Morphology, Multilocus Phylogeny, and Toxin Analysis Reveal *Amanita albolimbata*, the First Lethal *Amanita* Species From Benin, West Africa

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INTRODUCTION

Some species of *Amanita* Pers. are edible, whereas others are poisonous. All lethally poisonous *Amanita* species are in *A.* sect. *Phalloideae* that is characterized by a non-striate and non-appendiculate pileus and attenuate lamellulae, the presence of persistent annulus, a limbate volva on the bulbous stipe base, and amyloid basidiospores (Corner and Bas, 1962; Bas, 1969; Tulloss and Bhandary, 1992; Yang, 1997, 2005, 2015). Members of the section are responsible for greater than 90% of mushroom fatalities reported worldwide (Bresinsky and Besl, 1990; Unluoglu and Tayfur, 2003; Cai et al., 2014, 2016; Li et al., 2015). In Central Europe and North America, lethal amanitas such as *A. bisporigera* G.F. Atk., *A. phalloides* (Vaill.: Fr.) Link, *A. subhilarcaea* (Murrill) Murrill, *A. verna* (Bull.) Lam., and *A. virosa* Bertill. have caused dramatic human and animal poisoning cases (Wieland, 1973, 1986; Bresinsky and Besl, 1985). It has been reported that *A. exitialis* Zhu L. Yang and T. H. Li, *A. fuliginea* Hongo, *A. fuligineoides* P. Zhang and Zhu L. Yang, *A. pallidorosea* P. Zhang and Zhu L. Yang, *A. rimosa* P. Zhang and Zhu L. Yang, and *A. subjunquillea* S. Imai cause a lot of fungal...
Lethal Amanita Species From West Africa

poisoning cases in East Asia (Zhang et al., 2010; Deng et al., 2011; Chen et al., 2014; Li et al., 2014, 2020; Cai et al., 2016). The toxins in lethal amanitas are mainly amatoxins, phallotoxins, and virotoxins (Wieland et al., 1983; Wieland, 1986; Cai et al., 2016). Among them, amatoxins are 10–20 times more toxic than the other ones and represent the major toxins responsible for human poisoning (Li and Oberlies, 2005). These cyclopeptide toxins are able to resist high temperatures, and their consumption can cause severe liver and renal failure (Wieland, 1973, 1986; Chen et al., 2014).

Lethal amanitas have been extensively studied in Asia, Europe, and America, where more than 50 toxic taxa have been described (Zhang et al., 2010; Cai et al., 2014, 2016; Li et al., 2015; Yang, 2015; Thongbai et al., 2017; Cui et al., 2018). Although the genus Amanita is known worldwide, few taxa of the genus have been reported from tropical Africa (Walleyn and Verbeken, 1998; Eyi-Ndong et al., 2011; Yorou et al., 2014; Härkönen et al., 2015; De Kesel et al., 2017; Fraiture et al., 2019; Tulloss et al., 2020). Six species belonging to A. sect. Phalloideae are known from tropical Africa, of which three, including A. allobora Pat., A. murinacea Pat., and A. thejoleuca Pat., were described from Madagascar (Patrouillard, 1924; Fraiture et al., 2019). The other three species, A. bweyeyensis Fraiture, Raspé and Degreef, A. harkoneniana Fraiture and Saarmäki, and A. strophiolata Beeli were described from DR Congo (Beeli, 1927, 1935; Fraiture et al., 2019).

In this study, a new member of A. sect. Phalloideae from tropical West Africa is described. Its macro- and micromorphological characteristics, as well as its phylogenetic relationships with other Amanita species are discussed. In addition, the screening of the species for the known toxins occurring in Amanita is reported.

MATERIALS AND METHODS

Collections and Preservation

Specimens were opportunistically collected in Benin, West Africa (Figure 1), during the rainy season from June to September (2018-2019), especially in the forest dominated by Fabaceae/Leguminosae (Isoberlinia Craib and Stapf ex Holland, Anthonotha P. Beauv., Berlinia Sol. ex Hook. f.), Phyllanthaceae (Uapaca Baill.), and Dipterocarpaceae (Monotes A. DC.). Specimens were photographed in situ using a digital camera–type Canon EOS 60D. Macro- and micromorphological characteristics were recorded on fresh materials, according to Tulloss and Yang (2011). Color codes recorded from fresh materials follow Kornerup and Wanscher (1981). The fresh basidiomata were dried using an electric dryer Stockli Dorrex at 45°C for 1 day and stored thereafter as exsiccates with their label in sealable plastic bag–type minigrip. The dried specimens along with the duplicates of dried specimens and the isotype of the new species are conserved at the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Small pieces of fresh basidiomata were also stored in CTAB lysis buffer (2% cetyl trimethylammonium bromide, 100 mM Tris–HCl, 20 mM EDTA, 1.4 M NaCl) and dried with silica gel for molecular investigations. Nomenclature aspects, as well as authorities for scientific names, have been double checked against Index Fungorum and in Tulloss et al. (2020).

Micromorphological Investigations

Microscopic structures were studied from dried materials mounted in 5% KOH and stained with Congo red to depict all tissues. The Melzer's reagent was used to test the amyloidity of basidiospores. All measurements and line drawings were performed at 1,000× magnification, and a minimum of 20–30 basidiospores from each basidioma were measured in side view. Micromorphological investigations were performed by means of a microscope-type Nikon Eclipse 50i. The abbreviation (n/m/p) is used to describe basidiospores where n is the number of basidiospores from m basidiomata of p collections. The basidiospores dimensions are provided using the notation (a)–b)–c(d) with the range b–c containing a minimum of 95% of the measured values, a and d in the brackets showing the two extreme values. Q is used for the ratio length/width of a spore in side view; Qm is the average Q of all basidiospores ± sample standard deviation. The measurements for the basidiospores were analyzed with Piximetre v5.10 (Henriot and Cheyve, 2020). The descriptive terms are in accordance with Bas (1969), Yang (2005), Yang (2015), Cai et al. (2016), and Cui et al. (2018).

DNA Extraction, Amplification, and Sequencing

The total genomic DNA was obtained from materials preserved in CTAB or dried with silica gel following the modified CTAB procedure (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) amplification and sequencing were performed in accordance with those described in Cai et al. (2014) and Cui et al. (2018). The following primer pairs were used for PCR amplification and sequencing: ITS1F and ITS4 to amplify ITS region (White et al., 1990; Gardes and Bruns, 1993); LROR and LR5 (Vilgalys and Hester, 1990) for nrLSU region; EF1-983F and EF1-1567R (Rehner and Buckley, 2005) for the translation elongation factor 1α (tef1-α) region; ARP2B-6F and ARP2B-7R for RNA polymerase II second largest subunit (rpb2) region; and Am-b-tubulin F and Am-b-tubulin R (Cai et al., 2014) for beta-tubulin (β-tubulin) region.

Sequence Alignment and Phylogenetic Analyses

Thirty-four sequences (seven for ITS, eight for nrLSU, seven for rpb2, six for tef1-α, and six for β-tubulin) were newly generated for this study and deposited in GenBank (Table 1)2. Additional sequences were retrieved from previously published articles and GenBank (Table 1). The sequences were aligned using MAFFT v7.310 (Katoh and Standley, 2013) and edited manually when necessary using BioEdit v7.0.9 (Hall, 1999). The

1 http://www.mycology.net
2 http://www.ncbi.nlm.nih.gov/
poorly aligned portions and divergent regions were eliminated using Gblocks v0.91b (Castresana, 2000; Talavera and Castresana, 2007). A concatenated dataset (including ITS, nrLSU, rpb2, tef1-α, β-tubulin) comprising 312 sequences was constructed using Phyutility v2.2 (Smith and Dunn, 2008) and used for phylogenetic analyses. Before using the concatenated dataset for phylogenetic analyses, the Incongruence Length Difference test in PAUP v4.0a168 (Swofford, 2002) was performed to detect any conflicts between the gene regions. As no incongruence ($P = 0.363000$) was detected, the maximum likelihood (ML) and Bayesian inference (BI) were used on the concatenated alignment for phylogenetic tree inference. The ML analysis was performed using RAxML v7.9.1 (Stamatakis, 2006) under the GTR + GAMMA + I nucleotide substitution model and performing non-parametric bootstrapping with 1,000 replicates. The BI was performed in MrBayes v3.2 (Ronquist et al., 2012). The best substitution model was determined using the Akaike Information Criterion implemented in jModeltest v.2 on the CIPRES Science Gateway v3.1 (Miller et al., 2010). The BI was conducted with the following parameters: two runs, each with four simultaneous Markov chains, and trees were summarized every 1,000 generations. The analyses were completed after 20,000,000 generations when the average standard deviation of split frequencies was 0.002200 for the five-gene analysis, and the first 25% generations were discarded as burn-in. The phylograms from ML and BI analyses were visualized with


| Species name                  | Collection or collector no. | Country of origin | GenBank accession no. | ITS | nrLSU | rpb2 | tefl-α | β-tubulin |
|-------------------------------|-----------------------------|-------------------|-----------------------|-----|-------|------|--------|-----------|
| *Amanita alliaria*            |                            |                   |                       |     |       |      |        |           |
| *Amanita albolimbata*         | JEIC0707                   | Benin             | MT966936              | MT966943 | MT966959 | MT966956 | MT966951 |           |
| *Amanita albolimbata*         | JEIC0739                   | Benin             | MT966935              | MT966942 | MT966953 | MT966955 | MT966950 |           |
| *Amanita albolimbata*         | JEIC0667                   | Benin             | MT966932              | MT966939 | MT966958 | MT966953 | MT966947 |           |
| *Amanita albolimbata*         | JEIC0675                   | Benin             | MT966934              | MT966941 | MT966947 | MT966946 | MT966949 |           |
| *Amanita albolimbata*         | JEIC0653                   | Benin             | MT966933              | MT966940 | MT966941 | MT966954 | MT966948 |           |
| *Amanita albolimbata*         | JEIC0638                   | Benin             | HKAS94241             | MT966944 | MT966945 | –      | –      |           |
| *Amanita albolimbata*         | HKAS93847                  | Benin             | MT966937              | MT966945 | –      | –      | –      |           |
| *Amanita australis*           |                            |                   |                       |     |       |      |        |           |
| *Amanita biverticillata*      |                            |                   |                       |     |       |      |        |           |
| *Amanita brunneitoxicaria*    |                            |                   |                       |     |       |      |        |           |
| *Amanita bweyeyensis*         |                            |                   |                       |     |       |      |        |           |
| *Amanita djarilmari*          |                            |                   |                       |     |       |      |        |           |
| *Amanita eucalypti*           |                            |                   |                       |     |       |      |        |           |
| *Amanita exitialis*           |                            |                   |                       |     |       |      |        |           |
| *Amanita fuliginea*           |                            |                   |                       |     |       |      |        |           |
| *Amanita fuligineoides*       |                            |                   |                       |     |       |      |        |           |
| *Amanita gardneri*            |                            |                   |                       |     |       |      |        |           |
| *Amanita griseorosea*         |                            |                   |                       |     |       |      |        |           |
| *Amanita harkoneniana*        |                            |                   |                       |     |       |      |        |           |
| *Amanita krombholzi*          |                            |                   |                       |     |       |      |        |           |
| *Amanita mollis*              |                            |                   |                       |     |       |      |        |           |
| *Amanita molybdopholis*       |                            |                   |                       |     |       |      |        |           |
| *Amanita parviexitialis*      |                            |                   |                       |     |       |      |        |           |
| *Amanita phalloides*          |                            |                   |                       |     |       |      |        |           |

(Continued)
### RESULTS

#### Phylogenetic Data

The topologies of ML and BI phylogenetic trees obtained in this study are practically the same (Supplementary Figures S1, S2). In the combined dataset (ITS, nrLSU, rpb2, tef1-α, and β-tubulin), 312 sequences were included. The combined dataset contained 3,024 total characters, including 2,070 constant (proportion = 0.684524), 93 variable and parsimony-uninformative, and 861 parsimony-informative. The target species, *A. albolimbata*, forms a well-supported distinct lineage (MLB = 100%, BPP = 1.0) and is a close sister to the Asian species (*A. parviexitialis* Qing Cai, Zhu L. Yang and Yang-Yang Cui) (Figure 2 and see also Supplementary Figures S1–S7). Within the section, *A. albolimbata* is genetically distant from other African species such as *A. alliodora*, *A. bweyeyensis*, and *A. harkoneniana*. In the phylogenetic tree, the African and Australian species hold the basal positions.

#### Taxonomy

*Amanita albolimbata* J.E.I. Codjia, N.S. Yorou and Zhu L. Yang, sp. nov.

Mycobank: MB836777

Figures 3, 4
Etymology
“Albo” (white), “limbata” (limb bearing volva), meaning white limbate volva.

Type
BENIN. Donga Province: Bassila, 09°07′58″N, 2°07′43″E, Forest Reserve of Bassila, woodland of Uapaca togoensis Pax (Phyllanthaceae), date: 06 August 2019, leg. and det. Jean Evans I. CODJIA, Holotype JEIC0739 (UNIPAR), Isotype (Phyllanthaceae), date: 06 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0667, date: 11 September 2019, leg. and det. Jean Evans I. CODJIA, JEIC0653. Borgou Province: Okpara, Atacora Province: Kota, 10°12′39″N, 01°26′45.8″E, gallery forest of Kota, date: 08 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0638. Donga Province: Bassila, 09°07′58″N, 2°07′43″E, Forest Reserve of Bassila, date: 02 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0707. Borgou Province: Ndali, 09°14′31.93″N, 02°43′22.7″E, Forest Reserve of Ndali, date: 31 July 2019, leg. and det. Jean Evans I. CODJIA, JEIC0638. Borgou Province: Okpara, 09°16′34.8″N, 02°43′12.8″E, Forest Reserve of Okpara, date: 22 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0667, date: 11 September 2019, leg. and det. Jean Evans I. CODJIA, JEIC0675.

Distribution
Currently known from Benin, but likely occurs more widely in the region in similar vegetation. Additional specimens examined were BENIN. Colline Province: Ouessé, 08°26′34.4.0″N, 02°33′09.0″E, open forest, date: 02 July 2015, leg. B. Feng 1854 (HKAS94241), date: 03 July 2015, leg. G. Wu 1470 (HKAS93847). Borgou Province: Ndali, 09°14′31.93″N, 02°43′22.7″E, Forest Reserve of Ndali, date: 31 July 2019, leg. and det. Jean Evans I. CODJIA, JEIC0638. Donga Province: Bassila, 09°07′58″N, 2°07′43″E, Forest Reserve of Bassila, date: 02 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0707. Atacora Province: Kota, 10°12′39″N, 01°26′45.8″E, gallery forest of Kota, date: 08 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0653. Borgou Province: Okpara, 09°16′34.8″N, 02°43′12.8″E, Forest Reserve of Okpara, date: 22 August 2019, leg. and det. Jean Evans I. CODJIA, JEIC0667, date: 11 September 2019, leg. and det. Jean Evans I. CODJIA, JEIC0675.

Analysis of Toxins by LC-HRMS
Amanita albolimbata contains three cyclic peptides: α-amanitin, β-amanitin, and phallacidin (Figure 5). The formula of α-amanitin is C₃₉H₅₄N₁₆O₁₅S with a monoisotopic mass of 918.3541. The calculated mass of the [M + H]⁺ ion is 919.3614,
and the measured mass was 919.3609 with mass discrepancy of 0.59 ppm. The formula of \(\beta\)-amanitin is \(C_{39}H_{53}N_9O_{15}S\) with a monoisotopic mass of 919.3381. The calculated mass of the \([M + H]^+\) ion is 920.3455, and the measured mass was 920.3461 with mass discrepancy of 0.7 ppm. The formula of phallacidin is \(C_{37}H_{50}N_8O_{13}S\) with a monoisotopic mass of 846.3218. The calculated mass of the \([M + H]^+\) ion is 847.3291, and the measured mass was 847.3293 with mass discrepancy of 0.26 ppm.

The measured masses of the two other adduct ions for above cyclic peptides, \([M + Na]^+\) and \([M + K]^+\), are also included in Figure 5. No corresponding mass was identified for phalloidin.

**DISCUSSION**

**Species Delimitation**

Most of the fatal mushroom poisonings are caused by lethal amanitas belonging to A. sect. Phalloideae (Bresinsky and Besl, 1990; Uluoglu and Tayfur, 2003; Cai et al., 2014, 2016; Li et al., 2015; Cui et al., 2018). In tropical Africa, very few lethal amanitas have been reported (Walley and Verbeke, 1998; Fraiture et al., 2019, 2020; Tulloss et al., 2020). Only six species are known from tropical Africa including A. alliodora, A. murinacea, and A. thejoleuca described from Madagascar and A. bweyeyensis,
A. harkoneniana, and A. strophiolata described from DR Congo (central Africa). *Amanita albolimbata* represents a new lethal *Amanita* from tropical Africa and can be recognized by its white basidiomata with a convex or planate pileus without umbo, a limbate volva with inner part composed of abundant inflated cells, and broadly ellipsoid to ellipsoid basidiospores.

The multigene phylogenetic analyses revealed that *A. albolimbata* is an independent lineage in *A. sect. Phalloideae*. Surprisingly, the species is genetically distant from other African species such as *A. alliodora*, *A. bweyeyensis*, and *A. harkoneniana* that form a clade. Among lethal amanitas from tropical Africa, *A. albolimbata* is similar to *A. strophiolata* because of the white basidiomata, but unfortunately, we do not have any material of the latter species to test its phylogenetic relationship to other species. However, *A. strophiolata* presents some distinct morphological characteristics that clearly separate it from *A. albolimbata*. *Amanita strophiolata* was described by Beeli (1927, 1935) from DR Congo and is distinguished by its umbonate pileus, often yellowish at center, the absence of volval remnants on pileus, ellipsoid to elongate basidiospores (10–11 × 6–7 μm), a distinctive annulus in the form of a funnel, and larger basidiomata.

*Amanita albolimbata* also shows some similarities with Asian, European, and American species including *A. exitialis*, *A. bisporigera*, *A. molliuscula*, *A. parviexitialis*, and *A. virosa*, based on the white basidiomata. Those species have never been reported in tropical Africa and differ from the African species by morphological characteristics. *Amanita exitialis* was described from China (Yang and Li, 2001) and subsequently has been collected from India (Bhatt et al., 2003). The species is distinguished from *A. albolimbata* by the absence of volval remnants on pileus, globose-to-subglobose basidiospores (9.5–12 × 9–11.5 μm), 2-spored basidia, scarce inflated cells in the inner part of the volva, and larger basidiomata (Yang and Li, 2001; Cui et al., 2018). *Amanita bisporigera* was described from North America and characterized by the absence of volval remnants on pileus, a skirt-like annulus, globose-to-subglobose basidiospores (7.8–9.6 × 7–9 μm), 2-spored basidia and sometimes 4-spored, and larger basidiomata (Jenkins, 1986; Tulloss et al., 1995; Yang and Li, 2001; Yang, 2015). The species mainly has 2-spored basidia, in late spring and early summer; later in year, it may sometimes mainly or entirely have 4-spored basidia (Tulloss and Possiel, 2005). *Amanita molliuscula* was described from China and characterized by the absence of volval remnants on pileus, globose-to-subglobose basidiospores (7.5–9 × 7–9 μm), and larger basidiomata (Cai et al., 2016; Cui et al., 2018). *Amanita parviexitialis* was described from China and characterized by the absence of volval remnants on pileus sometimes slightly brownish at center, subglobose, rarely globose to broadly ellipsoidal basidiospores (7.5–9.5 × 7–9 μm), 2-spored basidia, and smaller basidiomata (Cai et al., 2016; Cui et al., 2018).
**Amanita virosa** is widely distributed across Europe and temperate to subtropical Asia (Neville and Poumarat, 2004; Zhang et al., 2010; Li et al., 2015; Yang, 2015; Cai et al., 2016; Cui et al., 2018). It has an umbo-bonate pileus, white, often cream at the center, the absence of volval remnants on pileus, the presence of globose-to-subglobose basidiospores (8–11 × 8–10 µm), scarce inflated cells in the inner part of the volva, and larger basidiomata (Cai et al., 2016; Cui et al., 2018). Generally, *A. albolimbata* sometimes has a patchy volval remnant on the pileus, which is typically absent for *A. exitialis*, *A. bisporigera*, *A. molluscula*, *A. strophiolata*, and *A. virosa* (Beeli, 1927, 1935). *A. exitialis*, *A. bisporigera*, *A. molluscula*, *A. parviexitialis*, and *A. virosa* occur in forests of Pinaceae and Fagaceae (Tulloss et al., 1995; Cai et al., 2016; Cui et al., 2018).

*Amanita albolimbata* is also distinct from other white species in *Amanita* sect. *Phalloideae* by its ecology. It occurs in woodland and gallery forests, associated with *U. guineensis* or *U. togoensis* (Phyllanthaceae) and *I. doka* (Fabaceae/Leguminosae), whereas *A. strophiolata* grows in swampy forests (Beeli, 1927, 1935). *Amanita exitialis*, *A. bisporigera*, *A. molluscula*, *A. parviexitialis*, and *A. virosa* occur in forests of Pinaceae and Fagaceae (Tulloss et al., 1995; Cai et al., 2016; Cui et al., 2018).

In the multigene phylogenetic tree, the African and Australian taxa are basal. This suggests that the lethal amanitas originated from the paleotropical areas. Cai et al. (2014) also suggested a possible paleotropical origin of lethal amanitas and highlighted the need for more molecular–phylogenetic studies on collections from the tropics and the Southern Hemisphere.

**Toxicity in Amanita**

For centuries, wild mushrooms have been consumed massively and popular in the human diet because of their matchless taste, protein content, and medicinal properties (de Román et al., 2006; Cheung, 2010). However, the high interest on wild mushroom collections and consumption could increase the risk of poisoning by lethal mushrooms. During picking, confusions could easily be made between edible and poisonous mushrooms because of their morphological similarities. Many mushroom poisoning cases have been reported worldwide and have mainly been caused by members of *A. sect. Phalloideae* (Zhang et al., 2010; Cai et al., 2014, 2016; Yang, 2015; Li et al., 2015, 2020; Thongbai et al., 2017). Consequently, much attention has been devoted to the species producing toxins within *A. sect. Phalloideae* (Chen et al., 2014; Li et al., 2014; Garcia et al., 2015; Cai et al., 2016).

The different toxins documented in those species are mainly amatoxins, phallotoxins, and virotoxins, which can cause severe damages, like liver and renal failure (Wieland, 1973, 1986; Chen et al., 2014). *Amanita exitialis*, *A. bisporigera*, *A. brunneitoxicaria*, *A. djarilmari*, *A. eucalypti*, *A. fuliginea*, *A. fulgineoides*, *A. gardeneri*, *A. marmorata*, *A. millsii*, *A. molluscula*, *A. ocreata*, *A. parviexitialis*, *A. strophiolata*, and *A. virosa*. *A. parviexitialis* and *A. strophiolata* produce amatoxins, phallotoxins, and virotoxins.
A. pallidorosea, A. parviexitialis, A. phalloides, A. rimosa, A. subbullacea, A. subjunquillea, A. verna, and A. virosa are known to contain those toxins and are distributed across Asia, America, Australia, and Europe (Wieland, 1973, 1986; Bresinsky and Besl, 1985; Chen et al., 2014; Li et al., 2014; García et al., 2015; Cai et al., 2016).
Until now, no lethal amanitas had been reported from West Africa. However, lethal amanitas have been documented from Central Africa and Madagascar (Fraiture et al., 2019). *Amanita albolimbata* represents the first lethal species of *A. sect. Phalloideae* known from West Africa. The most notorious toxins, α-amanitin, β-amanitin, and phallacidin, have also been detected in the species.

Numerous amanitoid taxa are harvested and consumed by local people in tropical Africa (Codjia and Yorou, 2014; Yorou et al., 2014; Boni and Yorou, 2015; De Kesel et al., 2017; Fadeyi et al., 2017; Milenge et al., 2018; Soro et al., 2019). Because of the whitish color of the basidiomata, *A. albolimbata* can be confused with *A. subviscosa* Beeli. *Amanita subviscosa* is commonly harvested and used as food by local people in Benin (Yorou et al., 2014; Boni and Yorou, 2015; Fadeyi et al., 2017, 2019). Still, *A. subviscosa* displays contrasting morphological characteristics with *A. albolimbata* by a slightly squamulose and viscous pileus, slightly striated margin, slightly bulbous, and slightly furfuraceous and hollow stipe, with a distinctive membranous volva (Beeli, 1935). The lack of *A. albolimbata* in various ethnomycological investigations (Yorou et al., 2014; Boni and Yorou, 2015; Fadeyi et al., 2017; Soro et al., 2019) attests that either local people are aware about its toxicity, or some fatal but unrecorded cases did occur within rural communities. However, it is important to educate local people on the best ways to discriminate morphologically close taxa in order to avoid the consumption of lethal *Amanita* species for an effective prevention of future poisoning incidents.

### DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

### AUTHOR CONTRIBUTIONS

ZLY, JEIC, and NSY conceived and designed the research. JEIC collected the species, performed the molecular phylogenetic analyses and the taxonomic studies, and wrote the first draft of the manuscript. JEIC and QC generated the DNA sequences. JEIC and SWZ carried out the cyclic peptide toxins analyses. QC, HL, MR, NSY, and ZLY critically revised and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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### FUNDING

This study was supported by the International Partnership Program of Chinese Academy of Sciences (No. 151853KYSB20170026), Yunnan Ten-Thousand-Talents Plan – Yunling Scholar Project, and the FORMAS Grant (No. 226-20141109).

### ACKNOWLEDGMENTS

We are grateful to Gang Wu and Bang Feng from Kunming Institute of Botany, CAS, for providing additional materials and images for this study. We thank Yang-Yang Cui, Pan Meng Wang, Si-Peng Jian, Xin Xu, and Kui Wu (Kunming Institute of Botany, CAS) for their kind assistance.

### SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.599047/full#supplementary-material

Supplementary Figure 1 | Phylogenetic tree inferred by Maximum Likelihood analysis based on combined dataset (ITS, nrLSU, rpb2, tef1-α, and β-tubulin). Bootstrap values ≥50% are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 2 | Phylogenetic tree inferred by Bayesian Inference analysis based on combined dataset (ITS, nrLSU, rpb2, tef1-α, and β-tubulin). Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 3 | Phylogenetic tree inferred by Maximum Likelihood analysis based on ITS sequences. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 4 | Phylogenetic tree inferred by Maximum Likelihood analysis based on nrLSU sequences. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 5 | Phylogenetic tree inferred by Maximum Likelihood analysis based on rpb2 sequences. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 6 | Phylogenetic tree inferred by Maximum Likelihood analysis based on tef1-α sequences. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

Supplementary Figure 7 | Phylogenetic tree inferred by Maximum Likelihood analysis based on β-tubulin sequences. Bootstrap values ≥50% and Bayesian posterior probabilities ≥0.90 are reported on branches. Sequences generated in this study are highlighted in red.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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