craseoderma* Bas (Bas 1978) from Brazil also have similar inflated cells in the pileipellis. However, the former belongs to section *Validae*, and the latter belongs to section *Vaginatae*.

Morphological variation within *A. rimosa* is unclear because only one specimen was collected. Thus, more collections need to be found and examined in the future.

Amanita pallidorosea P. Zhang & Zhu L. Yang, **sp. nov.** (Fig. 3, 10, 11)

MycoBank: MB 515099

Etymology: the epithet refers to the color of the pileus.

Pileus 5–8 cm latus, initio conicus, deinde convexus vel applanatus, umbonatus, pallide roseus ad centrum, albus vel albidus versus marginem, nudus, margine non striata, non appendiculata. Lamellae liberae, 5 mm latae, albae, confertae, lamellulis attenuatis. Stipes 8–15× 0.6–1.2 cm, albus vel pallide bubalinus, fibrilloso-squamulosus, solidus, annulatus, volvatus; bulbus subglobosus, 1.2–2.2 cm latus. Volva limbata, alba, tenuissima, ex hyphis filamentosis praecipue, composita. Annulus membranaceus, minute striatus, albus, subapicalis, ex hyphis filamentosis, et cellulis clavatis, lato-clavatis vel elliposoideis in permixtione partium subaequalium compositus. Caro alba. Basidia 4-sporigera, interdum 2-sporigera. Sporae (6.0) 6.5–8.0 (−10.0)× 6.0–7.5 (−9.5) μm, globosae vel subglobosae, amyloideae. Fibulae absentes. ***Holotypus:*** *B. Xiao 009 (HKAS 54164) 28. VI. 2008, Houhe Village, Nanchuan District, Chongqing Municipality, China.*

Basidioma (Fig. 3a, 10, 11) medium-sized. Pileus 5–8 cm in diameter, rounded conic when young, then convex, finally becoming applanate and umbonate at maturity, glabrous, pallid rose [9A2-4, 10A2-4; Hydrangea Pink, Pinkish Vinaceous, Congo Pink] in center, paler and becoming white to whitish toward margin, with innate pinkish radiating fibrils, sometimes the whole surface whitish to white, subviscid when wet, margin non-striate, non-appendiculate; trama white. Lamellae free, white to whitish [1A1, 1A2], crowded, up to 5 mm high; lamellulae attenuate, plentiful, in two to three ranks. Stipe 8–15×0.6–

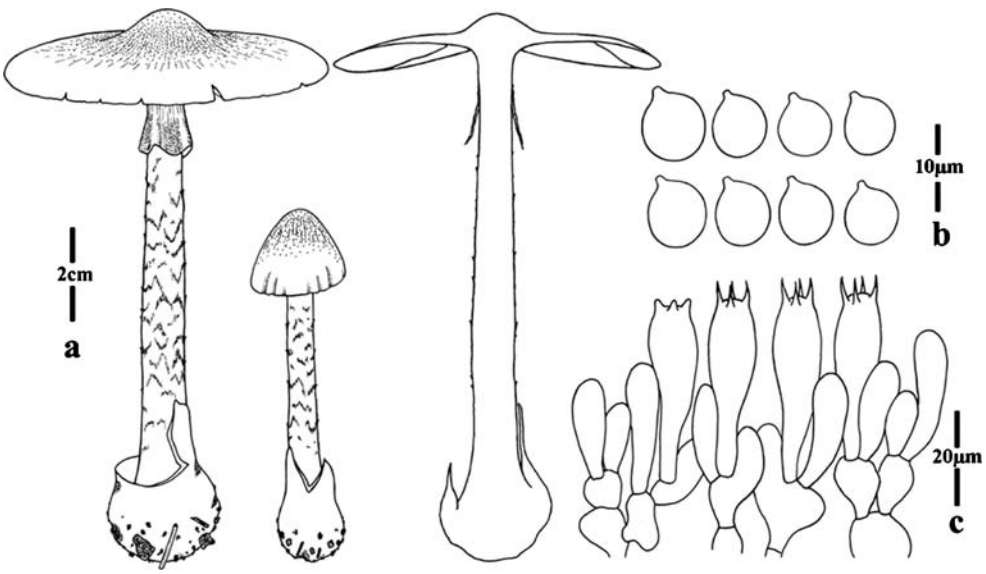

Fig. 3 *Amanita pallidorosea* (HKAS 54164, holotypus). **a** Basidioma. **b** Basidiospores. **c** Basidia and subhymenium

1.2 cm, subcylindric, slightly tapering upward, white to pale buff [5A2-3; Light Buff], solid, with finely fibrillose squamules; context white. Basal bulb subglobose, 1.2–2.2 cm wide. Volva limbate, membranous, rather firm, with free limb up to 1 cm high, both surfaces white. Annulus present, subapical, thin, membranous, white, persistent.

Lamellar trama bilateral: mediostratum 25–35 μm wide, made up of fairly abundant to abundant, ellipsoid to fusiform cells (25–45×10–15 μm), mixed with branching, interwoven, sometimes anastomosing, 2–5 μm wide hyphae; lateral stratum made up of fairly abundant to abundant, clavate to fusiform cells (30–60×10–20 μm), mixed with abundant, filamentous hyphae 3–6 μm wide. Subhymenium (Fig. 3c) 20–30 μm thick, with two to three layers of subglobose, ovoid to irregularly shaped cells (10–20×8–15 μm). Basidia (Fig. 3c) 30–45×9–11 μm, clavate, 4-spored, occasionally 2-spored; sterigmata 4–7 μm long. Basidiospores (Fig. 3b) [85/4/3] (6.0-) 6.5–8.0 (−10.0)× 6.0–7.5 (–9.5) μm [Q=1.0–1.15 (−1.23), **Q**=1.08±0.06], globose to subglobose, amyloid, colorless, hyaline, thin-walled, smooth; apiculus small. Lamellar edge consisting of numerous globose to clavate cells (10–25×8–10 μm), single or 2–3 in chain, thin-walled, colorless, mixed with filamentous hyphae. Pileipellis 60–100 μm thick: the upper layer (30–60 μm thick) gelatinized, made up of radially arranged, colorless, 4–6 (−15) μm wide filamentous hyphae; the lower layer (40–50 μm thick) made up of radially and compactly arranged non-gelatinized, filamentous hyphae 4–6 (15) μm wide, with dirty white intracellular pigment. Volval limb on the stipe base dominantly made up of interwoven, somewhat longitudinal, thin-walled to slightly thick-walled (≤0.5 μm thick), colorless, hyaline, sometimes anastomosing, 5–12 μm wide filamentous hyphae; inflated cells (50–80×15–25 μm) terminal, scattered, subfusiform to clavate; inner surface of the limb strongly gelatinized, made up of filamentous hyphae 2–5 μm wide; outer surface of the limb made up of colorless, hyaline, 4–8 μm wide filamentous hyphae. Annulus consisting of thin to slightly thick-walled, branching and anastomosing, 3–6 μm wide filamentous hyphae, mixed with abundant to very abundant (at upper surface and at edge of annulus), clavate (50–100×15–30 μm) to ellipsoid (20–50×10–20 μm) to subglobose (10–20×8–15 μm), colorless, hyaline, thin-walled inflated cells, terminal, single or 2–3 in chain; vascular hyphae rare. Clamp-connection absents in all tissues.

Habitat and Distribution: Solitary or scattered on ground in a forest dominated by Fagaceae. Presently known from northeastern, central and southwestern parts of China.

Specimen examined: China, Chongqing Municipality, Nanchuan District, Houhe Village, alt. 820 m, 28. VI. 2008, B. Xiao 009 (HKAS 54164, **holotype!**). Jilin Province, Huinan County, Yangzishao, alt. 380 m, 29. VIII. 2007, H. Y. Bao 002 (HKAS 52314). Hunan Province, Zhangjiajie National Forest Park, alt. 1,100 m, 12. VIII. 2002, P. Zhang 0338 (MHHNU 6838).

Notes: In August, 2007, a poisoning in Jilin Province, Northeastern China was caused by eating this mushroom. Four persons in a family ate the mushroom, two of them died. Our study showed that the poisonous species belong to the section *Phalloideae* and was new to science. It is described here as *A. pallidorosea*. *Amanita pallidorosea* is characterized by its pale rose pileus with a conspicuous umbo over the disc, and small, globose to subglobose basidiospores. Several white or whitish amanitas in East Asia, including *A. exitialis*, *A. virosa*, *A. oberwinklerana* and *A. subjunquillea* var. *alba* are somewhat similar to the present species on account of sometimes producing fruitbodies with a cream or light buff tint to the pileus (Imazeki and Hongo, 1987; Yang and Li, 2001), but none of them develops this rosy tone. Furthermore, the former three species can be separated from *A. pallidorosea* by their basidia or basidiospores. *Amanita. subjunquillea* var. *alba* possesses basidia and basidiospores similar to that of *A. pallidorosea*, but it differ from *A. pallidorosea* at least by its cap's lacking both a rose tint and an umbo.

Although the pallid rose pileus is an important character for *A. pallidorosea*, a purely white forms may also exist in this species. Specimen MHHNU 6838 is such an example (discussed below).

Molecular phylogeny

Phylogenetic analysis of the ITS data set

Aligned sequences of ITS were 809 sites long. Among these, 353 characters were constant, 112 variable characters were parsimony-uninformative, and 344 characters parsimony informative. Parsimony analysis of the ITS data set resulted in four equally parsimonious trees of 1,092 steps (CI=0.699, RI=0.807, RC=0.564). One of the most parsimonious trees was shown in Fig. 4. The result of the analysis strongly supported two major monophyletic clades (A and B), which corresponded respectively to a lethal group in section *Phalloideae* and to members of section *Lepidella* included in this study. Within clade A, six lineages were recovered: (1) the *A. fuliginea* lineage (Clade 1, bootstrap 96%), (2) the *A. virosa* lineage (Clade 2, bootstrap 99%), (3) the *A. rimosa* lineage (Clade 3), (4) the *A. fuligineoides-A. exitialis-A.* sp. 1 lineage (Clade 4, bootstrap less than 50%), (5) the *A. subjunquillea-A. subjunquillea* var. *alba-A. phalloides* lineage (Clade 5, bootstrap 99%) and (6) the *A. bisporigera-A. pallidorosea* lineage (Clade 6, bootstrap 97%).

Bayesian analysis of ITS sequences yielded a tree (not shown) similar to that from the MP analysis but with a

much stronger support (posterior probability 0.95) for clade 4 and different topological rearrangements within major clade B. Clades with Bayesian posterior probabilities value greater than 0.90 were indicated by thick branches in Fig. 4.

Phylogenetic analysis of the nLSU data set

Aligned sequences of nLSU were 684 sites long. Among these, 458 characters were constant, 72 variable characters were parsimony-uninformative, and 154 characters parsimony informative. Parsimony analysis of the nLSU data set resulted in three equally parsimonious trees of 480 steps (CI=0.582, RI=0.549, RC=0.320). One of the most parsimonious trees was shown in Fig. 5. The MP analysis

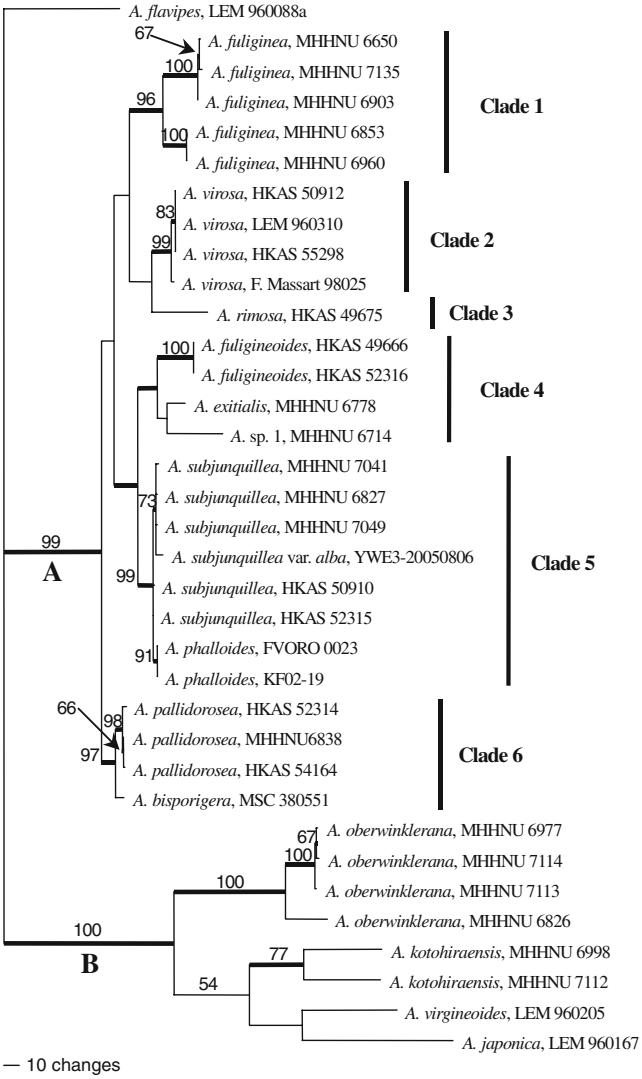

Fig. 4 One of the four most parsimonious trees generated for lethal amanitas based on ITS sequences. Two major clades are indicated by '*A*' and '*B*' below branches. Bootstrap support values greater than 50% are indicated above the branches. *Thick branches* indicate Bayesian posterior probabilities value is greater than 0.90 for that clade

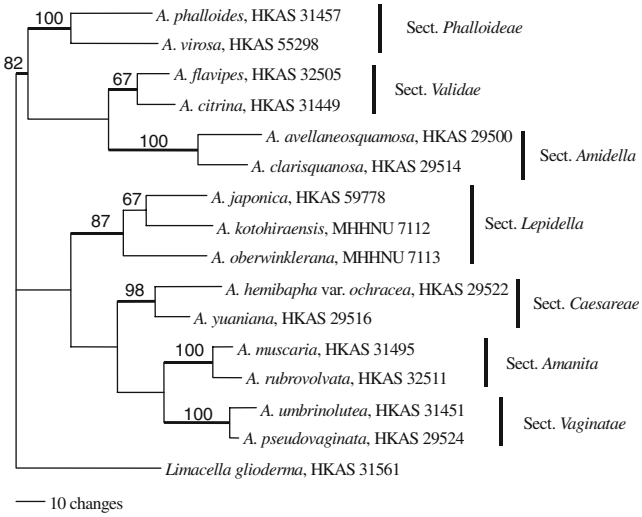

Fig. 5 One of the two most parsimonious trees of genus *Amanita* based on nLSU sequences. Bootstrap support values greater than 50% are indicated above the branch. *Thick branches* indicate Bayesian posterior probabilities value is greater than 0.90 for that clade

based on nLSU sequences supported the division of the genus *Amanita* into seven sections. A broader clade consisting sections *Phalloideae*, *Validae* and *Amidella* was well supported (bootstrap 82%), while three clades corresponding to sections *Amanita*, *Vaginatae* and *Caesareae* failed to cluster together. *Amanita oberwinklerana* and two members of section *Lepidella*, *A. japonica* and *A. kotohiraensis* were grouped in one clade with a strong bootstrap support (87%).

Bayesian analysis of nLSU sequences yielded a topology (not shown) somewhat congruent with that from the MP analysis, but with differences in the following aspects. First, a higher-level clade consisting sections *Amanita*, *Vaginatae* and *Caesareae* was recognized (posterior probability 0.98), which corresponds to subgenus *Amanita*. Secondly, sections *Phalloideae*, *Validae* and *Amidella* formed another well-supported higher-level clade (posterior probability 1.0), linking with section *Lepidella* at the base with weak support (posterior probability 0.72).

Discussion

Phylogeny of *A. subjunquillea*, *A. fuliginea* and *A. exitialis*

Our results suggested that nine lineages of lethal amanitas in East Asia could be recognized. Eight of them correspond to the taxa based on morphology, including *A. exitialis*, *A. fuliginea*, *A. fuligineoides, A. pallidorosea, A. rimosa*, *A. subjunquillea*, *A. subjunquillea* var. *alba*, and *A. virosa*. Among these eight taxa, the first seven are endemic to East Asia, while the last one is a broadly distributed Eurasian species.

Figs. 6–11 Basidiomata of three new species. **6, 7.** *Amanita fuligineoides* (HKAS 52316). **8, 9.** *Amanita rimosa* (HKAS 49675). **10.** *Amanita pallidorosea* (HKAS 52314). **11.** *Amanita pallidorosea* (HKAS 54164). **Bars**=2 cm

Amanita subjunquillea is found in Japan, South Korea, and China (Yang 2005). Nucleotide sequence divergence of the ITS regions within this species is very low, despite the fact that samples were collected from distant locations. For example, specimen HKAS 50910 was collected from Jilin Province, Northeastern China, while MHHNU 6827 was collected from Hunan Province, central-southern China. The ITS sequence difference between these two samples was only nine bases (divergence=1.32%), suggesting the molecular differences related to geographical distributions in this species were relatively insignificant.

Amanita fuliginea is common both in Japan and China. Two subclades with distinctive molecular differences were recognized in the ITS phylogenetic tree (clade 1). One subclade consists of three specimens collected from different locations and associated with different hosts (i.e. MHHNU 6650 from Changsha, Hunan Prov., associated with *Lithocarpus glaber* (Thunb.) Nakai; MHHNU 6903 from Chengbu, Hunan Prov, associated with *Castanopsis fargesii* Franch.; and MHHNU 7135 from Sanming, Fujian Prov., associated with *C. kawakamii* Hayata). Interestingly, all these three samples possessed quite homogenous ITS sequences (more than 99% nucleotide identity). Another subclade consists of two specimens located about three kilometers away from each other, and this subclade can be interpreted as a population that is genetically distinct from the three other *A. fuliginea* populations. The possibility that this subclade represents a cryptic species should not be excluded, considering that the ITS sequence changes between the two subclades is greater than the differences among other pairs of closely related species (e.g. *A. pallidorosea/A. bisporigera*; *A. phalloides/A. subjunquillea*).

Amanita exitialis is characterized by its white basidioma with an apical annulus and a firm limbate volva, and 2-spored basidia (Yang and Li 2001). This species has only been found in Guangdong Province, Southern China and

appears to be a tropical to subtropical species. This species or its affinity is likely to be found in other parts of Southern China and Southeast Asia in the future. It is interesting to note that *A. exitialis* was not clustered together with the North American white-colored, two-spored species, *A. bisporigera*.

The three white-colored lethal amanitas, *A. exitialis*, *A. subjunquillea* var. *alba*, and *A. virosa* didn't form a monophyletic group in our analyses, suggesting that the character "unpigmented pileus" has originated more than one time in section *Phalloideae*. By the same reasoning, "bearing 2-spored basidia" also seems to have originated more than once.

Phylogenetic placement of the three new species

Amanita fuligineoides and *A. fuliginea* are morphologically very similar to each other, but were clustered in different groups, suggesting that they are distinct species. Surprisingly, *A. fuligineoides* and *A.exitialis* together with *A.* sp. 1 (MHHNU 6714) are grouped as a clade (Clade 4). Although this clade is poorly supported in MP tree (less than 50% bootstrap), but well supported in Bayesian analysis (posterior probability 0.95). The conspicuous morphological differences suggest that they belong to three separate species. The phylogenetic relationships among them are still uncertain at present.

The pileipellis structure of *A. rimosa* is unique in section *Phalloideae*. Molecular analyses also suggest that, although grouped in the major clade representing the lethal group in section *Phalloideae*, it did not form a clade with any other taxon in the group, but fell in an individual clade alone.

Three specimens of *A. pallidorosea* collected from different locations in China formed a highly supported clade (Clade 6) with 98% bootstrap support, linking with *A. bisporigera* with a bootstrap of 97%, suggesting a close phylogenetic relationship between these two species. Among the three specimens of *A. pallidorosea* sequenced in this study, HKAS 52314 and HKAS 54164 possessed the pallid rose pileus, but MHHNU 6838 produced white fruitbody and lacking the roseate tinge unique to this species and morphologically appeared more similar to *A. subjunquillea* var. *alba*. The ITS sequence difference between MHHNU 6838 and HKAS 54164 (holotype of *A. pallidorosea*) was only 1 base pair, indicating that MHHNU 6838 was likely a white form of *A. pallidorosea*.

Phylogenetic position of *A. oberwinklerana*

Amanita oberwinklerana was originally described from Japan (Yang and Doi 1999) and later reported from many localities in China (Yang and Li 2001; Yang 2005). Due to its non-appendiculate pileal margin, membranous limbate volva on the bulbous stipe base and amyloid spores, this species was placed in section *Phalloideae*. However, the results of molecular analyses from both ITS and nLSU show that it's closely related to several species from section *Lepidella* such as *A. kotohiraensis*, *A. virgineoides* and *A. japonica*, with a very high bootstrap support. Although whether the entire section *Lepidella* is monophyletic is still unclear, a monophyletic group including these four taxa is present within this section. Some morphological and chemical evidences also support the new placement of *A. oberwinklerana*: a relatively friable volva with abundant inflated cells (Yang and Doi 1999; Yang and Li 2001); basidioma containing considerably lower amatoxin concentrations than in other lethal *Amanita* species (Chen et al. 2003), and possessing a manifest and nauseating smell like that of bleaching powder which has consistently been found in the courses of collection of *A. kotohiraensis* and *A. japonica*, and was regarded as a chemical character of several species of section *Lepidella* (Bas 1969). Modifications of the volva from 'pulverulent', 'wart-forming' to 'limbate' or 'saccate' exist among the members of section *Lepidella* (Bas 1969), and *A. oberwinklerana* can be regarded as an extreme example in this section, producing salient volva: it sometimes produces a short limbate volva and leaving membranous volval remnants on the pileus, while in most cases, it form a well-developed, limbate volva, making it impossible to distinguish it from *A. subjunquillea* var. *alba* or *A. exitialis*, unless microscopic examinations are conducted.

It is worth emphasizing that the intraspecific divergence of the ITS sequences in *A. oberwinklerana* is very high (up to 12%), though four collections of this species form a monophyletic clade with the maximal bootstrap value (100%). One of the four sequences (MHHNU 6826) has a high level of divergence with the other three sequences, but no remarkable morphological, ecological or geographical character was found about this collection. Phylogenetic structure of this species may be clarified when more materials from other loci are available.

Phylogenetic placement of East Asian species of sect. *Phalloideae* confused with *A. subjunquillea* var. *alba*

Recent advances of molecular techniques have provided a method of recognizing species on the basis of phylogenetic trees constructed from DNA data, i.e. phylogenetic species recognition (PSR). In many cases, PSR revealed phylogenetically different taxa that had not previously been distinguished using a morphological species concept (MSR) or the biological species concept (BSR) (Taylor et al. 2000).

Amanita subjunquillea var. *alba* is characterized by its white basidioma, small and globose to subglobose spores,

and yellow reaction to KOH solution. As mentioned above, the mushrooms with white basidioma in section *Phalloideae* might have evolved from different ancestors. The character of round and small spore is common for lethal amanitas in this region. Yellow reaction to KOH was observed on several white species in East Asia. As a consequence, many phylogenetically different collections fit the morphological concept of *A. subjunquillea* var. *alba* and have been labeled with this name. Indeed, two new species described above, *A. rimosa* and *A. pallidorosea* were also mis-regarded as *A. subjunquillea* var. *alba* at their first sight. Since *A. subjunquillea* var. *alba* is a variety of *A. subjunquillea*, it should has a close relationship with *A. subjunquillea* var. *subjunquillea*. According to morphological and anatomical characters, two specimens in this study (i.e. YWE3-20050806 and MHHNU 6714) could be identified as *A. subjunquillea* var. *alba*. However, based on ITS sequences, only specimen YWE3-20050806 was grouped together with *A. subjunquillea*, which probably represents the "true" *A. subjunquillea* var. *alba*, while MHHNU 6714 was grouped with *A. exitialis* and *A. fuligineoides* in a different clade and might have evolved from other ancestors. Although our molecular analysis reveals that MHHNU 6714 represents a unique ITS sequence type (temporarily labeled as *A.* sp. 1), no sound morphological evidences have been found to characterize it. The authors also observed several other materials morphologically similar to *A. subjunquillea* var. *alba*. These specimens were not included in this study because DNA sequences were unavailable. Further collection, field observation and laboratory research may yield more data to characterize the members of *"Amanita subjunquillea* var. *alba"* complex.

Biogeographic relationships between lethal amanitas of East Asia and Europe or North America

Biogeographic relationships between *Amanita* of East Asia and Europe or North America have been repeatedly discussed in the previous mycological literature (e.g. Weiß et al. 1998; Yang 2000; Zhang et al. 2004). In this study, we propose a few new insights.

Amanita virosa was often reported from East Asia and southern Asia in the last century (e.g. Imai 1933; Xie et al. 1986; Imazeki and Hongo 1987; Bhatt et al. 1999). However, detailed examinations suggested that some other white coloured amanitas including *A. oberwinklerana* and *A. subjunquillea* var. *alba* were misidentified as *A. virosa*, and thus its occurrence in East Asia was questioned (Yang 2000; Bhatt et al. 2003), and Yang (2005) did not include it in his work. In this study, a collection from Changbaishan, Northeastern China (HKAS 50912) showed no ITS sequence difference with two samples of *A. virosa* collected respectively from the Czech Republic (HKAS 55298) and Japan (LEM 960310), and these three collections clustered with a collection of *A. virosa* from France (F. Massart 98025) with a strong statistic support (bootstrap 99%). The basidiospores of HKAS 50912 were 9.0–11.0 (−11.5)×8.5–10.5 (−11.0) µm, within the range for *A. virosa* (Breitenbach and Kränzlin 1995; Chiusa 2000; Neville and Poumarat 2004). Based on our molecular analyses and morphological data, we conclude that *A. virosa* does occur in East Asia. Weiß et al. (1998) sequenced the nLSU of a specimen from southeastern China (HKAS 27133), which was labeled as *A.* cf. *virosa* at that time. Unfortunately, the ITS sequence of that specimen is unavailable. The nLSU sequence differences between that specimen and HKAS 55298 (*A. virosa* from Czech) was ten base pairs (divergence=1.6%), suggesting that HKAS 27133 was likely represented an independent taxon morphologically similar to *A. virosa*. *Amanita virosa* was also reported from North America (e.g. Coker 1917; Jenkins 1986; Phillips 2005). However, Tulloss et al. (1995) questioned its distribution in North America. As morphological characters among the group of white *Phalloideae* are often subtle and no sequence of North American material is available for comparison, its occurrence in North America needs to be clarified in the future.

Pringle and Vellinga (2006) suggested that *A. phalloides* was artificially introduced from Europe to North America, South America, Africa south of the Sahara, Australia and New Zealand with its host plant. This species was reported from China (e.g. Teng 1936, 1996; Tai 1979) and Japan (Imazeki and Hongo 1970; Hongo 1982; Hongo and Izawa 1994). Hongo and Izawa (1994) provided a photo of a specimen morphologically similar to *A. phalloides* from Japan. The voucher is not available for the authors to study. Currently, there is no evidence that *A. phalloides* occurs in China. However, several morphological similar species such as *A. subjunquillea*, *A. fuliginea*, *A. manginiana* sensu W. F. Chiu and *A. pseudoporphyria* were often misidentified as this species (Yang 2005). In Fig. 4, two collections of *A. phalloides* (i.e. FVORO 0023 and KF 02-19) from Europe were depicted as very closely related to the five collections of *A. subjunquillea* (i.e. MHHNU 6827, MHHNU 7041, MHHNU 7049, HKAS 50910 and HKAS 52315) from China. From a morphological point of view, both species usually produce yellowish basidiomata, though color variations of the basidiomata exist. *Amanita subjunquillea* differs from *A. phalloides* by its smaller spores, smaller basidia and smaller basidiomata. These two species may share a common ancestor. Because the ITS sequence divergence between these two species is very low (e.g. The ITS sequence differences between FVORO 0023 and HHHNU 7041 was 14 base pairs), it is conceivable that the segregation of these taxa occurred recently. In most

cases, genetic isolation and divergence seem to precede the differentiation of morphological character (Taylor et al. 2000). However, in this study, we found obvious morphological differences between *A. phalloides* and *A. subjunquillea*, though their ITS sequences are quite similar to each other. This may be due to the fact that only one gene was analyzed. Similarly, Ge et al. (2008) reported that a sample of *Flammulina* sp. collected from Tibet, China, would be regarded as a lineage of *Flammulina velutipes* according to molecular analysis, but possessed a hymeniform suprapellis, which was significantly different from that of the latter species. It is thus reasonable to assume that much more genetic differences between *A. phalloides* and *A. subjunquillea* may be revealed when multi-locus DNA analysis is applied. Among the five collections of *A. subjunquillea* included in this study, three of them (MHHNU 6827, MHHNU 7041, MHHNU 7049) together with *A. subjunquillea* var. *alba* (YWE3-20050806), formed a subclade with moderate support (bootstrap 73%) linking with the rest two (HKAS 50910 and HKAS 52315) at the base. The topology of the clade suggested that HKAS 50910 and HKAS 52315 represented the ancestral types in this species, while MHHNU 6827, MHHNU 7041 and MHHNU 7049 represented the derived types. Since both of the ancestral types were collected from Northeastern China, while three of the derived types were collected from south part of China, *A. subjunquillea* was probably original from Far East area, and subsequently dispersal toward south. Similar evolutional event was reported in *A. muscaria* (Geml et al. 2006).

Amanita bisporigera, a lethal species with white basidiomata and 2-spored basidia, was originally described from the United States. Morphologically, it is similar to *A. exitialis*, which also produces white basidiomata and 2-pored basidia. Unexpectedly, the American species was revealed to share a lineage with *A. pallidorosea*, a 4-spored species from China. *A. bisporigera* and *A. pallidorosea* can be regarded as paired species distributing in two different continents. According previous studies, similar paired species of *Amanita* between East Asia and North America are relatively common (Yang 2000; Zhang et al. 2004; Tulloss 2005).

Key to species of section Phalloideae in East Asia

1. Basidia 2-spores……..…… *A.exitialis*
1. Basidia 4-spores……2
2. Upper layer of Pileipellis contain abundant inflated cell (50–280×15–40 μm)……..…… *A. rimosa*
2. Upper layer of Pileipellis made of filamentous hyphae 4–10 μm wide……..……..…..3
3. Basidiospores 9.0–11.0 (−11.5)×8.5–10.5 (−11.0) μm…… *A. virosa*
3. Average length of basidiospores less than 9.0 μm……4
4. Pileus bright-coloured; white, yellow, pallid rose……..……5
4. Pileus dark-coloured; gray, grayish brown, fuliginous umber, fuliginous……7
5. Pileus pallid rose, umbonate at disc……*A. pallidorosea*
5. Pileus yellow or white, without umbo on disc……..……6
6. Pileus yellow……..……*A. subjunquillea*
6. Pileus white……...…….. *A. subjunquillea* var. *alba*
7. Stipe with grayish fibrils or squanules; annulus persistent……..8
7. Stipe with white fibrils or squanules; annulus often broken and attach on the margin of pileus……..…9
8. Fruitbody large; pileus fuliginous umber; bulb at the stipe base elongated downward, subclavate to napiform....*A. fuligineoides*
8. Fruitbody small; pileus dark gray to fuliginous; bulb at the stipe base not elongated, subglobose……...…….. *A. fuliginea*
9. Stipe base not elongated; volval with relatively fewer inflated cells; *Q*=(1.0-) 1.07–1.30 (−1.44)……...…
……*A. manginiana* sensu W. F. Chiu
9. Stipe base elongated downward; volval with abundant inflated cells; *Q*=(1.09-) 1.21–1.58 (−1.80)…….. *A. pseudoporphyria*

Acknowledgments The authors thank Dr. Xianghua Wang (Kunming Institute of Botany) for providing several valuable collections for this study. We are grateful to Dr. Wenqi Hu (Montreal, Canada) for revising an earlier version of the manuscript. Dr. Rodham E. Tulloss (Roosevelt, New Jersey) and Dr. Jianping Xu (McMaster University, Canada) are acknowledged for their critically reviewing the manuscript. The authors also thank Dr. Yanchun Li (Kunming Institute of Botany) for providing a couple of sequences. This study was supported by Postdoctoral Science Fund of China (No. 20070411171), the National Science Fund for Distinguished Young Scholars (No. 30525002) of the National Natural Science Foundation of China (NSFC), the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-YW-G-025), the NSFC (Nos. 30700005, 30871766 and U0836604) and the National Basic Research Program of China (No. 2009CB522300).

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