Two new species of *Amanita* section *Roanokenses* with a radicating basal bulb

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Research Article

Keywords: Morphology, Phylogenetic analyses, Taxonomy

Posted Date: February 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-193237/v1>

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Abstract

Two new species of *Amanita* sect. *Roanokenses* (Amanitaceae, Agaricales, Basidiomycota), *Amanita alboradicata* and *A. fulvopyramis* are proposed here on morphological and molecular evidence. The described and illustrated are based on morphological studies of collections from Jilin, Zhejiang and Hunan provinces in China. Nuclear ribosomal large subunit (nrLSU), RNA polymerase II second largest subunit (*rpb2*) and translation elongation factor 1- α (*tef1- α*) sequences analyses supported establishment of these two new species and revealed their phylogenetic positions. Both new species possess long radicating basal bulbs. *Amanita alboradicata* is characterized by a white or dirty white pileus covered with angular warts. *Amanita fulvopyramis* is characterized by the brown orange to light brown, pyramidal to verrucose volval remnants on the pileus, and light brown to brown lamellae. Holotypes are deposited in the Mycological Herbarium of Hunan Normal University.

Introduction

The family Amanitaceae includes five genera: *Amanita*, *Catatrama*, *Limacellopsis*, *Limacella*, and *Zhuliangomyces* (Yang et al. 2018; Redhead 2019). *Amanita* is the largest genus; it harbors 95% of the species in the family and includes more than 600 accepted species (Kirk et al. 2008; Cui et al. 2018). Many species of Amanitaceae have been extensively collected and studied since the 1990s in China, and there are more than 200 formally described taxa (Yang 1997, 2005, 2015; Zhang et al. 2010; Deng et al. 2014; Deng et al. 2016; Li and Cai 2014; Ariyawansa et al. 2015; Li et al. 2015; Cai et al. 2016; Liu et al. 2017; Cui et al. 2018; Jabeen et al. 2019; Ullah et al. 2019; Saba et al. 2019; Zhang et al. 2020).

Corner and Bas proposed splitting *Amanita* into two subgenera and six sections (Corner and Bas 1962; Bas 1967), whereas other mycologists proposed splitting *Amanita* into two subgenera and seven sections on the basis of molecular phylogenetic evidence and morphological data (Weiß et al. 1998; Yang 1997). With more molecular phylogenetic data, *Amanita* was divided into three subgenera and 11 sections: subgen. *Lepidella* (sect. *Lepidella*), subgen. *Amanita* (sect. *Amanita*, sect. *Amarrendiae*, sect. *Caesareae*, and sect. *Vaginatae*), and subgen. *Amanitina* (sect. *Amidella*, sect. *Arenariae*, sect. *Phalloideae*, sect. *Roanokenses*, sect. *Strobiliformes*, and sect. *Validae*) (Cui et al. 2018); sect. *Strobiliformes*, *Amarrendiae*, and *Arenariae* were newly introduced. Morphologically, sect. *Amarrendiae*, *Arenariae*, and *Caesareae* harbor both sequestrate and agaricoid basidiomata, and the other eight sections mainly accommodate species with agaricoid basidiomata (Cui et al. 2018). In some studies, sect. *Lepidella* sensu Bas (1969) was recovered as a polyphyletic rather than monophyletic group (Yang and Li 2001; Hosen et al. 2015). Economically, some species are edible and some species are poisonous or even deadly. Over 90% species are ectomycorrhizal symbionts, and only less than 10% of species are saprotrophic (Cui et al. 2018). The two new species studied in this paper are in sect. *Roanokenses*, which was effectively proposed by Singer in 1962, with *Amanita roanokenses* Coker as type species (Singer 1962a). *Amanita* subsect. *Limbatulae* Bas, which is a synonym of sect. *Roanokenses* (Bas 1969), has the noteworthy characters “limbate volva” and “layered volva,” however, molecular evidence shows that this characteristic has presumably evolved independently and has different characteristics in some species, such as *A. neo-ovoidea* Hongo. Thirty-six species of sect.

Roanokenses were recently recorded from China, which included 14 novel and 22 known species (Cui et al. 2018; Jo et al. 2020).

Materials And Methods

Specimen collection and morphological descriptions

The descriptions of the new species are based on morphological studies of fresh and dry materials. Fresh materials were photographed and recorded in the field, and then dried by a hot air fan with the temperature adjusted to 55–60°C for approximately 8 h. Dry materials were mounted in 5% (w/v) KOH and 1% (w/v) Congo red for microscopic examination and line drawings. Spores were mounted in Melzer's reagent to test for amyloidity. The color designations were from Korerup and Wanscher (1978). In basidiospore descriptions, the abbreviation $[n/m/p]$ represents n basidiospores measured from m basidiomata of p collections, and basidiospore dimensions are given using the notation $(a-)b-c(-d)$. The range $b - c$ contains a minimum of 90% of the measured values and extreme values (a and d) are given in parentheses. The ratio of basidiospore length to width is abbreviated as Q , and Q_m = average Q of all basidiospores measured $\pm$ sample standard deviation (Bas 1969). The collections of the two new species were deposited in the Mycological Herbarium of Hunan Normal University (MHHNU).

DNA Extraction, PCR Amplification, And DNA Sequencing

Materials for molecular studies were dried with silica gel. Genomic DNA was extracted from dried fruiting bodies using an Ezup cfDNA Extraction Kit (Sangon Biotech Co., Ltd., China). Primers LROR/LR5 were used for partial nuclear ribosomal large subunit (nrLSU) amplification (Vilgalys and Hester 1990); EF1-983F/EF1-1567R for the translation elongation factor 1- α (*tef1- α*) (Rehner and Buckley 2005); Am-6F/Am-7R for the RNA polymerase II second largest subunit (*rpb2*) (Cai et al. 2014). PCR amplifications were performed in a total volume of 25 μ L. Thermal cycle parameters of PCR amplification were: denaturation at 94°C for 5 min; followed by 31 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 1 min; and a final extension at 72°C for 7 min. Amplified PCR products were quantified by gel electrophoresis on a 1% agarose gel with voltage set to 122 V, and electrophoresis was conducted in 1 $\times$ TAE buffer for 12 min. The target bands were purified using a universal DNA purification kit (Sangon Biotech Co., Ltd., China). The purified PCR amplification products were sequenced on an ABI 3730 automated DNA sequencer (Perkin–Elmer Inc., USA). The same primers used for PCR were also used for sequencing reactions.

Phylogenetic Analyses

To determine the phylogenetic positions of the two new species, nrLSU, *tef1- α* and *rpb2* sequences of species of each *Amanita* section were analyzed with those of the new species. These sequences were aligned with MUSCLE v3.8.31 (Edgar 2004) using default parameters, and manually checked and modified when necessary using BioEdit v7.0.9.0 (Hall 1999). Bayesian inference (BI) analyses executed in MrBayes v3.2 (Ronquist and Huelsenbeck 2003) were run for 1,000,000 generations using four Metropolis–Coupled Monte Carlo Markov chains to calculate posterior probabilities. Maximum likelihood (ML) analysis was conducted in RAxML v7.2.6 (Stamatakis et al. 2004) with 1,000 replicates. The MrBayes analysis was automatically terminated using the stoprul and stopval commands when the standard deviation of the split

frequencies fell below 0.01. Chain convergence was further verified using Tracer v1.7.1 (<https://beast.community/tracer>) to confirm sufficiently large ESS values (> 200). Subsequently, the sampled trees were summarized after omitting the first 25% of trees as burn-in using the 'sump' and 'sumt' command implemented in MrBayes. MrModeltest v3.7 (Posada and Crandall 1998) was used to determine the optimal substitution model for the concatenated nrLSU and Multi-locus dataset (nrLSU, *tef1-a* and *rpb2*) with the Akaike information criterion; GRT + I + G was selected as the substitution model for the two dataset.

Fifteen sequences (five nrLSU, five *rpb2* and five *tef1-a*) of the two new species were newly generated for this study. Sequences of other Amanitaceae species were downloaded from GenBank (www.ncbi.nlm.nih.gov). Scientific names, GenBank accession numbers, and other relevant information for the specimens included in this study are listed in Table 1.

Table 1
Taxa and GenBank accession numbers of sequences used in phylogenetic analysis

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
<i>Amanita alboflavescens</i> Hongo	HKAS77235	Yunnan, China	MH486365 ^b	-	-
<i>A. alboradicata</i>	MHHNU10531 (T)	Jilin, China	MW016757^j	MW546614^j	MW546620
<i>A. alboradicata</i>	MHHNU10535	Jilin, China	MW016758^j	MW546615^j	MW546621^j
<i>A. arenaria</i> (O.K. Mill. & E. Horak) Justo	VPI363	Australia	GQ925384 ^c	-	-
<i>A. arenaria</i>	VPI679	Australia	GQ925382 ^c	-	-
<i>A. aspericeps</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS77783 (T)	Guangdong, China	MH486372 ^b	-	-
<i>A. atrobrunnea</i> Thongbai, Raspé & K.D. Hyde	MFLU 15-1415	Thailand	NG_057090 ^d	-	-
<i>A. avellaneifolia</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS79891	Yunnan, China	MH486378 ^b	MH485871 ^b	MH508679 ^b
<i>A. brunneofolia</i> J.W. Jo, H.S. Kim, Y.N. Kwag & C.S. Kim	KA19-0899	South Korea	MT385143 ^a	MT412396 ^a	MT412402 ^a
<i>A. brunneofolia</i>	KA19-0899-1 (T)	South Korea	MT385144 ^a	MT412397 ^a	MT412403 ^a
<i>A. brunneomaculata</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS68393	Yunnan, China	MH486410 ^b	-	-
<i>A. brunneostrobilipes</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS60291 (T)	Hainan, China	MH486415 ^b	-	MH508703 ^b
<i>A. brunneostrobilipes</i>	HKAS96787	Hainan, China	MH486416 ^b	MH485897 ^b	MH508704 ^b
<i>A. caesareoides</i> Lj.N. Vassiljeva	HKAS71021	Japan	MH486419 ^b	-	-

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
<i>A. caojizong</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS79673 (T)	Yunnan, China	MH486429 ^b	MH485908 ^b	MH508714 ^b
<i>A. castanopsis</i> Hongo	KA14-1020	South Korea	MT385139 ^a	MT412391 ^a	MT412398 ^a
<i>A. castanopsis</i>	KA14-1568	South Korea	MT385138 ^a	MT412392 ^a	MT412399 ^a
<i>A. cf. javanica</i>	HKAS101408	Hainan, China	MH486436 ^b	-	-
<i>A. cf. vestita</i>	HKAS80175	Anhui, China	MH486439 ^b	-	-
<i>A. cf. xanthogala</i>	HKAS84707	Yunnan, China	MH486441 ^b	MH485918 ^b	MH508723 ^b
<i>A. cinctipes</i> Corner & Bas	HKAS78465	Guangdong, China	MH486449 ^b	-	-
<i>A. cinereovelata</i> Hosen	HKAS81647 (T)	Bangladesh	NG_058570 ^e	KP259288 ^e	KP259289 ^e
<i>A. ejii</i> Zhu L. Yang	HKAS70229	Yunnan, China	MH486484 ^b	MH485963 ^b	MH508761 ^b
<i>A. elliptica</i> Q. Cai, Y.Y. Cui & Zhu L. Yang	HKAS79602	Hainan. China	MH486487 ^b	-	-
<i>A. excelsa</i> (Fr.) Bertill.	HKAS96169	Jilin, China	MH486492 ^b	-	-
<i>A. flavofloccosa</i> Nagas. & Hongo	HKAS101443	Sichuan, China	MH486515 ^b	-	-
<i>A. flavofloccosa</i>	HKAS92006	Yunnan, China	MH486516 ^b	-	-
<i>A. fuligineoides</i> P. Zhang & Zhu L. Yang	HKAS83694	Yunnan, China	MH486553 ^b	-	-
<i>A. fulva</i> Fr.	HKAS96168	Australia	MH486555 ^b	-	-
<i>A. fulvopyramis</i>	MHHNU8814(T)	Hunan, China	MT878535^j	MW546617^j	MW546623^j
<i>A. fulvopyramis</i>	MHHNU8841	Hunan, China	MT878536^j	MW546618^j	MW546624^j
<i>A. fulvopyramis</i>	MHHNU10581	Zhejiang, China	MW471097^j	MW546619^j	MW546625^j
<i>A. grandis</i> (Bougher) Justo	H7353	Australia	GQ925376 ^c	-	-
<i>A. griseofarinosa</i> Hongo	HKAS80926	Hubei, China	MH486559 ^b	MH486025 ^b	MH508830 ^b

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
<i>A. griseopantherina</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS82340 (T)	Sichuan, China	MH486571 ^b	MH486035 ^b	MH508840 ^b
<i>A. griseoverrucosa</i> Zhu L. Yang	HKAS57357	Yunnan, China	MH486581 ^b	-	-
<i>A. gymnopus</i> Corner & Bas	HKAS71618	Yunnan, China	MH486582 ^b	MH486044 ^b	MH508851 ^b
<i>A. heishidingensis</i> Fang Li	HKAS76122	Guangdong, China	NG_057019 ^f	-	-
<i>A. heishidingensis</i>	HKAS81484	Guangdong, China	KJ922993 ^f	MH486049 ^f	MH508854 ^f
<i>A. incarnatifolia</i> Zhu L. Yang	HKAS100593	Anhui, China	MH486594 ^b	-	-
<i>A. inculta</i> (Bougher) Justo	H7335	Australia	GQ925371 ^c	-	-
<i>A. japonica</i> Hongo ex Bas	TMI26147	Japan	KJ922990 ^f	-	-
<i>A. kotohiraensis</i> Nagas. & Mitani	HKAS100577	Anhui, China	MH486614 ^b	MH486068 ^b	MH508874 ^b
<i>A. luteofolia</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS81895 (T)	Hubei, China	MH486633 ^b	MH486083 ^b	MH508890 ^b
<i>A. macrocarpa</i> W.Q. Deng, T.H. Li & Zhu L. Yang	31939L (T)	South Korea	KC408378 ^g	-	-
<i>A. manginiana</i> Har. & Pat.	MHHNU30818	Hunan, China	MH605436 ^b	MH614263 ^b	MH614264 ^b
<i>A. miculifera</i> Bas & Hatan.	HKAS101425	Shenyang, China	MH486643 ^b	MH486093 ^b	MH508901 ^b
<i>A. minutisquama</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS100504 (T)	Hainan, China	MH486644 ^b	MH486095 ^b	MH508902 ^b
<i>A. modesta</i> Corner & Bas	HKAS78433	Guangdong, China	MH486650 ^b	MH486099 ^b	MH508907 ^b
<i>A. muscaria</i> (L.) Lam.	HKAS61888	Heilongjiang, China	MH486651 ^b	MH486100 ^b	MH508908 ^b
<i>A. neo-ovoidea</i> Hongo	HKAS89025	Yunnan, China	MH486656 ^b	MH486106 ^b	MH508913 ^b

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
<i>A. nouhrae</i> Truong, Kuhar & M.E. Sm.	MES1921	Argentina	KY053391 ^h	-	-
<i>A. oberwinkleriana</i> Zhu L. Yang & Yoshim. Doi	HKAS85832	Yunnan, China	MH486681 ^b	MH486118 ^b	MH508928 ^b
<i>A. pallidochlorotica</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS77280 (T)	Yunnan, China	KC429046 ^f	MH486158 ^b	MH508964 ^b
<i>A. pallidorozea</i> P. Zhang & Zhu L. Yang	HKAS82350	Shandong, China	MH486737 ^b	-	-
<i>A. parvicurta</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS100497	Fujian, China	MH486744 ^b	-	-
<i>A. parvipantherina</i> Zhu L. Yang, M. Weiss & Oberw.	HKAS67828	Yunnan, China	MH486750 ^b	-	-
<i>A. parvivirginea</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS79051 (T)	Guangdong, China	MH486753 ^b	-	-
<i>A. pseudomanginiana</i> Q. Cai, Y.Y. Cui & Zhu L. Yang	HKAS83470 (T)	Sichuan, China	MH486772 ^b	-	-
<i>A. pseudoporphyrina</i> Hongo	BZ-N10	Thailand	KY747473 ^d	KY656872 ^d	
<i>A. pseudoporphyrina</i>	HKAS89074	Yunnan, China	MH486785 ^b	MH486199 ^b	MH509012 ^b
<i>A. pyramidata</i> Zhu L. Yang, Y.Y. Cui & Q. Cai	HKAS87943 (T)	Yunnan, China	MH486795 ^b	MH486209 ^b	MH509021 ^b
<i>A. roseolifolia</i> Y.Y. Cui, Q. Cai & Zhu L. Yang	HKAS101403 (T)	Hainan, China	MH486807 ^b	MH486219 ^b	MH509032 ^b
<i>A. rubescens</i> Pers.	HKAS101398	France	MH486811 ^b	-	-
<i>A. rubiginosa</i> Q. Cai, Y.Y. Cui & Zhu L. Yang	HKAS52216 (T)	Yunnan, China	MH486817 ^b	MH486229 ^b	MH509045 ^b
<i>A. rufoferruginea</i> Hongo	HKAS79616	Guangdong, China	MH486842 ^b	-	-

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
<i>A. subfrostiana</i> Zhu L. Yang	HKAS100531	Shandong, China	MH486874 ^b	-	-
<i>A. solitaria</i> (Bull.) Fr.	RET 298-1	Gironde, France	HQ539741 ⁱ	-	-
<i>A. sphaerobulbosa</i> Hongo	KA14-1202	South Korea	MT385142 ^a	-	-
<i>A. subfrostiana</i> Zhu L. Yang	HKAS58750	Sichuan, China	MH486897 ^b	-	-
<i>A. timida</i> Corner & Bas	HKAS83228	Guangdong, China	MH486932 ^b	MH486323 ^b	MH509147 ^b
<i>A. umbrinolutea</i> (Secr. ex Gillet) Bataille	HKAS89201	Sichuan, China	MH486933 ^b	-	-
<i>A. vestita</i> Corner & Bas	HKAS78437	Guangdong, China	MH486942 ^b	MH486337 ^b	MH509163 ^b
<i>A. virgineoides</i> Bas	HKAS77278	Fujian, China	MH486945 ^b	MH486340 ^b	MH509166 ^b
<i>A. vittadinii</i> (Moretti) Vittad.	HKAS101430	Italy	MH486950 ^b	-	-
<i>A. yenii</i> Zhu L. Yang & C.M. Chen	HKAS87047	Yunnan, China	MH486951 ^b	MH486344 ^b	MH509171 ^b
<i>Limacellopsis</i> <i>asiatica</i> Zhu L. Yang, Q. Cai & Y.Y. Cui	HKAS76497	Gansu, China	KT833811 ^b	-	-
<i>L. asiatica</i>	HKAS82561 (T)	Sichuan, China	KT833812 ^b	-	-
Sequences produced in this study are indicated in bold, “-” = no information/sequence available, T = Type specimen					
^a sequences from the literature Jo et al. (2020)					
^b sequences from the literature Cui et al. (2018)					
^c sequences from the literature Justo et al. (2010)					
^d sequences from the literature Thongbai et al. (2017)					
^e sequences from the literature Hosen et al. (2015)					
^f sequences from the literature Li et al. (2014)					

Taxon	Specimens No.	Location	GenBank accession		
			nrLSU	RPB2	EF1
^g sequences from the literature Deng et al. (2014)					
^h sequences from the literature Truong et al. (2017)					
ⁱ sequences from the Genbank					
^j sequences obtained in this study					

Results

Phylogenetic analyses

nrLSU phylogenetic analysis of Amanitaceae

For *Amanita* species from sect. *Roanokenses* that lacked ITS sequences, we built an nrLSU phylogenetic tree. In this study, 75 nrLSU sequences of Amanitaceae, which included two for the newly reported taxa, were included in the nrLSU phylogenetic study. The nrLSU dataset contained 834 nucleotide sites after alignment (including gaps), 403 characters were variable, of which 318 characters were parsimony-informative. Tree topologies obtained by both ML and BI were almost identical; thus, only the BI tree is shown in Fig. 1. Representatives of the Amanitaceae species *Limacellopsis asiatica* were selected as the outgroup. Phylogenetic analysis indicated that the new species *A. alboradicata* and *A. fulvopyramis* clustered with the members of sect. *Roanokenses* with ML bootstrap (BS) = 100 % and BI posterior probability (PP) = 1.0. Moreover, *A. castanopsis* and *A. japonica* clustered with the new species *A. alboradicata*.

Multi-locus (nrLSU, tef1-α and rpb2) phylogenetic analysis of sect. Roanokenses

More than 40 species of sect. *Roanokenses* have been reported from China (Cui et al. 2018). For some species from sect. *Roanokenses* lack of ITS sequences, 30 species were selected for multi-locus (nrLSU, *tef1-α* and *rpb2*) phylogenetic analysis, which included the two new species (Fig. 2). The multi-locus dataset aligned sequences contained 1918 nucleotide sites (including gaps), 765 characters were variable, of which 612 characters were parsimony-informative. The results showed that the new species *A. alboradicata* clustered with *A. castanopsis* in the phylogenetic analysis based on the multi-locus (ML BS = 100 %, BI PP = 1). The other new species, *A. fulvopyramis*, clustered with *A. brunneofolia* in the phylogenetic analysis based on the multi-locus sequence with strong support (ML BS = 100 %, BI PP = 1).

Taxonomy

Amanita alboradicata P. Zhang, **sp. nov.** Figures 3 and 4

MycoBank: *****

Etymology: *alboradicata*, from *albus* = white, and *radicata*, from *radicatus* = rooted, is proposed because the species is white and has a radicating basal bulb.

Holotype: Tonghua county, Baijifeng Forest Park, Jilin province, China, in a temperate forest, altitude 740 m, 28 Aug. 2020, P. Zhang 4031 (TYPE, MHHNU10531, GenBank Acc. No.: nrLSU = MW016757, *tef1-α* = MW546614, *rpb2* = MW546620).

Basidiomata (Fig. 3) medium-sized to large. *Pileus* 7–9 cm in diam., globose when young, hemispherical when expanding, later convex to plano-convex, without umbo, with margin non-striate and often splitting with age, strongly appendiculate; surface pure white (1A1), usually decorated with big, white verrucose warts easily falling off with age or in rain, with large, angular, dirty white (1A2) warts on the center, 2–5 mm wide and 1–3 mm high; trama white (1A1), unchanging. *Lamellae* free, pure white (1A1), somewhat crowded; lamellulae attenuate. Stipe 23–28 cm long (with *radicatus*), 1.5–2 cm wide at apex, subcylindric or slightly attenuate upwards, surface white (1A1), upper part often covered with white floccose to farinose squamules, lower part often covered with pale white floccose recurving squamules; context white; basal bulb fusiform, strobiliform to long clavate, radicating, 2–3.5 cm in diam., recurving scaled volval remnants often arranged in circles on its surface. *Annulus* apical to subapical, floccose to pulverulent, fragile and fugacious. Spore print white. Odor nondistinct.

Lamellar trama bilateral. Mediostratum 40–60 μm wide, composed of abundant subfusiform, ellipsoid to clavate inflated cells (40–75 × 10–40 μm); filamentous hyphae abundant, 3–10 μm wide; vascular hyphae scarce. Lateral stratum composed of abundant subfusiform to ellipsoid inflated cells (25–45 × 7–14 μm), diverging at an angle of ca. 30° to 60° to mediostratum; filamentous hyphae abundant and 2–6 μm wide. *Subhymenium* 40–70 μm thick, with 2–3 layers of subglobose to ellipsoid or irregular cells, 12–28 × 10–20 μm. *Basidia* (Fig. 4-a) 36–60 × 8–13 μm, slenderly clavate, 4-spored, clamps present common, hyaline; 4 sterigmata, straight, up to 2–5 μm long; *basidiospores* (Fig. 4-b) [60/2/2] (9) 10–12(13) × 5.5–7 μm, $Q = (1.43)1.54–1.92(2.18)$, $Q_m = 1.77 \pm 0.41$, mostly ellipsoid, occasionally broadly ellipsoid or elongate, thin-walled, hyaline, smooth, with a small to medium-large apiculus, amyloid; *lamellar edge* appearing as a sterile strip, composed of subglobose to ellipsoid or sphaeropedunculate inflated cells (20–45 × 18–36 μm), single and terminal or in chains of 2–3, thin-walled, hyaline; filamentous hyphae abundant, 3–6 μm wide, irregularly arranged or ± running parallel to lamellar edge. *Pileipellis* 120–200 μm thick, lacking differentiation between upper and lower layer, nongelatinized or with upper layer slightly gelatinized, composed of radially arranged, thin-walled, hyaline to yellowish, filamentous hyphae 3–12 μm wide, vascular hyphae scarce. *Volval remnants* on pileus composed of inflated cells and filamentous hyphae (Fig. 4-c), more or less erectly arranged, inflated cells very abundant to dominant, up to 80 × 50 μm, subglobose, ovoid to ellipsoid, clavate, or sphaeropedunculate, with hyaline vacuolar; filamentous hyphae fairly abundant, 3–12 μm wide, frequently branching, with thin-walled and hyaline to dirty white vacuolar pigments; septa sometimes with clamps. *Volval remnants* on stipe base similar to that on pileus, but with more abundant filamentous hyphae. *Stipe trama* longitudinally acrophysalidic; acrophysalides 200–400 × 20–60 μm; filamentous hyphae scattered to abundant, 2–15 μm wide. *Annulus* composed of radially arranged elements: inflated cells very abundant, subglobose, fusiform to ellipsoid, 12–35 × 12–25 μm,

hyaline, thin-walled; filamentous hyphae abundant, 2–8 µm wide, hyaline, thin-walled. *Clamps* present in all parts of basidioma.

Habitat and distribution Solitary or scattered on soil in mixed forests, at 700–800 m alt. Presently known only from Baijifeng Forest Park in Jilin province, North China.

Additional specimens examined: Tonghua county, Baijifeng Forest Park, Jilin province, China, at 126°06'21.21"E, 41°56'54.92"N, alt. 740 m. 28 Aug. 2020, P. Zhang 4035 (MHHNU10535).

Commentary: *Amanita alboradicata* is characterized by its medium-sized to large basidioma with a white pileus covered with white to dirty white verrucose volval remnants, white lamellae, a whitish stipe covered with white floccose to farinose recurving squamules, a fusiform, strobiliform to long clavate radicating basal bulb, ellipsoid amyloid spores, and abundant clamps in all tissues. Although it is genetically very closely related to *A. castanopsis* and *A. japonica*, *A. alboradicata* can easily be distinguished. First, the basidiomata colors from the three *Amanita* are easy to distinguish; *A. alboradicata* is white to dirty white on whole basidiomata, whereas *A. japonica* has a grey pileus and *A. castanopsis* has a yellow to brown bulb at the stipe base (Imazeki et al. 1988). Moreover, *A. japonica* possesses a fusiform-rooting to subclavate basal bulb covered with floccose-pulverulent and grey volval remnants (Bas 1969), whereas *A. alboradicata* is covered with floccose-pulverulent and white volval remnants.

Amanita brunneostrobilipes Zhu L. Yang, Y.Y. Cui & Q. Cai, which share a white basidioma with a radicating basal bulb from sect. *Roanokenses*, however, *A. brunneostrobilipes* differs from *A. alboradicata* by its brown, recurved squamules on the basal bulb and the absence of clamps. *Amanita virgineoides* is also similar to *A. alboradicata*. They both have white basidiomata, white volval remnants, clamps present, and an appendiculate margins. However, *A. virgineoides* annulus has a verrucose lower surface, whereas that of *A. alboradicata* is floccose to pulverulent.

***Amanita fulvopyramis* P. Zhang, sp. nov.** Figures 5 and 6

MycoBank: *****

Etymology: *fulvopyramis*, from *fulvo* = brown to gray-brown, and *pyramis* = pyramidal, is proposed because its volval remnants on pileus conical, subconical to pyramidal, brownish.

Holotype: Yizhang county, Mangshan National Nature Reserve, Hunan province, China, in a mixed forest with Fagaceae and Pinaceae, alt. 1400 m, 27 Jul. 2016, P. Zhang 2314 (TYPE, MHHNU8814, GenBank Acc. No.: nrLSU = MT878535, *tef1-α* = MW546617, *rpb2* = MW546623).

Basidiomata (Fig. 5) medium-sized to large. *Pileus* 9–10 cm in diam., globose when young, hemispherical when expanding, later plano-convex to applanate, without umbo, with margin non-striate and often splitting with age, strongly appendiculate; surface grey yellow (3B5) when young, then light yellow (4A5) to dark yellow (3B3–3B4), usually decorated with big, acute pyramidal, verrucose warts, 2–3 mm wide and 2–4 mm high in the center of the pileus, becoming minute towards margin, grey orange (5B4), brown orange (5C6) to light brown (5D4–5), or brown (5E4), easily falling off, leaving yellowish (4A2–3) to light brown

(5D4) prints or scars with age; trama pale yellow (2A3), unchanging. *Lamellae* adnexed, light brown (5D3) to brown (5E4), somewhat crowded; lamellulae attenuate, plentiful. Stipe 18–25 cm long (with radicans), 0.5–1.7 cm wide at apex, subcylindric or slightly attenuate upwards, surface white to yellowish white (4A1–4A2), with silk luster, upper part often covered with pale yellow (4A3) to pale orange (5A3) floccose to farinose squamules, lower part often covered with brown orange (5C5) to brownish yellow (5C7) pulverulent, floccose to tomentose squamules; context white to pale yellow (4A2); basal bulb strobiliform to long clavate, radicating, 2–3.5 cm in diam., covered with subfelted to subtomentose volval remnants, sometimes exhibiting some longitudinal splitting with age. *Annulus* apical, subapical to fugacious, thick, with cream-colored (3A2) and silk luster at upper surface, lower surface matt yellow (3B3–4) with floccose to farinose warts. Spore print dirty white (1A2) to creamy yellow (4A2). Odor nondistinct.

Lamellar trama bilateral. Mediostratum 30–60 µm wide, composed of abundant subfusiform, ellipsoid to clavate inflated cells (50–80 × 12–40 µm); filamentous hyphae abundant, 4–9 µm wide; vascular hyphae scarce. Lateral stratum composed of abundant subfusiform to ellipsoid inflated cells (25–45 × 6–15 µm), diverging at an angle of ca. 30° to 45° to mediostratum; filamentous hyphae abundant and 2–5 µm wide. *Subhymenium* 30–55 µm thick, with 2–3 layers of subglobose to ellipsoid or irregular cells, 10–25 × 10–20 µm. *Basidia* (Fig. 6–a) 45–60 × 10.5–13.5 µm, slenderly clavate, 4-spored, clamps present, hyaline to yellow; sterigmata 5–6 µm long; *basidiospores* (Fig. 6–b) [60/2/2] (9)9–11(11.5) × (7)7–8.5(8.5) µm, $Q = (1.20) 1.25–1.50 (1.64)$, $Q_m = 1.36 \pm 0.28$, mostly ellipsoid, occasionally broadly ellipsoid or elongate, thin-walled, hyaline, pale-yellow, smooth, with a small to medium-large apiculus, amyloid; *lamellar edge* sterile, composed of subglobose to ellipsoid or sphaeropedunculate inflated cells 25–40 × 15–30 µm, single and terminal or in chains of 2–3, thin-walled, hyaline; filamentous hyphae abundant, 3–5 µm wide, irregularly arranged or ± running parallel to lamellar edge. *Pileipellis* 150–200 µm thick, lacking differentiation between upper and lower layer, nongelatinized or with upper layer slightly gelatinized, composed of radially arranged, thin-walled, hyaline to yellowish, filamentous hyphae 3–10(–12) µm wide. *Volval remnants* on pileus composed of inflated cells and filamentous hyphae (Fig. 6–c), more or less arranged in erectly chains; inflated cells very abundant to dominant, up to 80 × 50 µm, subglobose, ovoid to ellipsoid, clavate or sphaeropedunculate, with hyaline to yellowish vacuolar pigments; septa with clamps; Inner part of volval remnants on pileus often with conspicuous vascular hyphae. *Volval remnants* on stipe base similar to that on pileus, but with more abundant filamentous hyphae and fairly abundant vascular hyphae. *Stipe trama* longitudinally acrophysalidic; acrophysalides 200–400 × 25–50 µm; filamentous hyphae scattered to abundant, 3–15 µm wide. *Annulus* composed of radially arranged elements: inflated cells very abundant, subglobose, fusiform to ellipsoid, 15–40 × 15–25 µm, hyaline, thin-walled; filamentous hyphae abundant, 2–7 µm wide, hyaline, thin-walled. *Clamps* present in all parts of basidioma.

Habitat and distribution Solitary or scattered on soil in mixed forests, at 800–1400 m alt. Only known from Mangshan National Nature Reserve in Hunan province and Qianjiangyuan National Park in Zhejiang province, China.

Additional specimens examined: Yizhang county, Mangshan National Nature Reserve, Hunan province, China, at 112°56'14.21"E, 24°57'13.58"N, alt. 1400 m. 28 Jul. 2016, P. Zhang 2341 (MHHNU8841); same location, 29 Jul. 2019, P. Zhang 3691 (MHHNU10191); Kaihua county, Qianjiangyuan National Park, Zhejiang

province, China, at 118°8' 39.86"E, 29°15'4.51"N, alt. 874 m, 11 Sep. 2018, Jin-Bao Pu (HKAS106468); same location, at 118°14'01.67"E, 29°24'72.12"N, alt. 846 m, 16 Jul. 2020, Jin-Bao Pu & Wei-Qing Liang, P. Zhang 4081 (MHHNU10581).

Commentary: *Amanita fulvopyramis* is characterized by its medium-sized to large basidioma with a light yellow to dark yellow pileus covered with brownish pyramidal to verrucose volval remnants, a subclavate, napiform to wedged and radicating basal bulb, ellipsoid amyloid spores, and abundant clamps in all tissues. *Amanita brunneofolia* J.W. Jo, H.S. Kim, Y.N. Kwag & C.S. Kim is the most similar species morphologically, but molecular evidence proves that it represents different species. *Amanita brunneofolia* is characterized by a yellowish white to greenish white pileus, covered with brownish, floccose pyramidal volval remnants, an appendiculate margin, reddish brown lamellae, a long radicating stipe, and ellipsoid to elongate amyloid basidiospores ($8.2\text{--}9.6 \times 5.2\text{--}5.9 \mu\text{m}$) (JO et al. 2020a). *A. fulvopyramis* has a yellowish white to dark yellow pileus, and a relatively larger basidiospores ($9\text{--}11 \times 7\text{--}8.5 \mu\text{m}$). They are very easy to confuse because there are few differences; however, *A. brunneofolia* is only found in South Korea, and *A. fulvopyramis* is only found in subtropical China.

Amanita fulvopyramis is related to *A. macrocarpa* W.Q. Deng, T.H. Li & Zhu L. Yang and *A. xanthogala* Bas. However, *A. macrocarpa* has a larger basidioma with a ca. 15–24 cm pileus (Deng et al. 2014). *Amanita xanthogala* releases an oily yellow latex when injured, whereas *A. fulvopyramis* does not release any latex or discolor on bruising; moreover, *A. xanthogala* has a whitish or yellowish to pale fulvo-cinnamomeus pileus (Bas 1969). Furthermore, *A. fulvopyramis* has a light brown to brown lamellae, whereas *A. macrocarpa* and *A. xanthogala* have yellowish to cream lamellae.

Discussion

The two new species assigned to *Amanita* sect. *Roanokenses* both have verrucose to floccose volval remnants on the pileus surface and stipe base, and the pileus margin is non-striate and appendiculate; moreover, they both possess a long radicating basal bulb. Singer (1962a) provided a brief Latin diagnosis of sect. *Roanokenses*, “Pileo stipiteque pulvere deterisili obtectis”, which means pileus or stipe covered with powder or deterisile volval remnants. Cui et al. (2018) described a more detailed diagnosis of sect. *Roanokenses*, he state that stipe with bulbous base. However, molecular phylogenetic analyses show that the two new species belong to sect. *Roanokenses*, we compared the characters of the two new species with Singer’s Latin diagnosis and Cui’s description, and we speculate that the main characteristics of sect. *Roanokenses* as follows: pileus margin non-striate, appendiculate; stipe with bulbous base or with a radicating basal bulb; volval remnants often as cones, verrucae, pyramids, powder, floccules to patches on pileus surface and stipe base; or volval remnants absent on pileus and then leaving a limbate volva on stipe base; annulus membranous to fugacious or absent; basidiospores amyloid; clamps present or absent (Singer 1962a; Cui et al. 2018). Most members of sect. *Roanokenses* possess all kinds of volval remnants on the pileus and stipe base, and a few members do not have volval remnants on the pileus but possesses a limbate volva, such as *A. pseudoporphyria* Hongo and *A. oberwinklerana* Zhu L. Yang & Yoshim. Doi.. Additionally, most members of sect. *Roanokenses* have a bulbous stipe base, but a few members have a long radicating basal bulb, such as *A. japonica*, *A. ejii* Zhu L. Yang, *A. brunneofolia*, and the two new

species. Thus, it would be necessary to seek more section-specific diagnoses to distinguish the members of sect. *Roanokenses*.

Mushroom poisoning cases caused by *Amanita* have been frequently reported in recent decades (Chen 2016). Multi-locus phylogenetic analysis of Amanitaceae revealed that the species in *Amanita* sect. *Phalloideae* could be separated into three subclades, one of which is a lethal *Amanita* subclade (Cui et al. 2018). *Amanita caojizong* Zhu L. Yang, Y.Y. Cui & Q. Cai, *A. modesta* Corner & Bas, *A. oberwinklerana* and *A. pseudoporphyrina* were previously arranged in *Amanita* sect. *Phalloideae* together with the lethal *Amanita*, but now belong to sect. *Roanokenses*. Chen (2016) suggested that *A. oberwinklerana* and *A. pseudoporphyrina* may cause acute kidney failure. Therefore, we speculate that some *Amanita* in sect. *Roanokenses* may be poisonous. In addition, three members of sect. *Roanokenses*, *A. gymnopus* Corner & Bas, *A. kotohiraensis* Nagas. & Mitani, and *A. neoovoidea* may also cause the same symptoms as *A. oberwinklerana* and *A. pseudoporphyrina*, these species may potentially contain 2-amino-4,5-hexadienoic acid. Because of the low 2-amino-4,5-hexadienoic acid content in *Amanita*, there is no effective method to detect this substance. Therefore, whether the new species also causes symptoms of renal failure remains unknown.

We found that the closely related species *A. castanopsis* and *A. rubiginosa* both have an enlarged stipe base. In fact, most members of sect. *Roanokenses* have a bulbous stipe base or have a long radicating basal bulb. According to our molecular research and morphological comparison in this study, we speculate that there may be a close genetic relationship between species having a globose or subglobose basal bulb and species with a radicating basal bulb.

The radicata is an extension of the stipe, not a rhizoid. In fungi, rhizoids are small branching hyphae that grow downwards from the stolons that anchor the fungus to the substrate, where they release digestive enzymes and absorb digested organic material; this is why fungi are called heterotrophs by absorption. Whereas, the radicating basal bulb extends deep into the substrate to transport nutrients and provide better support to the basidiocarp.

Declarations

Acknowledgments

The authors thank Mallory Eckstut, PhD, from Liwen Bianji, Edanz Editing, China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript. This study was financially supported by the National Natural Science Foundation of China (No. 31750001; No. 31670015), and the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China, 2019HJ2096001006.

Funding: This study was financially supported by the National Natural Science Foundation of China (No. 31750001; No. 31670015), and the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China, 2019HJ2096001006.

Conflict of interest: The authors declare that they have no conflicts of interest.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: DNA sequence data that support the findings of this study have been deposited in GenBank repository. Other data generated or analysed during this study are included in this published article.

Code availability: Not applicable.

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Juan Zhong, Gui-Wu Li, Jin-Bao Pu, Zuo-Hong Chen, Ping Zhang. The first draft of the manuscript was written by Juan Zhong and Ping Zhang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Consensus Amanitaceae phylogenetic tree generated from nrLSU sequences using MrBayes Bayesian inference (BI) and RAXML maximum likelihood (ML) analyses. BI posterior probabilities over 0.90 and ML bootstrap values over 50% are reported at nodes (BI/ML). New species are shown in bold text. Only the BI tree is shown.

Figure 2

Phylogeny of sect. Roanokenses inferred from multi-locus (nrLSU, tef1- α and rpb2) sequences using MrBayes Bayesian inference and RAxML maximum likelihood. BI posterior probabilities over 0.90 and ML bootstrap values over 50% are reported at nodes (BI/ML). New species are shown in bold text. Only the BI tree is shown.

Figure 3

Basidiomata of *Amanita alboradicata*. a and b. Mature basidiomata, MHHNU10531 c. A long radi-cating basal bulb of basidiomata, MHHNU10535. Scales bars = 2 cm.

Figure 4

Microscopic characters of *Amanita alboradicata* (type, MHHNU10531) a. Hymenium and sub-hymenium; b. basidiospores; c. volval remnant on pileus.

Figure 5

Basidiomata of *Amanita fulvopyramis*. a. Young basidiomata, MHHNU8841. b. Mature basidio-mata, MHHNU8814. c. Lamellae of basidiomata, MHHNU8814 (holotype). Scales bars = 2 cm.

Figure 6

Microscopic characters of *Amanita fulvopyramis* (type, MHHNU8814) a. Hymenium and subhymenium; b. basidiospores; c. volval remnant on pileus.