



A new species and four new records of *Amanita* (Amanitaceae; Basidiomycota) from Northern Thailand

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Abstract

Mushrooms belonging to the genus *Amanita* were collected during a fungal biodiversity study in northern Thailand in 2012–2014. Morphological characteristics and molecular phylogenetic analyses were used to identify the mushrooms to species. *Amanita castanea* is described as new to science and compared with phenetically and phylogenetically similar species. It is assignable to *Amanita* stirps *Citrina* within *Amanita* series *Mappae*. Four other species, *A. concentrica*, *A. rimosa*, *A. cf. rubromarginata* and *A. zangii* are first reports for Thailand; detailed morphological and molecular data are provided for the Thai material.

Key words: *Amanitaceae*, ectomycorrhizal fungi, morphology, phylogeny

Introduction

Amanita Pers. is an important genus of mushrooms that includes several species that are widely recognized as the most toxic mushrooms in the world. The *Amanitaceae* is defined as those agarics having bilateral, divergent lamellar trama and a longitudinally acrophysalidic stipe context (Tulloss & Yang 2016e, Tulloss *et al.* 2016). *Amanita* is concisely characterized as the set of species of the *Amanitaceae* that exhibit schizohymenial development in their agaric and secotioid species (Tulloss & Yang 2016e, 2016f, Tulloss *et al.* 2016). Most species in the genus are considered to be ectomycorrhizal (EcM) and their distribution in forests and heaths including (among others) *Betulaceae*, *Dipterocarpaceae*, *Fabaceae*, *Myrtaceae*, *Pinaceae*, and *Salicaceae* suggests that they play a critical role in forest ecosystems worldwide (Yang 1997, Weiß *et al.* 1998, Zhang *et al.* 2004).

As of 15 October 2016, *Amanita* comprised approximately 900 taxa of which 540 had validly published names; 305 were known by provisional names or temporary codes; and the remainder was known by misapplied names or had invalid or illegitimate names (Tulloss & Yang 2016a). *Amanita* is divided into two subgenera and seven sections (Tulloss & Yang 2016f, 2016g). Subgenus *Amanita* is characterized by basidiospores that lack an amyloid (dark blue) color change in iodine solutions (e.g. Melzer's reagent). Subgenus *Amanita* is divided into three sections: sect. *Amanita*, sect. *Caesareae* Singer and sect. *Vaginatae* sensu Yang (1997). Subgenus *Lepidella* is characterized by basidiospores that are amyloid in iodine solutions, and is divided into four sections: sect. *Amidella* (E.-J. Gilbert) Veselý, sect. *Lepidella* sensu Bas (1969), sect. *Phalloideae* (Fr.) Quél. and sect. *Validae* (Fr.) Quél.

The evolution and diversity of *Amanita* species from Southeast Asia has been largely understudied (Moncalvo *et al.* 2000, Yang 2015, Tulloss *et al.* 2015). However, amanitas from China have been quite extensively studied (e.g., Chen *et al.* 2014, Deng *et al.* 2014, Li & Cai 2014, Zhang *et al.* 2015). A recent monograph of *Amanita* species of

China (Yang 2015) reported approximately 130 taxa, while from Guangdong Province alone Li & Cai (2014) reported more than 30 taxa. In contrast, there have been few studies on *Amanita* in Thailand, with only 25 *Amanita* taxa documented from northern Thailand (Sanmee *et al.* 2008). Recent fungal biodiversity studies in evergreen *Fagaceae* and deciduous dipterocarp forests in the Mekong subregion, which includes border areas of northern Thailand, China (Yunnan Province), Laos, and Myanmar, have revealed high species diversity, including in ectomycorrhizal genera (Sysouphanthong *et al.* 2010, Zhao *et al.* 2010, Zhao *et al.* 2011, Ye *et al.* 2014, Chen *et al.* 2015, Cui *et al.* 2015, Wisitrassameewong *et al.* 2015), demonstrating the need for further study of this genus. In the present paper, morphological descriptions and distribution data for four taxa discovered for the first time in Thailand, and for a new species, *Amanita castanea*, belonging to *Amanita* [section *Validae*, series *Mappae*] stirps *Citrina* are provided. Species identifications and the new taxon proposal are supported by both morphological and phylogenetic analyses.

Materials and methods

Collections

Specimens were collected mainly in forests dominated by *Fagaceae* (*Castanopsis*, *Lithocarpus*, *Quercus*) and/or *Dipterocarpaceae* (*Dipterocarpus*, *Shorea*) in Chiang Mai and Chiang Rai provinces in northern Thailand during the rainy season from June to August 2012–2014. Fresh specimens were examined, photographed, described and dried using a food dehydrator. Tissue samples for later DNA analyses were taken from fresh or dried specimens. Herbarium codes follow Index Herbariorum (Thiers 2016) with the exception of “RET”, which is the code adopted for R.E. Tulloss’ Herbarium Rooseveltensis Amanitorum. The examined specimens are deposited in the Mae Fah Luang University Herbarium (MFLU) and Biotec Bangkok Herbarium (BBH). All author citations of species rank not included in the main body of the text are located in Table 1.

Morphological study

Macro-morphological features were described from fresh specimens. Color codes are according to Kornerup & Wanscher (1978). Microscopic features such as basidia, basidiospores, pileipellis, partial veil and universal veil were studied from dried tissue mounted in H₂O and KOH 5% aqueous solution. Congo red was used for highlighting all tissues, and amyloidity of basidiospores was observed using Melzer’s reagent. All microscopic features were photographed using a Nikon Eclipse 80i compound microscope fitted with a Cannon 600D digital camera. Dimensions of microscopic characters were measured using Image Frame Work (Tarosoft®, Thailand). In the description of basidiospore measurements, the following notation was used: “[*n/m/p*]” indicating *n* basidiospores were measured from *m* basidiomata of *p* collections with a minimum of 25–50 basidiospores from each basidiome. Spore length and width are measured in side view not including the apiculus. Size and shape of basidiospores are presented in a form following the description of ranges for biometric variables according to Tulloss (2016a) (*a*–) *b*–*c* (–*d*), in which *b* represents the 5th percentile, *c*, the 95th percentile, while *a* and *d* are the lowest and highest extreme values measured, respectively. The range of length/width ratio of basidiospores (Q) is provided. Summary data are also provided where appropriate following the method of Tulloss (2016a). In addition to Tulloss’ standard format, standard deviation is provided for Q’ (the mean of all Q values computed for a single taxon). The description of the universal veil on the stipe base is taken from the interior tissue only without regard to the surfaces. Bas (1969) observed that in *Amanita*, if clamps are not found on the bases of basidia they will not be found in any other tissue of the basidiome. Tulloss’ experience over decades supports Bas’ hypothesis. Hence, absence of clamps only with relation to basidia are reported. This situation arises in all sections of *Amanita*. Clamps are absent from bases of basidia for all taxa in the following *Amanita* sections of concern in this paper: *Vaginatae* sensu Zhu L. Yang, *Phalloideae*, and *Validae*. *Amanita* [section *Lepidella*] stirps *Hesleri* is characterized in part by absence of clamps (Tulloss *et al.* 2016). Faces of Fungi and Index Fungorum numbers are provided as explained in Jayasiri *et al.* (2015), Index Fungorum (2016) and Mycobank (2016).

TABLE 1. Taxa of *Amanita* included in molecular phylogenetic analysis. Newly generated sequences of taxa from Thailand are highlighted in bold.

Species	Collection	Locality	GenBank accession no.	
			ITS	LSU
<i>Amanita altipes</i> Zhu L. Yang, M. Weiss & Oberw.	HKAS 58805	USA	JN943175	JN941158
<i>A. areolata</i> T. Oda, C. Tanaka & Tsuda*	FB-30251 (CBM)	Japan	AB167727	—
<i>A. bisporigera</i> G.F. Atk.	RET 377-9	USA	KJ466374	KJ466434
<i>A. brunnescens</i> G.F. Atk.	RET 637-7	USA	KT006762	KT006766
<i>A. caesarea</i> (Scop.) Pers.	RET 450-3	Italy	JX844686	KF877208
<i>A. castanea</i>	MFLU 15-1424	Thailand	KU904823	KU877539
<i>A. citrina</i> (Schaeff.) Pers.	Z.L. Yang D 33	German	—	AF024446
<i>A. cf. citrina</i>	HKAS 34170	China	AY436449	AY436489
<i>A. citrina</i>	KA 12-1612	Korea	KF245909	KF245893
<i>A. citrina</i>	LEM 960298	Japan	AB015679	—
<i>A. citrina</i> *	JM96/61	USA	—	AF097378
<i>A. citrina</i> *	EMF1	China	JF273504	—
<i>A. citrina</i> var. <i>grisea</i> (Hongo) Hongo	LEM 970501	Japan	AB015680	—
<i>A. citrina</i> var. <i>grisea</i>	HKAS 32506	China	—	AF024447
<i>A. concentrica</i>	MFLU 15-0128	Thailand	KU904816	KU877534
<i>A. congolensis</i> (Beeli) Tulloss, B. E. Wolfe, K. W. Hughes, Kudzma & Arora	RET 346-6	D. R. Congo	KR919753	HQ539736
<i>A. cf. esculenta</i> Hongo & I. Matsuda	TRTC 150410	Thailand	JX844709	KF877229
<i>A. excelsa</i> (Fr. : Fr.) Bertill.	HKAS 31510	Germany	AY436453	AY436491
<i>A. exitialis</i> Zhu L. Yang & T.H. Li	HKAS 75774	China	JX998027	JX998052
<i>A. flavipes</i> S. Imai	KA 12-1517	Korea	KF245912	KF245896
<i>A. flavoconia</i> G.F. Atk.	ANDES F408CV3	Colombia	FJ890029	FJ890041
<i>A. flavorubescens</i> G.F. Atk.	F:PRL 6062	USA	GQ166902	—
<i>A. cf. fritillaria</i> Sacc.	HKAS 56832	China	KJ466372	KJ466479
<i>A. fuliginea</i> Hongo	HKAS 75780	China	JX998023	JX998048
<i>A. fuligineoides</i> P. Zhang & Zhu L. Yang	LHJ 140722-18	China	KP691686	KP691697
<i>A. fulva</i> (Schaeff.) Fr.	KA 12-1406	Korea	KF017933	KF021672
<i>A. hemibapha</i> (Berk. & Broome) Sacc.	RET 342-8	India	JX844716	KF877233
<i>A. ibotengutake</i> T. Oda, C. Tanaka & Tsuda	FB 30966 (CBM)	Japan	AB080985	—
<i>A. cf. jacksonii</i> Pomerl.	FCME 21652	Mexico	JX844720	KF877255
<i>A. japonica</i> Hongo ex Bas	TMI 26147	Japan	KJ922994	KJ922990
<i>A. lavendula</i> (Coker) Tulloss, K. W. Hughes, Rodrig. Cayc. & Kudzma	RET 639-7	USA	KP866163	KR865979
<i>A. modesta</i> Corner & Bas	HKAS 75405	China	KJ466379	KJ466439
<i>A. morrisii</i> Peck	RET 271-7	USA	KT213441	KT213442
<i>A. muscaria</i> (L.) Lam.	RET 320-1	USA	EU071911	EU071984
<i>A. novinupta</i> Tulloss & J. Lindgr.	RET 060-2	USA	KF561974	KF561978
<i>A. ocreata</i> Peck	HKAS 79686	USA	KJ466381	KJ466442
<i>A. orientifulva</i> Zhu L. Yang, M. Weiss & Oberw.	KA 12-0642	Korea	KF017940	KF021679

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TABLE 1. (Continued)

Species	Collection	Locality	GenBank accession no.	
			ITS	LSU
<i>A. orientigemmata</i> Zhu L. Yang & Yoshim. Doi	HKAS 38345	China	AY436465	AY436497
<i>A. parvipantherina</i> Zhu L. Yang, M. Weiss & Oberw.	HKAS 38297	China	AY436467	AY436499
<i>A. phalloides</i> (Fr. : Fr.) Link	HKAS 75773	USA	JX998031	JX998060
<i>A. ponderosa</i> Malençon & R. Heim	B05	Portugal	KJ950797	KJ950780
<i>A. porphyria</i> Alb. & Schwein.: Fr.	HKAS 31531	China	AY436471	AY436500
<i>A. porphyria</i>	RET 079-1	Switzerland	KP866181	KP866192
<i>A. porphyria</i> *	RET 370-10	Switzerland	—	KP866187
<i>A. rimosa</i>	MFLU 14-0064	Thailand	KU904819	KU877532
<i>A. rimosa</i>	MFLU 15-0153	Thailand	KU904820	KU877533
<i>A. rubescens</i> Pers.:Fr.	KA 12-1221	Korea	KF245919	KF245903
<i>A. cf. rubromarginata</i>	MFLU 15-01418	Thailand	KU904821	KU877537
<i>A. cf. rubromarginata</i>	MFLU 15-01420	Thailand	KU904822	KU877538
<i>A. rubrovolvata</i> S. Imai	HKAS 56744	China	JN943181	JN941156
<i>A. sepiacea</i> S. Imai	HKAS 38716	China	AY436473	AY436501
<i>A. solitaria</i> sensu NCL	HKAS 31459	Germany	AY436475	—
<i>A. sp.</i>	HKAS 77321	China	KJ466416	KJ466481
<i>A. sp.1</i>	HKAS 38419	China	AY436474	AY436502
<i>A. sp.1</i> Yang	RET 327-10	USA	KJ466396	KJ466458
<i>A. sp.2</i> Yang	HKAS 77350	China	KJ466400	KJ466462
<i>A. subglobosa</i> Zhu L. Yang	HKAS 58837	China	JN943177	JN941152
<i>A. subjunquillea</i> S. Imai	HKAS 75771	China	JX998032	JX998063
<i>A. vaginata</i> (Bull. : Fr.) Lam.	KA 12-0962	Korea	KF017950	KF021689
<i>A. virginiana</i> (Murrill) Murrill	RET 374-8	Japan	JX844750	KF877305
<i>A. virosa</i> (Fr.) Bertill.	HKAS 71040	China	KJ466429	KJ466496
<i>A. volvata</i> (Peck) Lloyd	KA 12-1367	Korea	KF245923	KF245907
<i>A. yuaniiana</i> Zhu L. Yang	HKAS 29516	China	AY436479	AF024488
<i>A. zangii</i>	MFLU 15-0130	Thailand	KU904817	KU877535
<i>A. zangii</i>	MFLU 15-0144	Thailand	KU904818	KU877536
<i>Limacella glioderma</i> (Fr.) Maire	19-X-1998	Netherlands	AY176451	AY176452
<i>L. cf. glioderma</i>	7-VIII-2000	USA	AY176453	AY176454

* indicates sequences from GenBank appearing in BLAST search results, but not used in phylogenetic tree inference.

DNA isolation, amplification and sequencing

Specimens were processed for molecular analyses at several core facilities including Southwest Forestry University, China (SWFU), the Botany Department, University of Wyoming, USA, and Botanic Garden Meise, Belgium, using a variety of methodologies for extraction of genomic DNA, PCR and sequencing. Genomic DNA from dried specimens processed at SWFU was extracted using a commercial DNA extraction kit (E. Z. N. A. Forensic Kit, D3591-01, Omega Bio-Tek), and PCR protocols were performed using previously described methods of White *et al.* (1990) with some modifications described in Zhao *et al.* (2010). Sequencing was performed on an ABI Prism Genetic analyzer (Applied Biosystems) or on ABI 3730 XL DNA analyzer (Applied Biosystems) at Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China. Genomic DNA extractions at the University of Wyoming were performed using a CTAB protocol with phenol-chloroform-isoamyl alcohol purification, followed by cleaning with a silica-matrix binding procedure,

as described in Miller (2004). At Botanic Garden Meise, DNA extractions were performed using a slightly different CTAB protocol. PCR amplification of ITS (nuclear ribosomal Internal Transcribed Spacer) and LSU regions was performed using the primer pairs ITS5/ITS4, ITS1/ITS4 and LR0R/LR5, respectively. Purified products were then sequenced using the same primer combinations as for PCR at the Nucleic Acid Exploration Facility at the University of Wyoming on an ABI 3130 XL DNA analyzer (Applied Biosystems), or at Macrogen Europe (Amsterdam) using an ABI 3730 XL DNA analyzer (Applied Biosystems). Forward and reverse reads were assembled and edited with Geneious Pro 5.1.7 (Biomatters Ltd., Auckland, New Zealand).

TABLE 2 Results of GenBank BLAST searches for ITS and LSU sequences from four new records of *Amanita* taxa from Thailand. S=Similarity and QC= Query Cover.

Species	Collection	Locality	GenBank accession no.		References
			ITS	LSU	
<i>A. concentrica</i> T. Oda, C. Tanaka & Tsuda	FB 24901 (CMB) (holotype)	Japan	NR119387 S=100%, QC=100%	—	Oda <i>et al.</i> 2002a
<i>A. rubromarginata</i> Har. Takh.	RET 383-1 (isotype)	Japan	KP662538 S=99.1%, QC=100%	KF877279 S=99.6%, QC=89%	Sánchez-Ramírez <i>et al.</i> 2014
<i>A. rimosa</i> P. Zhang & Zhu L. Yang	HKAS 49675 (holotype)	China	FJ176728 S=99.8%, QC=100%	—	Zhang <i>et al.</i> 2010
<i>A. rimosa</i>	HKAS 75777	China	JX998018 S=99.8%, QC=98%	KJ466499 S=99.8%, QC=94%	Cai <i>et al.</i> 2014
<i>A. rimosa</i>	HKAS 75779	China	JX998020 S=99.7%, QC=100%	KJ466500 S=99.8%, QC=94%	Cai <i>et al.</i> 2014
<i>A. rimosa</i>	HKAS 77279	China	KJ466392 S=100%, QC=100%	KJ466454 S=100%, QC=95%	Cai <i>et al.</i> 2014
<i>A. zangii</i> Zhu L. Yang, T.H. Li & X.L. Wu	HKAS 77331 (holotype)	China	KJ466432 S=100%, QC=99%	KJ466499 S=99.9%, QC=95%	Yang <i>et al.</i> 2001
<i>A. zangii</i>	GDGM 29241	China	KJ466433 S=99.8%, QC=99%	KJ466500 S=100%, QC=96%	Cai <i>et al.</i> 2014

DNA sequence dataset assembly

Thirty sequences of collections from Thailand were newly generated for this study and deposited in GenBank (GB) (<http://www.ncbi.nlm.nih.gov/>; Table 1). Additional LSU and ITS sequence data used in the analyses were retrieved from GenBank (Table 1). Initial BLAST searches (<http://blast.ncbi.nlm.nih.gov/>) of both LSU and ITS1+5.8S+ITS2 sequences were performed to estimate similarity with *Amanita* sequences already in GenBank. Terminal motifs for 18S, 5.8S and 28S loci were determined based on sequences catalogued by Rodríguez-Caycedo *et al.* (2016).

Phylogenetic analyses

A total of 68 ITS and 64 LSU sequences from *Amanita* species from Thailand and from GenBank were used for phylogenetic analyses. The quality of the sequences was considered in selecting sequences from GenBank for use in the analyses. The data matrix was initially aligned with MAFFT v.7.0 (Katoh *et al.* 2005) using the E-INS-i iterative refinement algorithm, with minimal manual adjustment in BioEdit v.7.0.9 (Hall 1999). For ITS, only the positions corresponding to ITS1 and ITS2 were kept in the alignment. The program Gblocks v0.91b (Castresana 2000) was used to exclude poorly aligned positions of the alignment with the following parameter settings: maximum number of contiguous non-conserved positions = 10 bp, minimum block size = 6 bp, and gaps allowed within selected blocks. Phylogenetic tree inference was performed using both Maximum Likelihood (ML) and Bayesian Inference (BI). The ML analyses were performed using RAxML-HPC2 (Stamatakis 2014) on the CIPRES Science Gateway V. 3.3 (Miller *et al.* 2009), with default settings except the number of bootstrap replicates was set to 1,000 for both single-gene and combined gene analyses. For the latter a mixed model partition was used. BI was performed using MrBayes v. 3.2.1 (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002, Ronquist & Huelsenbeck 2003). The best substitution model (GTR+I+G) was determined for the LSU and ITS datasets separately using the Akaike Information Criterion (AIC) with MrModeltest v. 3.7 (Posada 2008) and used in both single-gene and combined-gene analyses. The Bayesian analyses were conducted with six simultaneous Markov chains and trees were summarized every 100th generation. The analyses were stopped after 1,000,000 generations, when the standard deviation of split frequencies was below 0.01. The burn-in phase (20%) was estimated by checking the stationarity in the plot generated by the sump command. The remaining 8,000 trees were used to generate a majority-rule consensus tree. In both ML and BI analyses, *Limacella glioderma* (Fr.) Maire and *Limacella* cf. *glioderma* sequences from GB were selected as the outgroup. Phylograms

were visualized with FigTree ver. 1.3.1 (Rambaut 2009). The tree topologies obtained from ML and BI were identical, therefore only the ML combined gene tree is shown (Figure 7). The aligned combined gene dataset has been deposited in TreeBASE (TB2: S19318). We do not consider this tree to be a useful phylogenetic hypothesis for the whole genus *Amanita*. The restricted phylogenetic utility of ITS and LSU were discussed briefly by Tulloss *et al.* (2016), who also provided references to relevant literature. Figure 7 is intended to show, in a single figure, some strictly local relations of taxa considered in this article to other taxa for which sequences are known.

Results

DNA sequence analyses

Since *A. castanea* appears to be a new species on morphological grounds, both ITS and LSU sequences of *A. castanea* were subjected to a BLAST search against both GenBank and R.E. Tulloss' personal database, which contains unpublished sequences from yet to be described species. Details are presented with all other data in the following section.

Taxonomy

Amanita castanea Thongbai, Tulloss, Raspé & K.D. Hyde, *sp. nov.* (Figure 1 and 2)

Index Fungorum number: IF552009; *Mycobank:* MB 818356; *Facesoffungi number:* FoF 02074

Etymology:—'castanea' refers to the chestnut color of the pileus.

Holotype:—THAILAND, Chiang Mai Province, Doi-saket District, Thep-sadet Subdistrict, elev. 1300 m, 30 June 2014, B. Thongbai (MFLU15-1424!).



FIGURE 1. Basidiomata of *Amanita castanea*. a young and mature basidiomata. b–d basidiospores in 5% KOH. f–h basidiospores in congo red. i–k basidiospores in Melzer's reagent. l–o basidia and subhymenium at different stages of development. (a–o: BZ201405, holotype) (scale bar: a = 2 cm, b–k = 6 μ m, l–o = 10 μ m).

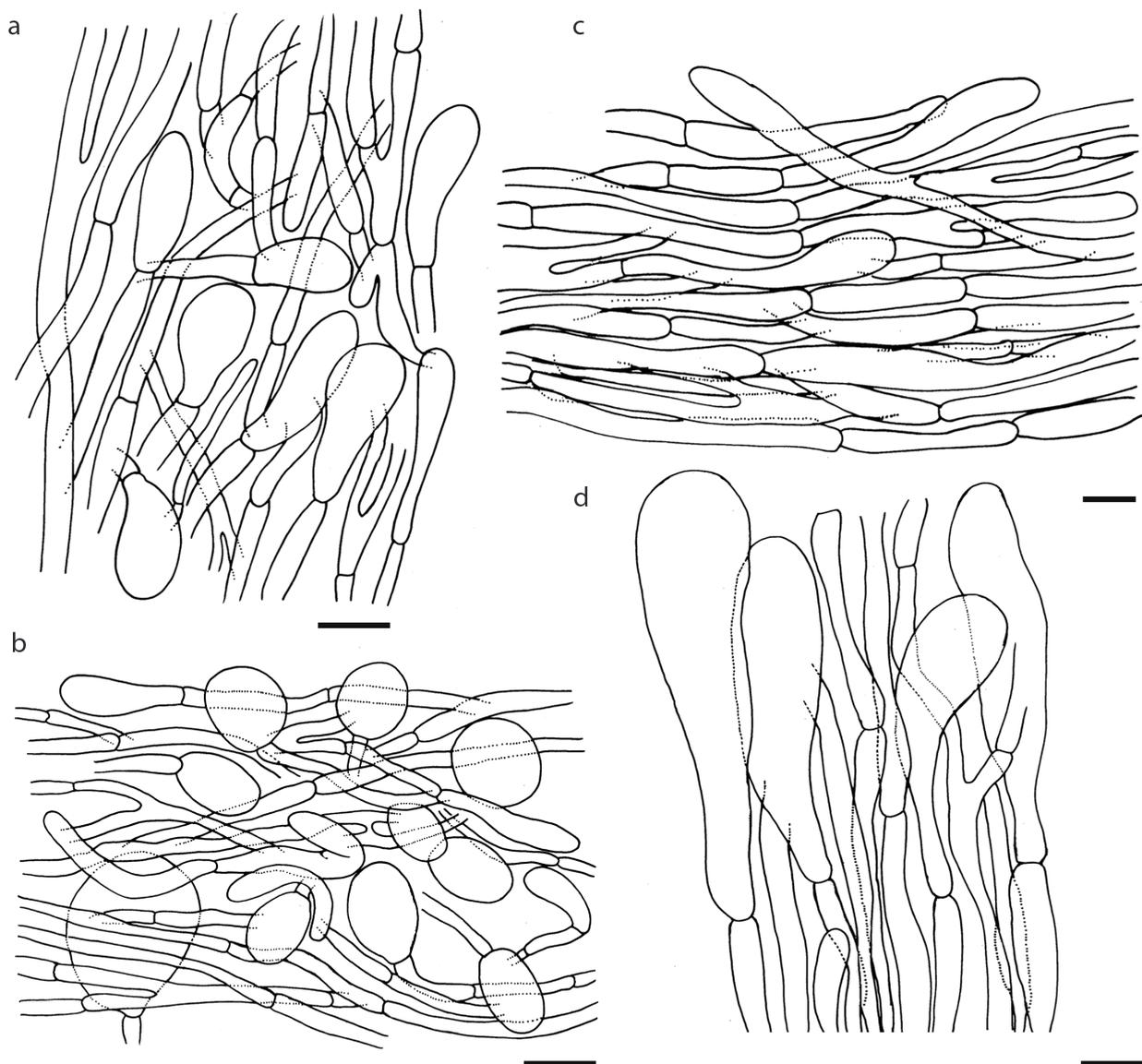


FIGURE 2. Microcharacters of *Amanita castanea*. a section of outer layer from partial veil. b section of velar remnants from pileus. c section of outer layer from pileus. d longitudinally acrophysalidic stipe trama. (a–d: BZ201405, holotype) (scale bars: a–d = 5 μ m).

Basidiomata [Fig. 1(a)] small to medium-sized. **Pileus** 50–75 mm wide, parabolic to hemispheric when young, convex to plane at maturity, sometimes depressed at center, dry, slightly viscid when moist, with shiny, sericeous or silky surface, chestnut to dark brown colored, darker at center, light brown to brownish orange (7D6–7C5) towards margin, with universal veil mostly towards margin, rarely over disc, as scattered gray to brownish gray, reddish brown to grayish brown warts or small floccose patches; margin incurved to flaring upward, non-striate, non-appendiculate; context 2–4 mm thick above stem, soft to slightly hard; pale yellowish or cream (4A2–3). **Lamellae** 4–6 mm broad, free to nearly free, subdistant when mature, yellowish white (3A2–3) to very pale greenish white (30A2–3); lamellulae of 3–6 lengths, attenuate to nearly truncate. **Stipe** 50–95 \times 10–15 mm (length includes bulb), nearly cylindrical or slightly tapering upwards, bulbous, white to dull white ground becoming brown (6D5, 6E5) at maturity, often covered with light orange grayish orange (6B3–6C3) squamules or fine flocculae, often with brownish ciliate scales formed from the outer layer of stipe below the partial veil in mature material (note: this is a common environmental effect seen in many amanitas), turning slightly yellow brown when bruised; context stuffed to nearly hollow, thin, yellowish white (4A2). **Bulb** compressible, subglobose to hemispheric, marginate, up to 28 mm wide, white to dirty white (1A1–2). **Universal veil on stipe base** as very short volval limb on bulb margin, cottony, greenish gray (30A1–2). **Partial veil** subapical, membranous, robust, skirt-like, greenish gray or greenish yellow (3B2), darker grayish green at thickened edge. **Odor** potato-like.

Lamellar trama bilateral, divergent; mediostratum 30–40 μm wide; filamentous hyphae 1.8–6 μm wide, branching, hyaline, with slightly inflated elements; vascular hyphae not observed. **Subhymenium** [Fig. 1(l)] 22–35 μm thick; inflated cells dominating, in 3–4 layers, subglobose, ovoid, 10–20 \times 8–12 μm , subtended by concatenated partially inflated hyphal segments. **Basidia** [Fig. 1(l–o)] 32–39(–44) \times 11–13(–14) μm , narrowly clavate to clavate, mostly 4-, occasionally 2-spored, with sterigmata up to 5 μm long; clamps absent. **Basidiospores** [Fig. 1(b–k)] [53/1/1] (7.2–) 7.3–9.4 (–9.7) \times (6.3–) 6.5–8.7 (–9.4) μm , (L' = 8.2 μm ; W' = 7.6 μm ; Q = 1.0–1.19 (–1.25); Q' = 1.09 \pm 0.06), smooth, hyaline, colorless, thin-walled, amyloid, globose to subglobose, infrequently broadly ellipsoid, occasionally adaxially flattened; apiculus rather variable, sublateral, very prominent to rather small, cylindrical to truncate-conic; contents monoguttulate or occasionally granular; white in deposit. **Lamellar edge** sterile; filamentous hyphae 3–7 μm wide, hyaline, colorless or pale yellow, thin-walled; inflated cells dominating, mostly globose to subglobose and sometimes ovoid, 9–22 \times 9–18(–22) μm , colorless, thin-walled. **Pileipellis** [Fig. 2(c)] up to 200 μm thick, 1-layered; loose, sometime branching, filamentous hyphae with slightly swollen tips, 4–10(–20) μm wide, minimal gelatinization limited to the pileipellis surface, thin-walled, with intracellular pale yellow pigment. **Universal veil on pileus** [Fig. 2(b)] filamentous hyphae, 2.5–6.2 μm wide, branching, with slightly inflated elements, with terminal subfusiform to narrowly clavate cells, 30–53 \times 15–21 μm , hyaline, thin-walled; inflated cells, globose to subglobose to ovoid to elongate, dominating, 9–22 \times 9–18(–22) μm ; vascular hyphae occasional. **Universal veil on stipe base** filamentous hyphae 3–8.5 μm wide, more abundant than on pileus, branching, hyaline, or with intracellular pale brown pigment; inflated cells similar in form to but less frequent than those on pileus; vascular hyphae occasional, 7–8.5 μm wide. **Stipe trama** [Fig. 2(d)] longitudinally acrophysalidic; filamentous hyphae 2.2–8.8 μm wide; acrophysalides subfusiform to clavate, 120–310 \times 25–45 μm ; vascular hyphae rare. **Partial veil** [Fig. 2(a)] filamentous hyphae 2–5 μm wide, gelatinized, branching, hyaline, inflated cells terminal, thin-walled, subclavate to clavate, 114–120 \times 25–36 μm , occasionally with intracellular pale brown pigment; vascular hyphae rare.

Habitat: scattered on the ground in forest of *Fagaceae*.

Specimen examined: THAILAND, Chiang Mai Province, Doi-saket District, Thep-sadet Subdistrict, elev. 1,300 m, 30 June 2014, B. Thongbai BZ201405 (holotype MFLU15-1424!, isotype BBH 40844!).

Known distribution: Only reported from northern Thailand.

Remarks: *Amanita castanea* belongs to subgenus *Lepidella*, section *Validae*, which is characterized by amyloid spores, non-appendiculate pileus margin and non-membranous universal veil (Corner & Bas 1962, Bas 1969). Within sect. *Validae*, *A. castanea* fits in the stirps *Citrina* (Singer 1975) of series *Mappae* (Drehmel *et al.* 1999), which is characterized by a compressible, marginate to submarginate bulb at the stipe base with universal veil remnants as a low and irregular marginal rim or limb (Tulloss & Yang 2016d). The species of series *Mappae* frequently have a potato- or radish-like odor (Tulloss *et al.* 2001). Molecular phylogenetic analyses also support our view that *A. castanea* is a novel species belonging to series *Mappae* because *Amanita castanea* is included in a well-supported clade comprised only of taxa from series *Mappae*. *Amanita castanea* is easily recognized by its chestnut colored pileus, small to medium-sized basidiomata, easily detachable universal veil in the form of scattered gray to brownish-gray, reddish-brown to grayish brown warts or small patches; a subglobose to hemispheric bulb with pale green tinge on top of the volval limb; and greenish gray or greenish yellow skirt-like partial veil that is darker grayish green at the thicker free edge. Morphologically, *A. castanea* is most closely related to *A. citrina* var. *grisea* and *A. porphyria*. *Amanita citrina* var. *grisea*, originally described from Japan, and later reported from southwestern China, has larger gray to dark gray basidiomata, and yellowish felty universal veil on the pileus (Tulloss & Yang 2016b). *Amanita porphyria*, originally described from Germany, has a virgate pileus that is dull red to grayish dull red, grayish purple, or pale brownish gray to violaceous brown, and is darkest in the center, and has a gray, thin, skirt-like partial veil. *Amanita porphyria* differs from *A. castanea* by having violaceous gray longitudinal fibers below the partial veil (Albertini & Schweinitz 1805, Neville & Poumarat 2004). Another brown-capped species in stirps *Citrina*, *A. solaniolens* (Stewart & Grund 1974), was originally described from eastern Canada and is now understood to occur at least as far south as Costa Rica (Tulloss *et al.* 2016a) and probably Andean Colombia. It is distinguished by a virgate cap that is dull-yellow to light blond at the margin and abruptly darkening to olive-brown at the center or largely brown, reminiscent of the cap of a tiny *A. brunnescens*. *Amanita solaniolens* also differs from *A. castanea* by having a creamy white to yellowish white universal veil comprised of flat patches on the pileus and a white to cream stipe with a partial veil that is white to pale yellow.

Initial BLAST searches on the ITS data in both GenBank and Tulloss' personal database, indicated the most similar sequences were from the following (author citations, source collections, and countries of collection in Table 1): *A. citrina* var. *grisea*—GB AB015680 with 94.1% similarity and 97% query cover (Oda *et al.* 1999); material possibly misidentified as *A. citrina*—GB JF273504 with 94.1% similarity and 100% query cover.

- 1) *A. porphyria*—GB KP866181 with 91.7% similarity and 99% query cover.

The BLAST search on the LSU sequence from *A. castanea* was also performed against both GenBank and Tulloss' personal database. The most similar sequences were from:

- 1) material probably misidentified as *A. citrina*—GB AF097378 with 98.3% similarity and 100% query cover (Drehmel *et al.* 1999).
- 2) *A. lavendula*—GB KR865979 with 98.3% similarity and 99% query cover (Tulloss *et al.* 2015).

Amanita series *Mappae* was proposed by Drehmel *et al.* (1999) on genetic and morphological grounds. Drehmel's clade is reproduced in general form at the top of Figure 7, although some names associated with GenBank sequences appear to be misapplied, *A. mappa* (Batsch) Fr. (= *A. citrina*), *A. porphyria*, *A. citrina* var. *grisea*, and *A. lavendula* are all classic taxa of stirps *Citrina* and are presented as a group sister to *A. brunnescens* as in the tree of Drehmel *et al.* (1999). In a study of long branch attraction in LSU trees involving 230 species of the *Amanitaceae* (Tulloss unpub. data, briefly discussed in Tulloss *et al.* 2016), a topology of the *Validae* similar to that in Figure 7 was achieved. In both cases, a small group of taxa including species such as *A. congolensis* and *A. media* Dav. T. Jenkins (Jenkins 1983; Tulloss 2016g) (not treated in the present study) are shown as sister to Series *Mappae* or appear as earlier divergent than the *Mappae*. This seems improbable to us, Series *Mappae* includes all the soft, globose to subglobose-bulbed taxa of the *Validae* that are known. This characteristic type of bulb is shared with a large number of the taxa of section *Phalloideae*, which is commonly shown as a sister group to the *Validae* as in Figure 7. *Amanita congolensis* and *A. media* have firm, fusiform bulbs in contrast with the *Mappae* and have evolved longer and proportionately narrower (elongate to cylindrical) spores than are to be found in most of the *Phalloideae* and *Validae*. Hence, it is desirable to have more information before concluding that such taxa could be among the earliest diverging in the *Validae*. Insufficient sampling, particularly in subsaharan Africa, southern Asia, and Australia may be contributing to error.

Amanita concentrica T. Oda, C. Tanaka & Tsuda, *Mycoscience* 43 (1): 81, 2002a (Figure 3)

Basidiomata [Fig. 3(a)] medium-sized. **Pileus** ca. 115 mm wide, hemispheric or parabolic when young, then convex to plane at maturity, dry, slightly shiny when moist, white to yellowish white (3A1-2), with pale yellow or butter yellow at center (4A2-3), covered with distinctive universal veil remnants as pyramidal warts up to 4 mm high, crowded over disc, becoming floccose patches toward margin, whitish to light orange, grayish orange to brownish orange (4C3-5A3); margin slightly striate, not appendiculate with universal veil, sometimes appendiculate with fragments of partial veil; context 4–9 mm thick above stem, soft to slightly hard, white to orange white (5A1-2). **Lamellae** 4–8 mm broad, free, crowded, white to yellowish white (3A1-2); lamellulae of 3–6 lengths, truncate to subtruncate. **Stipe** 95–125 × 1.5–2.3 mm (length includes bulb), tapering upwards, white to pale yellow (1A2-3A1-3), squamulose to scaly, light yellow (3A1-2) at apex, decorated with grayish orange to brownish orange (6B3-6D4) scales at base; context stuffed to hollow, white to yellowish white (1A1-3A1-2). **Bulb** subclavate to subnapiform, marginate, up to 35 mm wide, white to yellowish white (3A1-2). **Universal veil on stipe base** 3–5 concentric rings of whitish, grayish yellow to brownish orange (6B3-6D4) scales on the upper part of the bulb. **Partial veil** subapical, membranous, white to yellowish white (1A2-3A1-2). **Odor** not recorded.

Lamellar trama bilateral, divergent; mediostratum 40–60 µm wide, filamentous hyphae 2–8 µm wide, frequently branching, hyaline, colorless, with terminal cell slightly clavate to subfusiform, 45–130 × 10–25 µm, thin-walled; vascular hyphae rare. **Subhymenium** [Fig. 3(k)] 20–45 µm thick; filamentous hyphae 4–15 µm wide, with some intercalary inflated elements; inflated cells dominating, in 2–3 layers, broadly ellipsoid to ellipsoid to occasionally subfusiform, 9–26 × 8–22 µm. **Basidia** [Fig. 3(k–m)] 43–55 × 6–14 µm, clavate, 4- or (occasionally) 2-spored, with sterigmata up to 6 µm long; clamps present. **Basidiospores** [Fig. 3(b–j)] [50/1/1] 7.4–9.7 (–11.4) × (5.7–) 6.5–8.4 (–8.8) µm, (**L'** = 8.5 µm; **W'** = 7.4 µm; **Q** = (1.02–) 1.05–1.30 (–1.56); **Q'** = 1.16 ± 0.11), smooth, hyaline, colorless, thin-walled, inamyloid, subglobose to broadly ellipsoid, occasionally globose or ellipsoid, occasionally adaxially flattened; apiculus rather variable, sublateral, prominent, cylindrical to truncate-conic; contents monoguttulate; white in deposit. **Lamellar edge** sterile; filamentous hyphae 3–8 µm wide, hyaline, colorless, thin-walled, with slightly inflated segments; inflated cells dominating ovoid to subglobose to ellipsoid, and occasionally broadly clavate, 18–40 × 8–22 µm, hyaline or occasionally with intracellular pale brown pigment. **Pileipellis** 150–420 µm thick, 2-layered; upper layer 70–220 µm thick, filamentous hyphae 3–5 µm wide, strongly gelatinized, branching, hyaline, colorless, thin-walled; lower layer about 80–200 µm thick, filamentous hyphae 2–4 µm wide, branching, hyaline, thin-walled. **Universal veil on pileus** filamentous hyphae 4–7 µm wide, branching, hyaline, colorless or occasionally with intracellular pale brown



FIGURE 3. Basidiomata of *Amanita concentrica*. a young and mature basidiomata. b–d basidiospores in 5% KOH. e–g basidiospores in Congo red. h–j basidiospores in Melzer's reagent. k–m basidia and subhymenium at different stages of development; showing clamps at the bases of basidia. (a–m: BZ201326) (scale bars: a = 5 cm, b–j = 5 μ m, k = 20 μ m, l–m = 10 μ m).

pigment, thin-walled; inflated cells ovoid to ellipsoid dominating, 20–62 \times 10–38 μ m; vascular hyphae not observed. **Universal veil on stipe base** filamentous hyphae 2–7 μ m wide dominating, hyaline, colorless or occasionally with intracellular pale brown pigment; ovoid to ellipsoid cells, 30–62 \times 15–32 μ m; vascular hyphae not observed. **Stipe trama** longitudinally acrophysalidic; filamentous hyphae 2–9 μ m wide; acrophysalides clavate to pyriform, 120–210 \times 25–45 μ m; vascular hyphae not observed. **Partial veil** filamentous hyphae 2–5 μ m wide, branching, hyaline, colorless.

Habitat: scattered on the ground in forest of *Fagaceae*.

Specimens examined: THAILAND, Lampang Province, along the road number 1252, 18.935, 99.390833, elev. 1,450 m, 5 July 2013, O. Raspé & B. Thongbai BZ201326 (MFLU 15-0128, BBH 40576).

Known distribution: This species was originally described from Japan. It has also been found in China, Nepal, northern India (Tulloss & Yang 2016h) and now Thailand.

Remarks: *Amanita concentrica* belongs to *Amanita* [sect. *Amanita*, series *Amanita*] stirps *Concentrica* (Tulloss & Yang 2016a). In the field, outstanding morphological characteristics of *A. concentrica* are the whitish to brownish orange, grayish orange to orange pyramidal warts on a white to pale-yellowish pileus. The presence of these prominent, pointed warts and concentric rows of warts on the upper bulb can lead to taxonomic confusion with members of *Amanita* [sect. *Lepidella*] subsect. *Solitariae* (e.g., *A. eijii* Zhu L. Yang [2002]). However, the inamyloid basidiospores clearly place *A. concentrica* into subgenus *Amanita*, for which there is genetic support. The size of basidiomata as well as the size and shape of basidiospores of the Thai collection is very similar to the original description from Japan (Oda *et al.* 2002a, 2002c). The holotype was reported from an area with elevation of 300 m, in an evergreen broad-leaved forest with *Castanopsis cuspidata* (Thunb. ex Murray) Schottky var. *sieboldii* (Makino) Nakai and *Quercus glauca*

Thunb. The Thai specimen was collected at an elevation of 1,450 m in a fagaceous forest with, e.g., *Castanopsis* spp. No LSU sequences from the holotype of *A. concentrica* have been accessioned in GenBank.

The orangish or rusty stains on the basidiomata collected in Thailand are reminiscent of similar stains on normally white amanitas that Tulloss has seen in Costa Rica and (more rarely) in North America. It is likely that the phenomenon is superficial and can be moved by water. (See the vertical streaks on the stems in Fig. 3.) This suggests that the phenomenon is not genetically determined by the amanita, but is due to some other organism's presence on the mushroom. The study of the surface ecology of amanitas is largely unpursued except for, to some degree, in the cases of *Hypomyces hyalinus* (Schwein.) Tul. & Tul. and *Mycogone rosea* Link. There are other phenomena that appear to be related to organisms "infecting" amanitas that have been the cause of naming "infected" mushrooms as new or provisional taxa—sometimes mistakenly—for example, the "yellowing syndrome" [*A. crassifolia* Bas nom. prov. (Bas 1969, Tulloss 2016d) and other taxa]; the "cheese odor syndrome" [*A. alexandri* Guzmán (Guzmán 1975, 1980; Morales-Torres *et al.* 1999; Tulloss 2016c)]; and blackening of the volva and adjacent tissues [*A. muscaria* var. *fuligineoverrucosa* Neville & Poumarat (Neville & Poumarat 2002, 2004; Tulloss 2005, 2016e) and *A. brunneolocularis* Tulloss, Ovrebo & Halling (Tulloss *et al.* 1992, 2016f)].

Amanita rimosa P. Zhang & Zhu L. Yang, *Fungal Diversity* 42: 124, 2010 (Figure 4)

Basidiomata [Fig. 4(a)] small-sized. **Pileus** 50–62 mm wide, hemispheric when young, convex to applanate at maturity, dry, slightly subviscid, shiny when moist, minutely rimose around margin, pale yellow to cream (4A2-3) at center, white to yellowish white (3A1-2) towards margin; margin non-striate, non-appendiculate; context 2–3 mm thick above stem, soft, white (1A1). **Lamellae** 4–5 mm broad, free, crowded, white to whitish (1A1); lamellulae of 2–3 lengths, attenuate. **Stipe** 70–90 × 15–20 mm (length includes bulb), subcylindric, slightly tapering upwards, bulbous, covered with minute, white to whitish (1A1-2) squamules, turning slightly yellowish white when bruised; context solid, white to whitish (1A1-2). **Bulb** globose to subglobose, marginate, 16–20 mm wide. **Universal veil on stipe base** as volval limb on bulb margin up to 10 mm high, white to whitish (1A1-2); limbus internus membranous, white (1A1). **Partial veil** subapical, membranous, thin, skirt-like, slightly striate, white (1A1). **Odor** not recorded.

Lamellar trama bilateral, divergent; mediostratum 35–40 µm wide, filamentous hyphae 3–5 µm wide, branching, hyaline, thin-walled, with terminal cells ellipsoid to fusiform, 30–75 × 10–28 µm thin-walled; vascular hyphae not observed. **Subhymenium** (Fig. 4k) 25–32 µm thick; inflated cells in 2–3 layers, subglobose to ovoid, dominating, 15–25 × 10–20 µm. **Basidia** [Fig. 4(k–m)] 38–42 × 15–18 µm, clavate, 4-spored, with sterigmata 4–6 µm long; clamps absent. **Basidiospores** [Fig. 4(b–j)] [50/2/2] (6.0–) 6.3–8.3 (–8.6) × (5.6–) 5.8–7.4 (–8.2) µm, (L^* = 7.0 µm; W^* = 6.6 µm; Q = 1.01–1.16 (–1.18); Q^* = 1.06 ± 0.05), smooth, hyaline, colorless, thin-walled, amyloid, globose to subglobose, occasionally broadly ellipsoid, often adaxially flattened; apiculus sublateral, small, cylindrical to truncate-conic; contents granular or rarely monoguttulate; white in deposit. **Lamellar edge** sterile; inflated cells dominating, subglobose to broadly ellipsoid, single or 2–3 in chain, 20–25 × 15–20 µm, hyaline, colorless, thin-walled. **Pileipellis** 130–190 µm thick, 2-layered; upper layer 80–90 µm thick, filamentous hyphae 2–6 µm wide, slightly gelatinized, hyaline, colorless, thin-walled, with terminal cells ellipsoid to clavate to fusiform, 70–270 × 25–38 µm; lower layer 50–100 µm thick, filamentous hyphae 2–5 µm wide, non-gelatinized, hyaline or occasionally with intracellular pale yellow pigment, thin-walled. **Universal veil on stipe base** filamentous hyphae 2–4 µm wide, no inflated cells observed, gelatinized, branching, hyaline, thin-walled. **Stipe trama** longitudinally acrophysalidic; filamentous hyphae dominating, 1–5 µm wide; acrophysalides clavate, 120–310 × 25–45 µm; vascular hyphae rare. **Partial veil** filamentous hyphae 2–7 µm wide, branching, hyaline, colorless, thin-walled; inflated cells, clavate to ellipsoid, abundant, 20–45 × 10–35 µm, occasionally subglobose (12–25 × 10–20 µm); vascular hyphae rare.

Habitat: solitary or scattered on the ground in forest of *Fagaceae*.

Specimens examined: THAILAND, Chiang Mai Province, Mae-taeng District, Pha-daeng Temple, elev. 1,075 m, 09 June 2012, B. Thongbai, BZ201264 (MFLU 14-0064, BBH 40274); Chiang Rai Province, Chiang-khong District, Huay-sor Subdistrict, Nensomburn Village, 30 August 2014, B. Thongbai, BZ201386 (MFLU 15-0153, BBH 40581).

Known distribution: China (Zhang *et al.* 2010) and now Thailand.

Remarks: *Amanita rimosa* is a member of *Amanita* [subgenus *Lepidella*] sect. *Phalloideae*. In the field, *A. rimosa* can be recognized by the pileus that is white to yellowish white towards the margin, the appearance of fine radial raised ridges on the pileus (lens sometimes required) and the minutely rimose pileus margin surface. The main microscopic characteristic is the slightly gelatinized upper layer of the pileipellis with abundant inflated hyphae, which is unique among species in sect. *Phalloideae* (Zhang *et al.* 2010). The size of basidiomata, as well as size and shape of basidiospores of the Thai collections is very similar to the original description from China (Zhang *et al.* 2010). The

holotype and the Thai specimens were collected from evergreen broad-leaved forest with *Fagaceae* at elevations of 1,300 m and 1,075 m, respectively. The combined genes phylogenetic analyses indicated Thai *A. rimosa* sequences clustered with *A. rimosa* sequences from China. *Amanita rimosa* is a member of *Amanita* [subgenus *Lepidella*] sect. *Phalloideae* with 100% BS, PP = 1.0 (Figure 7).



FIGURE 4. Basidiomata of *Amanita rimosa*. a young and mature basidiomata. b–d basidiospores in 5% KOH. e–g basidiospores in Congo red. h–j basidiospores in Melzer's reagent. k–m basidia and subhymenium at different stages of development. (a–m: BZ201386) (scale bar: a = 5 cm, b–j = 5 μ m, k–m = 15 μ m).

Amanita cf. rubromarginata Har. Takah., *Mycoscience* 45 (6): 372, 2004 (Figure 5)

Basidiomata [Fig. 5(l–m)] small to medium-sized. **Pileus** 60–75 mm wide, hemispherical when young then convex to plano-convex to applanate at maturity, dry, subviscid when moist, with pale, short projecting hairs at center, carrot red to deep orange (6B7-8) to brownish orange (6C8) at center, grayish orange or apricot (5B5-6) toward margin; margin non-appendiculate, with marked long sulcate striations on outer half of pileus; context 3–7 mm thick above stem, yellowish white (2A2). **Lamellae** 5–10 mm broad, free, crowded, subventricose, yellowish (3A2) with margin markedly darker, red to reddish orange (7B7-8), fimbriate; lamellulae of 3–4 lengths, truncate. **Stipe** 50–110 \times 4–14 mm, subcylindrical, slightly tapering upwards, yellowish white (3B2), above partial veil covered with minute reddish orange (7B7-8) fibrils or squamules forming irregular transverse zones; context broadly fistulose to slightly chambered, yellowish white (3A2). **Universal veil on stipe base** as saccate volva, sheathing, up to 25 mm high, sticky, robust, thick, white (1A1). **Partial veil** subapical, membranous, pendant, large, thick, margin striate, reddish orange (7B7-8). **Odor** not recorded.

Lamellar trama bilateral, divergent; mediostatum 35–40 μ m wide, filamentous hyphae 2–5 μ m wide, branching, hyaline, thin-walled, with terminus clavate to subfusiform, 90–120 \times 25–30 μ m; vascular hyphae rare. **Subhymenium** [Fig. 5(j)] 30–40 μ m thick; 2–3 layers of subglobose to ellipsoid cells, 15–20 \times 10–15 μ m. **Basidia** [Fig. 5(k)] 25–30 \times 10–15 μ m, clavate to pyriform, 4-spored, with sterigmata 4–6 μ m long; clamps present at base. **Basidiospores** [Fig.



FIGURE 5. Basidioma of *Amanita* cf. *rubromarginata*. a–c basidiospores in 5% KOH. d–f basidiospores in congo red. g–i basidiospores in Melzer’s reagent. j–k basidia and subhymenium at different stages of development; showing clamps at the bases of basidia. l–m mature basidioma. (l–m: BZ201425) (scale bars: a–b = 5 µm, c = 10 µm, d–i = 5 µm, j–k = 10 µm, l–m = 3 cm).

5(a–i)] [50/2/2] (7.0–) 7.4–9.9 (–10.2) × (5.4–) 5.6–7.0 (–7.7) µm, (**L**’ = 8.8 µm; **W**’ = 6.3 µm; **Q** = (1.15–) 1.30–1.60 (–1.66); **Q**’ = 1.41±0.10), smooth, hyaline, colorless, thin-walled, inamyloid, ellipsoid, rarely broadly ellipsoid and elongate, adaxially flattened; apiculus rather variable, sublateral, very prominent to rather small, cylindric to truncate-conic; contents mostly monoguttulate, occasionally granular; white in deposit. **Lamellar edge** sterile; filamentous hyphae 2–7 µm wide, with inflated elements, colorless or occasionally with intracellular pale brown pigment, thin-walled; subglobose to globose cells dominating, 22–25 × 18–20 µm, colorless, or occasionally with intracellular pale brown pigment, thin-walled. **Pileipellis** 70–100 µm thick, 2-layered; upper layer 30–50 µm thick, filamentous hyphae 3–8 µm wide, strongly gelatinized, hyaline, branching, thin-walled; lower layer 40–50 µm thick, filamentous hyphae 7–14 µm wide, non-gelatinized, branching, hyaline or occasionally with intracellular yellowish pigment,

thin-walled. **Universal veil on stipe base** filamentous hyphae 5–8 μm wide, branching, hyaline or occasionally with intracellular yellow pigment, thin-walled; ellipsoidal to subglobose cells 20–28 \times 18–26 μm , hyaline or occasionally with intracellular yellow pigment, thin-walled. **Stipe trama** longitudinally acrophysalidic; filamentous hyphae 2–6 μm wide, branching, hyaline, thin-walled; acrophysalides not described. **Partial veil** filamentous hyphae 2–8.5 μm wide, hyaline, occasionally with intracellular yellow pigment, thin-walled, with terminal clavate to ellipsoid cells, 27–65 \times 16–52 μm .

Habitat: solitary or scattered on ground in forest of *Fagaceae*.

Specimens examined: THAILAND, Chiang Mai Province, Mae-on District, near Chiang Mai/ Lampang border, elev. 1,517 m, 02 July 2014, B. Thongbai, BZ201423 (MFLU 15-01418, BBH 40585); Lampang Province, Meuangpan District, elev. 1,170 m, 03 July 2014, B. Thongbai, BZ201425 (MFLU15-01420, BBH 40586).

Known distribution: southwestern Japan (Har. Takah. 2004) and now Thailand.

Remarks: *Amanita rubromarginata* is a member of *Amanita* sect. *Caesareae* falling in the 'jacksonii' clade of Sánchez-Ramírez *et al.* (2014 Figure 2). In the field, the distinguishing morphological characteristics of *A. rubromarginata* include distinctive long-sulcate striations on the margin of the pileus, glabrous to subviscid surface texture when moist, carrot red to deep orange to brownish orange, grayish orange or apricot colors toward the margin, long and thick reddish orange partial veil and white saccate volva. The distinctly reddish orange, fimbriate margin of the lamellae is unique among those similar species with an orange to brownish orange to reddish pileus, e.g., *A. hemibapha* var. *hemibapha* (Berk. & Broome) Sacc. var. *hemibapha*, *A. hemibapha* var. *ochracea* Zhu L. Yang, and *A. javanica* (Corner & Bas) T. Oda, C. Tanaka & Tsuda as well as all known species in the Americas and Europe. We are hesitant to firmly assign these collections to *A. rubromarginata* at this time because according to illustrations in the recent book of SW Japanese fungi (Terashima *et al.* 2016), as the mushroom ages, the center of the cap can become nearly black; and the margin, olive. We did not observe these characteristics in either of the Thai collections. The size of basidiomata as well as the size and shape of basidiospores of the Thai collections is similar to dimensions given in the original description from Japan (Takahashi 2004). The holotype was collected in an evergreen, broad-leaved forest with *Quercus miyagii* Koidz. and *Castanopsis cuspidata* (Thunb. ex Murray) Schottky var. *sieboldii* (Makino) Nakai. No elevation was recorded. The Thai specimens were collected at elevations of 1,170–1,517 m in fagaceous forest with *Castanopsis* spp.

In part, Figure 7 reproduces (see the clade marked "Sect. *Caesareae*") the separation of apparently earlier diverging white-, gray-, and brown-capped taxa from those with brighter colors as in the large sample, multilocus study of Sánchez-Ramírez *et al.* (2014). In the latter study, an isotype of *A. rubromarginata* was included in the sample. In Figure 7, the similarity of the Thai collections of the present taxon suggests a close relationship to *A. rubromarginata*.

Amanita zangii Zhu L. Yang, T.H. Li & X.L. Wu, *Fungal Diversity* 6: 160, 2001 (Figure 6)

Basidiomata [Fig. 6(a–c)] small to medium-sized. **Pileus** 50–80 mm wide, hemispherical when young, convex to broadly convex to applanate at maturity, dry, subviscid to viscid when moist, dirty white to milk white (1A2), minutely fibrillose, covered with felty to subfibrillose to flat verrucose universal veil remnants, greyish brown to dark brown, dark grey to blackish (6E2-3, 6F7); margin non-striate, appendiculate; context 4–7 mm thick above stem, soft to slightly hard, white (1A1). **Lamellae** 7–11 mm broad, free, subcrowded to crowded, white to pale cream (1A1, 4A1-3); lamellulae of 3–5 lengths, attenuate. **Stipe** 50–85 \times 10–15 mm (length includes bulb), subcylindric, slightly tapering downwards, with irregular, sub-bulbous, rooting base, whitish to yellowish white (2A1-2), minutely farinose at apex, whitish grey (4A1) at base; context solid, white (1A1). **Bulb** subclavate to subglobose, sometimes irregularly radicating, up to 30 mm wide, white to whitish gray (1A1, 4A1). **Universal veil on stipe base** as covering of farinose to floccose remnants, greyish white (4A1). **Partial veil** subapical to superior, submembranous-subfibrillose, white to whitish above (1A1), below concolorous with universal veil. **Odor** not recorded.

Lamellar trama bilateral, divergent; mediostatrum 30–55 μm wide, filamentous hyphae 4–8 μm wide, branching, hyaline, thin-walled; inflated cells terminal, ellipsoid to fusiform and 45–90 \times 10–25 μm ; vascular hyphae rare. **Subhymenium** [Fig. 6(m)] 25–55 μm thick; 2–4 layers of subglobose, ovoid or rarely broadly ellipsoid cells, 16–22 \times 8–20 μm . **Basidia** [Fig. 6(m–o)] 32–55 \times 10–12 μm , 4-spored, with sterigmata up to 4 μm long; clamps absent. **Basidiospores** [Fig. 6(d–l)] [50/2/2] 9.4–12.0 (–12.4) \times (6.5–) 6.6–8.8 (–8.9) μm , (L' = 10.7 μm ; W' = 7.6 μm ; Q = (1.23–) 1.30–1.61 (–1.71); Q' = 1.41 \pm 0.10), smooth, hyaline, colorless, thin-walled, amyloid, ellipsoid, rarely broadly ellipsoid to elongate, rarely adaxially flattened; apiculus rather variable, sublateral, proportionately small or rarely larger, cylindric to truncate-conic; contents monoguttulate or rarely granular; white in deposit. **Lamellar edge** sterile; filamentous hyphae 4–8 μm wide, branching, hyaline, colorless, thin-walled; inflated cells broadly clavate, dominating,



FIGURE 6. Basidiomata of *Amanita zangii*. a–b young and mature basidiomata. c subfibrillose velars remnants when young. d–f basidiospores in 5% KOH. g–i basidiospores in congo red. j–l basidiospores in Melzer's reagent. m–o basidia and subhymenium at different stages of development. (a–o: BZ201344) (scale bars: a–c = 30 mm, d–l = 6 μ m, m = 20 μ m, n–o = 15 μ m).

20–32 \times 13–18 μ m, hyaline, colorless, thin-walled. **Pileipellis** 50–80 μ m thick, 1-layered to hardly differentiated; filamentous hyphae 2–7 μ m wide, gelatinized, hyaline, occasionally with intracellular yellow to pale brown pigment, thin-walled. **Universal veil on pileus** filamentous hyphae 4–6 μ m wide, frequently hyaline and colorless, occasionally with intracellular brown pigment, slightly thick-walled, with terminal subcylindric to subfusiform cells, 70–100 \times 25–38 μ m, with occasional elongate inflated cells, dominating subglobose to broadly ovoid cells, singly or 2–3 in chain, 16–32 \times 11–17 μ m. **Universal veil on stipe base** filamentous hyphae 2–5 μ m wide, hyaline or sometimes with intracellular yellow pigment, thin-walled; inflated cells broadly clavate, ovoid or subglobose, singly or 2–3 in chain, dominating, 28–60 \times 15–50 μ m. **Stipe trama** longitudinally acrophysalidic; filamentous hyphae 1–7 μ m wide, hyaline, sometime with intracellular pale yellow pigment; acrophysalides slightly clavate, 150–300 \times 30–45 μ m; vascular hyphae rare. **Partial veil** filamentous hyphae 2–5 μ m wide, branching, hyaline, colorless, thin-walled, with inflated terminal segments, 12–25 μ m wide; vascular hyphae not observed.

Habitat: solitary or scattered on ground in forest of *Fagaceae* or deciduous dipterocarps.

Specimens examined: THAILAND, Chiang Mai Province, 3 km down road from Tharn Thong Lodges Resort, Mae-on District, Huai-kaeo Subdistrict, elev. 720 m, 11 June 2013, B. Thongbai, BZ201356 (MFLU 15-0130, BBH 40578); Lampang Province, along road number 1252, elev. 1420 m, 15 June 2013, B. Thongbai, BZ201344 (MFLU 15-0144, BBH 40580).

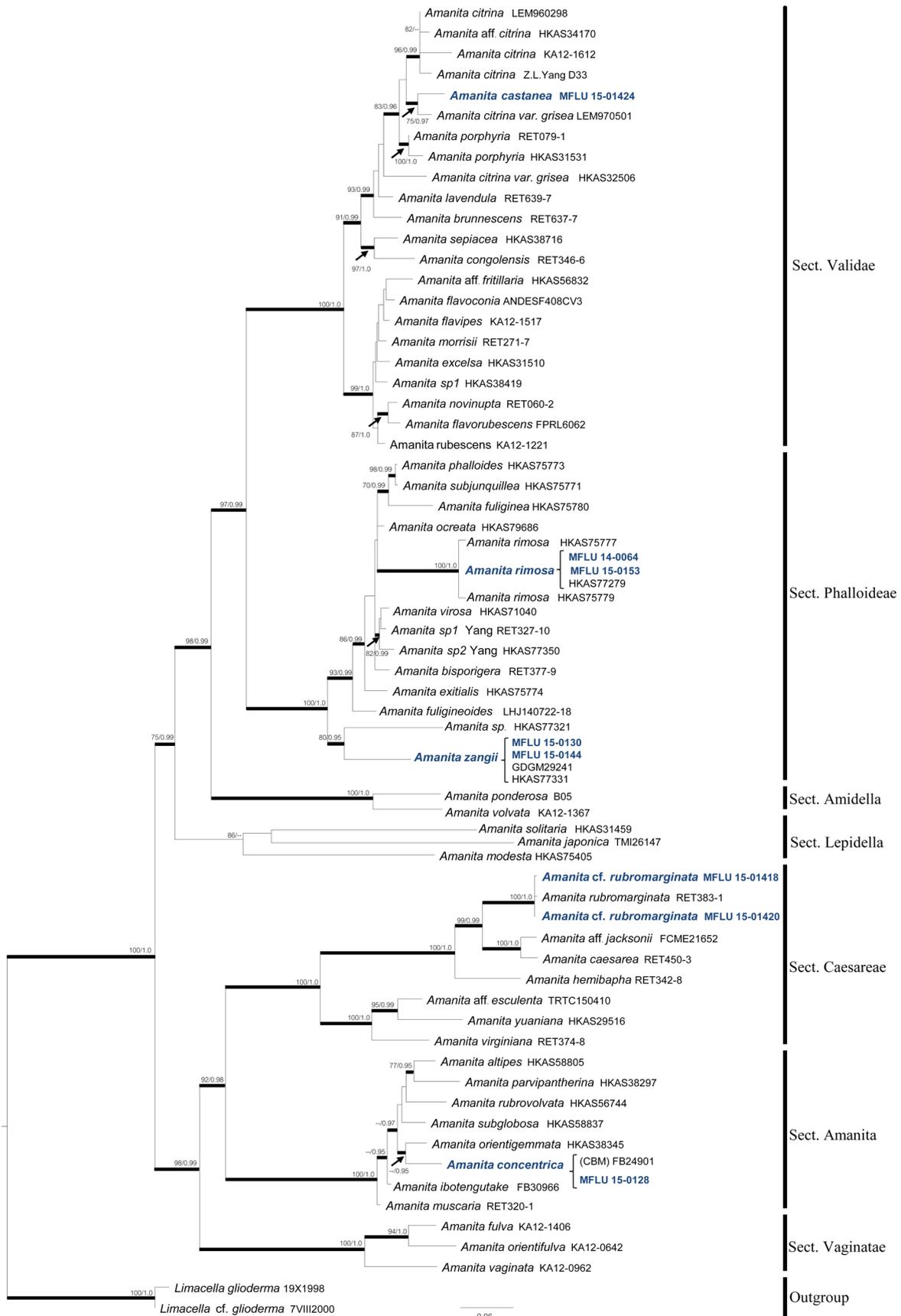


FIGURE 7. Phylogenetic relationships of *Amanita* species inferred from combine gene tree (LSU and ITS) sequences using Maximum Likelihood (ML). Posterior Probabilities (PP) from Bayesian Inference ≥ 0.95 are indicated as thick branches and bootstrap value $\geq 70\%$ are shown above the branches at nodes. *Amanita* species collected from Thailand are highlighted in bold. Voucher collection identifiers are provided after each species name.

Known distribution: China, Japan (Yang *et al.* 2001) and now Thailand.

Remarks: Basidiocarps of *A. zangii* have dark grey to blackish, felty to subfibrillose universal veil remnants on the pileus. The volval tissue is dominated by chains of elongate inflated cells. In addition, an appendiculate pileus margin is characteristic of the species. *Amanita zangii* is a member of Bas' stirps *Hesleri* (Bas 1969) along with *A. hesleri* Bas (eastern North America; Bas 1969) and *A. veldiei* D. A. Reid & Eicker ex Redhead (South Africa; Reid & Eicker 1991, Redhead 2016, Tulloss *et al.* 2016). Based on the data of Wolfe *et al.* (2012) and evidence that the members of the stirps are mycorrhizal in contrast to those amanitas with a putative or established amycorrhizal mode of acquiring carbon [attributed to the remainder of the subsection *Vittadiniae* Bas (Bas 1969)], Tulloss *et al.* (2016) segregated stirps *Hesleri* from subsect. *Vittadiniae*.

Vizzini *et al.* (2012) introduced the new combination *Aspidella zangii* based on their assumption that the present species is one of the amycorrhizal amanitas. This error was not repeated by Redhead *et al.* (2016). Taxa in stirps *Hesleri* have been repeatedly collected in forest habitats in association with members of the *Fagaceae*, *Pinaceae* and *Dipterocarpaceae*, which suggests they are ectomycorrhizal (Tulloss 2016b, Tulloss & Possiel 2016, Tulloss & Yang 2016c). *A. hesleri*, a close relative to *A. zangii* on morphological and genetic grounds, is clearly segregated from the supposedly amycorrhizal group in the large, four gene phylogeny of Wolfe *et al.* (2012). Existing data indicate that *A. hesleri* and *A. zangii* are sister taxa (Tulloss *et al.* 2016).

Thai specimens were collected from forest dominated by *Fagaceae* or in deciduous dipterocarp forest. Morphological comparisons between the Thai collections and those originally described from China indicate similarity in size of basidiomata and in size and shape of basidiospores. The stipe was clearly bulbous in the holotype (Yang *et al.* 2001) whereas the Thai collection showed an irregular, sub-bulbous and rooting stipe base. However, in this regard, it is worth noting that the bulb of *A. hesleri* can take multiple forms as shown in the illustrations of Tulloss (2016b).

A BLAST search on the ITS sequence of our *A. zangii* material indicated 100% similarity and 100% query cover with a sequence from the holotype of *A. areolata* (Oda *et al.* 2002b)—a synonym of *A. zangii* (Tulloss & Yang 2016c). No LSU sequence was present for *A. areolata* in GenBank. The combined genes phylogenetic analyses indicated that *A. zangii* clustered with the *A. zangii* sequences from GenBank; the cluster appears to be early diverging in sect. *Phalloideae* with 100% BS, PP = 1.0 (Figure 7). Cai *et al.* (2014) also showed a clade including *A. zangii* and *A. sp.* (HKAS77321) as early diverging in the *Phalloideae*. They explain why the putative relationship of *A. zangii* is odd due to highly contrasting morphologies. We agree with Cai *et al.* (2014) that further investigation into the matter would be useful.

Discussion

In the present study, we document a novel species and four first records of *Amanita* species in northern Thailand. Species circumscriptions and identification are supported by both phylogenetic and morphological evidence. One interesting exception might be *Amanita cf. rubromarginata*. The characteristics of the Thai collections, especially the colors of the pileus, agree well with the type description. However, additional collections made in southwestern Japan indicate that the colors of aging *A. rubromarginata* might be more different from the Thai collections than originally thought. According to illustrations in the recent book of SE Japanese fungi (Terashima *et al.* 2016), the center of the cap can be nearly black and the margin, olive. These colors were never observed in the Thai material. Therefore, more collections should be made from Thailand to assess the color variation.

Further molecular studies with multiple genes that avoid loci of the nuclear ribosomal DNA cistron and expanded taxon sampling are needed to resolve the tree topology for some *Amanita* sections, e.g. *Amanita* sect. *Caesareae* (Sánchez-Ramírez *et al.* 2014, 2015), *A. sect. Validae* series *Mappae* (Drehmel *et al.* 1999, Hughes *et al.* 2013, Tulloss & Yang 2016d), *A. sect. Lepidella* sensu Bas (Tulloss *et al.* 2016) and *A. sect. Phalloideae* (Cai *et al.* 2014, Tulloss *et al.* 2016). A possible set of four protein-coding loci was proposed by Sánchez-Ramírez *et al.* (2014) and utilized in (Sánchez-Ramírez *et al.* 2015).

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