Amanita sect. Phalloideae: two interesting non-lethal species from West Africa

Jean Evans I. Codjia, Pan Meng Wang, Martin Ryberg, Nourou S. Yorou, Zhu L. Yang

Abstract
The members of Amanita sect. Phalloideae (Fr.) Quél. are responsible for many fatalities worldwide. However, some species in this section have previously been reported as non-lethal and lacking deadly toxins. Sequences of five genes (ITS, nrLSU, RPB2, TEF1-α, TUB2) of species belonging to the section from tropical Africa, America, Asia, Australia, and Europe were included in this study to elucidate the phylogenetic relationships among the species. The results indicated that the lethal species are in one clade (subclade I) while the non-lethal species are divided into two clades (subclades II and III) within the section. Moreover, two non-lethal species from tropical Africa, namely A. ballerinoides and A. bulbulosa are newly described based on both morphology and molecular approaches. Phylogenetically, they cluster in the same subclade III with other known non-lethal amanitas, including A. ballerina, A. chuformis, A. franzii, A. levistriata, and A. pseudogemmata. Neither amatoxins nor phallotoxins were detected in A. ballerinoides and A. bulbulosa by LC-HRMS, which agrees with their placement in the non-lethal subclade III within A. sect. Phalloideae. Finally, a key to the West African species of Amanita sect. Phalloideae is provided.

Keywords Amanita · Multigene phylogeny · Taxonomy · LC-HRMS · Tropical Africa

Introduction
Amanita Pers. (Agaricales, Basidiomycota) is a cosmopolitan genus comprising about 1000 species names in the literature, with about 700 species presently accepted worldwide (Bas 2000; Yang 2005; Yang et al. 2018; Cui et al. 2018). It contains both choice edible and deadly poisonous species (Cui et al. 2014; Li et al. 2015; Cui et al. 2018; Codjia et al. 2020). Most of the species form ectomycorrhizal associations with vascular plants, which play important roles in the maintenance of the ecosystem (Reid 1980; Pegler and Shah-Smith 1997; Wood 1997; Yang 1997, 2005; Tulloss 2005; Wolfe et al. 2012; Corrales et al. 2018; Davison et al. 2021). The modern classification, that is based on morphological characters and molecular approaches, split the genus into three subgenera comprising eleven sections (Cui et al. 2018).

Lethal Amanita species are accommodated mostly within A. sect. Phalloideae, having the following characteristics: a non-striate and non-appendiculate pileus, attenuate lamellulae, the presence of persistent annulus, a limbate volva on the bulbous stipe base, and amyloid basidiospores (Comer and Bas 1962; Bas 1969; Tulloss and Bhandary 1992; Yang 1997, 2005, 2015; Davison et al. 2017; Cui et al. 2018; Tulloss et al. 2021). The section encompasses more than 50 deadly poisonous species described worldwide (Zhang et al. 2010; Cai et al. 2014, 2016; Yang 2015; Li et al. 2015; Davison et al. 2017; Thongbai et al. 2017; Fraiture et al. 2019; Codjia et al. 2020; Tulloss et al. 2021). However, very few taxa have been

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reported from tropical Africa (Walley and Verbeke 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; Codjia et al. 2020). Until now, seven species belonging to A. sect. Phalloideae were described from tropical Africa (Piepenbring et al. 2020). Three species, A. alliodora Pat., A. murinacea Pat., and A. thejoleuca Pat., were reported from Madagascar (Patrouillard 1924; Fraiture et al. 2019). Three other species, A. bweyeyensis Fraiture, Raspé & Degreer, A. harkomeniana Fraiture & Saarimäki, and A. strophiolata Beeli, were described from DR Congo (Beeli 1927, 1935; Fraiture et al. 2019). In addition, A. albolimbata J.E.I. Codjia, N.S. Yorou & Zhu L. Yang has been described from Benin (Codjia et al. 2020).

Recent studies have reported that some species in A. sect. Phalloideae, A. ballerina Raspé, Thongbai & K.D. Hyde, A. bweyeyensis Fraiture, Raspé & Degreer, A. franzii Zhu L. Yang, Y.Y. Cui & Q. Cai, A. pseudogemmata Hongo, and A. zangii Zhu L. Yang, T.H. Li & X.L. Wu do not produce neither amatoxins nor phallotoxins (Cai et al. 2014; Thongbai et al. 2017; Fraiture et al. 2019; Cui et al. 2018). Among those species, A. bweyeyensis represents a particular case. The species belongs to the lethal group of A. sect. Phalloideae and does indeed contain the genes responsible for producing the toxins. However, these genes are not expressed, and the species does not produce the toxins (Fraiture et al. 2019). The lack of toxins in that species has also been confirmed by the fact that local people in Rwanda know the species and eat it without any problem (Fraiture et al. 2019). In this study, we re-examined the phylogenetic positions of taxa of A. pseudogemmata Hongo, and A. zangii Zhu L. Yang, T.H. Li & X.L. Wu do not produce neither amatoxins nor phallotoxins (Cai et al. 2014; Thongbai et al. 2017; Fraiture et al. 2019; Cui et al. 2018). Among those species, A. bweyeyensis represents a particular case. The species belongs to the lethal group of A. sect. Phalloideae and does indeed contain the genes responsible for producing the toxins. However, these genes are not expressed, and the species does not produce the toxins (Fraiture et al. 2019). The lack of toxins in that species has also been confirmed by the fact that local people in Rwanda know the species and eat it without any problem (Fraiture et al. 2019). In this study, we re-examined the phylogenetic positions of taxa of A. sect. Phalloideae by adding specimens from tropical Africa. Their macro- and micromorphological characteristics, phylogenetic relationships with other Amanita species are discussed along with a key to the West African species of Amanita sect. Phalloideae. The screening of the new species for the known toxins occurring in Amanita is also reported.

Materials and methods

Collections and preservation

Collections were made in five (05) West African countries (Benin, Guinea, Ivory Coast, Mali, Togo) during the rainy seasons from June to September (2018–2020) (Fig. 1). Basidiomata were collected from forests dominated by Fabaceae/Leguminosae (Isoboeilinia Craib & Stapf ex Holland, Anthoantha P. Beauv., Berlinia Sol. ex Hook. f.), Phyllanthaceae (Uapaca Baill.) and Dipterocarpaceae (Monotes A. DC.). A digital camera type Canon EOS 60D was used to photograph the specimens in situ. The description of macro-morphological characteristics on fresh basidiomata follows Tulloss and Yang (2011). Color codes from fresh basidiomata are reported according to Kornerup and Wanscher (1981). The fresh basidiomata were air-dried using an electric dryer Stöckli Dörrex at 45°C during 24 h thereafter stored as exsiccates with their label in scalable plastic bags type minigrip. Holotypes and isotypes of the newly described species are deposited in the Mycological Herbarium of the University of Parakou (UNIPAR). Duplicate specimens are conserved at the Herbarium of Cryptogams at the Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). For molecular investigations, small pieces of fresh basidiomata were also stored in CTAB lysis buffer (2% cetyltrimethylammonium bromide, 100 mM Tris–HCl, 20 mM EDTA, 1.4 M NaCl) and dried with silica gel. The nomenclature aspects and authorities for scientific names were double-checked against Index Fungorum (www.mycology.net) and on the Studies in the Amanitaceae website (Tulloss and Yang 2021).

Micromorphological studies

Micromorphological data were recorded from dried materials and examined at 1000× magnification by means of a microscope type Zeiss Axiostar-40. Microscopic characteristics, measurements, and line drawings were obtained from slide preparations mounted in 5% KOH and stained with Congo Red. Melzer’s reagent was used to test the amyloidity of basidiospores. A minimum of 20–30 basidiospores from each basidioma were measured in side view. In addition, basidia, hyphal elements of the subhymenium, pileipellis, and stipe trama, volval elements, were measured from each type specimen. In the descriptions of basidiospores, the term (n/m/p) means n basidiospores from m basidiomata of p collections. The dimensions of basidiospores are provided with the notation (a)b–(d). The range b–c contains a minimum of 90% of the measured values. Extreme values, i.e., a and d, are provided in parentheses. Q is used for the ratio length/width of a spore in side view; $Q_m$ is the average of all basidiospores ± sample standard deviation. The statistical analysis of measurements for the basidiospores was conducted with Pixime tre v5.10 (Henriot and Cheype 2020). The descriptive terms follow Bas (1969), Yang (2005, 2015), Cai et al. (2016), Cui et al. (2018, 2021), and Codjia et al. (2020).

DNA extraction, PCR, and sequencing

Genomic DNA was obtained from materials preserved in CTAB or dried with silica gel using the modified CTAB method (Doyle and Doyle 1987). Polymerase chain reaction (PCR) amplification was performed at the nuclear ribosomal internal transcribed spacer region (ITS), using the primer pair ITS1F/ITS4 (White et al. 1990; Gardes and Bruns 1993), and the large subunit of nuclear ribosomal RNA gene region (nrLSU), using the primer pair LROR/LR5 (Vilgalys and
Protein-coding genes, including translation elongation factor 1-α (TEF1-α), RNA polymerase II second largest subunit (RPB2), and beta-tubulin (TUB2), were amplified using the primer pairs EF1-983F/EF1-1567R (Rehner and Buckley 2005), ARPB2-6F/ARPB2-7R (Codjia et al. 2020), and Am-b-tubulin F/Am-b-tubulin R (Cai et al. 2014; Codjia et al. 2020), respectively. The PCR reactions were as follows: pre-denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 50–54 °C for 40 s, elongation at 72°C for 60 s (ITS, RPB2, TEF1-α, TUB2) or 120 s (nrLSU), and final elongation at 72 °C for 8 min. Purified PCR products were then sequenced on an ABI-3730-XL DNA Analyzer (Applied Biosystem, Foster City, CA, USA) using the same primer combinations as for PCR. The generated sequences were assembled using Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan).

**Phylogenetic analyses**

In this study, all newly generated sequences were submitted to GenBank, and accession numbers are shown in Table 1. Additional sequences were retrieved from previously published papers and GenBank (Table 1). Species within A. sect. *Lepidella*, including *Amanita flavofloccosa* Nagas. & Hongo and *Amanita manicata* (Berk. & Broome) Pegler, were used as outgroup (Table 1). Alignments were performed using MAFFT v7.310 (Katoh and Standley 2013), edited manually when necessary in BioEdit v7.0.9 (Hall 1999).
Table 1  Taxa of *Amanita* included in molecular phylogenetic analyses. The new sequences generated in this study are highlighted in black bold.

| Species name               | Collection or collector no. | Country of origin | GenBank accession no. | Associated publication                      |
|----------------------------|------------------------------|-------------------|-----------------------|--------------------------------------------|
|                            |                              |                   | ITS                   | nLSU | RPB2 | TEF1-α | TUB2 | |
| **Subgenus Amanitina**     |                              |                   |                       |      |      |        |      | |
| **Sect. Phalloideae**      |                              |                   |                       |      |      |        |      | |
| *Amanita albolimbata*      | JEIC0739                     | Benin             | MT966935              | MT966942 | MT966963 | MT966955 | MT966950 | Codjia et al. (2020) |
| *Amanita albolimbata*      | HKAS93847                    | Benin             | MT966937              | MT966945 | –      | –      | –      | Codjia et al. (2020) |
| *Amanita alliodora*        | DSN062                       | Madagascar        | KX185611              | KX185612 | –      | –      | –      | Direct Submission   |
| *Amanita amerivirosa*      | RET 480-1                    | USA               | KJ466399              | KJ466461 | KJ466630 | KJ481965 | KJ466544 | Cai et al. (2014)   |
| *Amanita amerivirosa*      | RET 397-8                    | Canada            | KJ466398              | KJ466460 | KJ466629 | KJ481964 | KJ466543 | Cai et al. (2014)   |
| *Amanita ballerina*        | OR1026                       | Thailand          | KY747467              | MH157079 | KY656884 | –      | KY656865 | Thongbai et al. (2017) |
| *Amanita ballerina*        | OR1014                       | Thailand          | KY747466              | –      | KY656883 | –      | KY656864 | Thongbai et al. (2017) |
| *Amanita ballerinoides*    | JEIC0611                     | Ivory Coast       | –                     | –      | OK510846 | OK510827 | OK510834 | This study          |
| *Amanita ballerinoides*    | JEIC0422_2                   | Benin             | OK510853              | –      | OK510845 | OK510823 | OK510833 | This study          |
| *Amanita ballerinoides*    | JEIC0479                     | Guinea            | OK510854              | OK510856 | OK510847 | OK510824 | OK510835 | This study          |
| *Amanita ballerinoides*    | JEIC0466                     | Mali              | –                     | OK510857 | –      | OK510825 | OK510836 | This study          |
| *Amanita ballerinoides*    | SYN3950                      | Benin             | –                     | OK510855 | –      | OK510826 | OK510838 | This study          |
| *Amanita ballerinoides*    | SYN3961                      | Benin             | –                     | OK510858 | –      | –      | OK510837 | This study          |
| *Amanita bisporigera*      | RET 642-6                    | USA               | –                     | –      | K919771 | –      | –      | Direct Submission   |
| *Amanita bisporigera*      | RET 632-7                    | Canada            | KX827614              | KX827615 | –      | –      | –      | Direct Submission   |
| *Amanita brunneitoxicaria* | BZ2015-01                    | Thailand          | KY747462              | –      | KY656879 | –      | KY656860 | Thongbai et al. (2017) |
| *Amanita brunneitoxicaria* | BZ2015-02                    | Thailand          | KY747463              | –      | KY656880 | –      | KY656861 | Thongbai et al. (2017) |
| *Amanita bulbosa*          | JEIC0522                     | Guinea            | –                     | OK510864 | –      | –      | OK510841 | This study          |
| *Amanita bulbosa*          | JEIC0436                     | Benin             | –                     | OK510863 | OK510852 | OK510831 | OK510840 | This study          |
| *Amanita bulbosa*          | JEIC0512                     | Guinea            | –                     | OK510861 | OK510850 | OK510830 | OK510842 | This study          |
| *Amanita bulbulosa*        | JEIC0426_2                   | Benin             | –                     | OK510862 | OK510851 | –      | OK510843 | This study          |
| *Amanita bulbulosa*        | JEIC0432_2                   | Benin             | –                     | OK510859 | OK510848 | OK510829 | OK510839 | This study          |
| *Amanita bulbulosa*        | JEIC0762                     | Benin             | –                     | OK510860 | OK510849 | OK510832 | –      | This study          |
| *Amanita bweyeyensis*      | JD 1304                      | Rwanda            | MK570920              | MK570927 | MK570937 | MK570940 | MK570916 | Fraiture et al. (2019) |
| *Amanita bweyeyensis*      | JD 1257                      | Rwanda            | MK570919              | MK570926 | –      | –      | –      | Fraiture et al. (2019) |
| *Amanita choforinii*       | HKAS94075                    | China             | MT395378              | MT395380 | –      | MT364256 | –      | Cui et al. (2021)   |
| *Amanita choforinii*       | HKAS101028                   | China             | MT395379              | MT395381 | MT364258 | MT364257 | –      | Cui et al. (2021)   |
| *Amanita djirilnai*        | PERTH08776067                | Australia         | KY977732              | KY977704 | MF000755 | MF000750 | MF000742 | Davison et al. (2017) |
| *Amanita djirilnai*        | PERTH08776040                | Australia         | –                     | KY977708 | MF037234 | MF000743 | –      | Davison et al. (2017) |
| *Amanita eucalypti*        | PERTH8809860                 | Australia         | –                     | –      | MF000757 | –      | MF000745 | Davison et al. (2017) |
| *Amanita eucalypti*        | PERTH8809763                 | Australia         | –                     | KY977709 | –      | MF000747 | –      | Davison et al. (2017) |
| *Amanita exitialis*        | HKAS75775                    | China             | JX998026              | JX998053 | KJ466592 | JX998002 | KJ466504 | Cai et al. (2014)   |
| *Amanita exitialis*        | HKAS75776                    | China             | JX998025              | JX998051 | KJ466593 | JX998003 | KJ466505 | Cai et al. (2014)   |
| *Amanita franzii*          | HKAS91231                    | China             | MH508358              | MH486525 | MH485994 | MH508801 | MH485516 | Cai et al. (2018)   |
| Species name                  | Collection or collector no. | Country of origin | GenBank accession no. | Associated publication |
|------------------------------|-----------------------------|-------------------|-----------------------|------------------------|
| Amanita franzii              | HKAS79559                   | China             | –                     | MH486523 – MH508799 MH485514 | Cui et al. (2018) |
| Amanita fuliginea            | HKAS75780                   | China             | JX998023 JX998048 KJ466595 KJ997995 KJ466507 | Cai et al. (2014) |
| Amanita fuliginea            | HKAS75782                   | China             | JX998022 JX998049 KJ466597 KJ997996 KJ466509 | Cai et al. (2014) |
| Amanita fuligineoides        | HKAS83694                   | China             | – MH486553 MH486020 MH508824 MH485540 | Cai et al. (2018) |
| Amanita fuligineoides        | HKAS52727                   | China             | JX998024 JX998047 KJ466599 – KJ466511 | Cai et al. (2014) |
| Amanita gardneri             | PERTH08776121               | Australia         | KY057387 KY977712 MF000756 MF000752 MF000748 | Davison et al. (2017) |
| Amanita griseorosea          | HKAS89004                   | China             | – KU168387 KU168388 KU168386 KU168389 | Cai et al. (2016) |
| Amanita griseorosea          | HKAS75782                   | China             | JX998022 JX998049 KJ466597 KJ997996 KJ466509 | Cai et al. (2014) |
| Amanita harkoneniana         | P Pirot SN                  | Madagascar        | MK570922 MK570929 MK570938 MK570941 | Fraiture et al. (2019) |
| Amanita harkoneniana         | TS 1061                     | Tanzania          | MK570923 MK570930 – – – | Fraiture et al. (2019) |
| Amanita harkoneniana         | RET 155-1                   | USA               | – HQ539701 – – –       | Wolfe et al. (2012) |
| Amanita levistriata          | FLAS-F-61208                | USA               | MH211813 MH620278 – – – | Direct Submission |
| Amanita marmorata            | PW SN                       | Australia         | MK570924 MK570931 MK570939 MK570942 | Fraiture et al. (2019) |
| Amanita marmorata            | RET 623-7                   | Australia         | KP757875 KP757874 – – – | Direct Submission |
| Amanita milii                | HO581533                    | Australia         | KY977714 KY977713 MF000753 MF000759 MF000760 | Davison et al. (2017) |
| Amanita mollisscula          | HKAS77324                   | China             | KJ466409 KJ466472 KJ466639 KJ481974 KJ466553 | Cai et al. (2014) |
| Amanita mollisscula          | HKAS75555                   | China             | KJ466408 KJ466471 KJ466638 KJ481973 KJ466552 | Cai et al. (2014) |
| Amanita ocreata              | 7002                        | USA               | GQ250404 GQ250419 – – – | Direct Submission |
| Amanita ocreata              | HKAS79686                   | USA               | KJ466381 MH486688 KJ466607 KJ481947 KJ466518 | Cai et al. (2018) |
| Amanita pallidorosea         | HKAS82350                   | China             | MH508485 MH486737 MH486163 MH508971 MH485668 | Cai et al. (2018) |
| Amanita pallidorosea         | HKAS77349                   | China             | KJ466389 KJ466649 KJ466628 KJ481961 KJ466540 | Cai et al. (2014) |
| Amanita parvexitialis        | HKAS79049                   | China             | – KT971342 KT971345 KT971343 KT971342 KT971346 | Cai et al. (2016) |
| Amanita parvexitialis        | HKAS79601                   | China             | – – – – – – – – – – | Cai et al. (2016) |
| Amanita phalloides           | HKAS75773                   | USA               | JX998031 JX998060 KJ466612 – KJ466523 | Cai et al. (2014) |
| Amanita phalloides           | Qs6                         | France            | – – – – – – EU886739 – | Pringle et al. (2009) |
| Amanita pseudogemmata        | HKAS84744                   | China             | – MH486767 MH486185 MH508994 MH485691 | Cai et al. (2018) |
| Amanita pseudogemmata        | HKAS85889                   | China             | – MH486768 MH486186 MH508971 MH485692 | Cai et al. (2018) |
| Amanita rimosa               | HKAS101393                  | China             | – MH486806 MH486218 MH509031 MH485722 | Cai et al. (2018) |
| Amanita rimosa               | HKAS77279                   | China             | KJ466392 KJ466454 KJ466620 KJ481956 KJ466531 | Cai et al. (2014) |
| Amanita sp. 0595             | IEIC0595                    | Togo              | – OK510865 OK510828 OK510844 This study |
| Amanita suballiacea          | RET491-7                    | USA               | KJ466421 KJ466486 KJ466602 KJ481942 KJ466514 | Cai et al. (2014) |
| Amanita suballiacea          | RET490-1                    | USA               | KJ466420 KJ466485 KJ466601 KJ481941 KJ466513 | Cai et al. (2014) |
| Amanita subfuliginea         | HKAS77326                   | China             | KJ466404 KJ466467 KJ466636 KJ481971 KJ466550 | Cai et al. (2014) |
| Amanita subfuliginea         | HKAS77347                   | China             | KJ466405 KJ466468 KJ466637 KJ481972 KJ466551 | Cai et al. (2014) |
| Amanita subjunquillea        | HKAS77577                   | China             | JX998033 JX998061 KJ466655 JX997998 JX466573 | Cai et al. (2014) |
| Amanita subjunquillea        | HKAS77325                   | China             | KJ466425 KJ466490 KJ466565 KJ481988 KJ466574 | Cai et al. (2014) |
| Amanita subpallidorosea      | LHJ140923-41                | France            | KJ466425 KJ466490 KJ466565 KJ481988 KJ466574 | Cai et al. (2014) |
ambiguously 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, TUB2) comprising 370 sequences were constructed in Geneious v7.0.2 (Kearse et al. 2012) and used for phylogenetic analyses. The concatenated alignment was submitted to TreeBASE (submission ID 29481). The Incongruence Length Difference (ILD) test in PAUP v4.0a169 (Swofford 2002) was performed to determine if there were any incongruence between genes before concatenating them. As no incongruence ($P$-value = 0.884000) was detected, the maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic tree inference. The ML and BI were performed using IQ-TREE v1.6.12 (Nguyen et al. 2015) and MrBayes v3.2 (Ronquist et al. 2012), respectively. Nucleotide substitution models, as well as the

| Species name | Collection or collector no. | Country of origin | GenBank accession no. | Associated publication |
|--------------|-----------------------------|-------------------|----------------------|------------------------|
| **Amanita subpallidorosea** | LHIJ140923-55 | China | KP691680 KP691693 KP691702 KP691671 KP691712 | Li et al. (2015) |
| **Amanita virosa** | HKAS56694 | Finland | – – KJ466664 – KJ466583 | Cai et al. (2014) |
| **Amanita virosa** | HKAS71040 | Japan | KJ466429 KJ466496 KJ466665 KJ481997 KJ466584 | Cai et al. (2014) |
| **Amanita zangii** | HKAS99663 | China | MH508655 MH486958 MH486351 MH509178 MH485855 | Cui et al. (2018) |
| **Amanita zangii** | GDGM29241 | China | KJ466432 KJ466499 KJ466668 KJ482000 KJ466588 | Cai et al. (2014) |
| **Amanita griseoverrucosa** | HKAS57357 | China | MH508392 MH486581 MH486043 MH508850 MH485662 | Cui et al. (2018) |
| **Amanita ravenellii** | RET459-10 | USA | KJ466432 KJ466499 KJ466668 KJ482000 KJ466588 | Cai et al. (2014) |
| **Amanita strobiliformis** | MB-001177 | Germany | MH508614 MH486895 MH486298 MH509117 MH485798 | Cui et al. (2018) |
| **Amanita arenaria** | VPI 551 | Australia | – GQ925381 – – – | Justo et al. (2010) |
| **Amanita arenaria** | VPI 679 | Australia | – GQ925382 – – – | Justo et al. (2010) |
| **Amanita arenaria** | VPI 412 | Australia | GQ925393 GQ925383 – – – | Justo et al. (2010) |
| **Amanita citrina** | HKAS53467 | Germany | MH508312 MH486457 MH485937 MH508733 MH485461 | Cui et al. (2018) |
| **Amanita orsonii** | HKAS101426 | China | MH508472 MH486796 MH486210 MH509022 MH485719 | Cui et al. (2018) |
| **Amanita porphyria** | MB-100156 | Germany | MH508507 MH486712 MH486144 MH508949 – | Cui et al. (2018) |
| **Amanita rubescens** | HKAS92040 | China | MH485859 MH486816 MH486227 MH509043 MH485732 | Cui et al. (2018) |
| **Amanita avellaneosquamosa** | HKAS100602 | China | MH508258 MH486379 MH485873 MH508681 | Cui et al. (2018) |
| **Amanita bruneomaculata** | HKAS70032 | China | MH508279 MH486411 MH485893 MH508699 – | Cui et al. (2018) |
| **Amanita lanigera** | HKAS89030 | China | MH508420 MH486621 MH486074 MH508880 – | Cui et al. (2018) |
| **Amanita parvicurta** | HKAS101215 | China | MH485890 MH486745 MH486169 MH508978 – | Cui et al. (2018) |
| **Amanita avellaneifolia** | HKAS79891 | China | – MH486377 MH485871 MH508679 MH485409 | Cui et al. (2018) |
| **Amanita luteofolia** | HKAS81895 | China | – MH486633 MH486083 MH508890 MH485596 | Cui et al. (2018) |
| **Amanita pyramidata** | HKAS87943 | China | MH508535 MH486795 MH486209 MH509021 MH485718 | Cui et al. (2018) |
| **Amanita rubiginosa** | HKAS56959 | China | MH508562 MH486818 MH486230 MH509046 MH485735 | Cui et al. (2018) |
| **Amanita flavofloccosa** | HKAS101443 | China | – MH486515 MH485986 MH508791 MH485507 | Cui et al. (2018) |
| **Amanita flavofloccosa** | HKAS92006 | China | – MH486516 MH485987 MH508792 MH485509 | Cui et al. (2018) |
| **Amanita manicata** | PDD88353 | New Zealand | – MH486638 MH486087 MH508895 MH485600 | Cui et al. (2018) |
| **Amanita manicata** | HKAS90174 | China | – KT833801 KT833818 KT833831 – | Cui et al. (2018) |
best partitioning schemes, were determined using PartitionFinder2 v2.1.1 (Lanfear et al. 2017) implemented in PhyloSuite v1.2.2 (Zhang et al. 2020). For the ML analysis, the best partitioning schemes, the ultrafast bootstrap replicates at 1000, and the Shimodaira-Hasegawa and approximate likelihood ratio test (SH-aLRT) test with 1000 replicates (Guindon et al. 2010; Hoang et al. 2018) were used. The BI was conducted with the following parameters: 2 runs, each with four simultaneous Metropolis-Coupled Markov chains, and trees were sampled every 1000 generations. The analyses were completed after 20,000,000 generations when the average standard deviation of split frequencies was 0.017963 for the five-gene analysis, and the first 25% generations were discarded as burn-in. The phylogenetic trees inferred from ML and BI analyses were visualized with FigTree v1.4.4 (Rambaut 2018) and then edited in Adobe Illustrator CS6.

**Analysis of toxins by LC-HRMS**

Dried basidiomata of the target taxa were analyzed for the most notorious toxins, namely α-amantin, β-amantin, phalloidin, and phallacidin (standards provided by Sigma Chemical Co, USA). The liquid chromatography–high-resolution mass spectrometry (LC-HRMS) using 1290 Infinity II HPLC systems coupled with 6540 UHD precision mass Q-TOF instruments was applied to screen the compounds. Toxin extraction, as well as LC-HRMS analyses, follow the method of Codjia et al. (2020).

**Results**

**Phylogenetic analyses**

Forty-three sequences (2 for ITS, 11 for nrLSU, 8 for RPB2, 10 for TEF1-α, and 12 for TUB2) were newly generated in this study and deposited in GenBank (Table 1). The combined dataset (ITS, nrLSU, RPB2, TEF1-α, and TUB2) comprises 370 sequences. It contained 3008 total characters including 1662 constant (proportion = 0.552527), 135 variable and parsimony-uninformative and 1211 parsimony-informative. No topological incongruence was detected between ML and BI (Supplementary Figs. S1–S2). The multigene phylogenetic analyses revealed the monophyly of the subgenus *Amanitina* with full bootstrap support (SH-aLRT = 100 %, ML ultrafast bootstrap = 100 %, BPP = 1.0) (Fig. 2). Six clades were identified to correspond to sections in the subgenus *Amanitina* (sect. Amidella, sect. Arenariae, sect. Phalloideae, sect. Roanokenses, sect. Strobiliformes, and sect. Validae). In the section *Phalloideae*, three clades can be distinguished (Fig. 2); based on that, the lethal species form one clade (subclade I) while the non-lethal species are distributed between two clades (subclade II and subclade III). The non-lethal subclade II, which includes *A. hesleri* Bas and *A. zangii* is sister to the lethal subclade I (ML ultrafast bootstrap = 95 %), and the non-lethal subclade III, which includes *A. ballerina*, *A. ballerinooides*, *A. bulbulosa*, *A. chiformis*, *A. franzii*, *A. levi striata* D.T. Jenkins, *A. pseudogemmata*, *Amanita* sp. 0595, is sister to the clade that combines both subclade I and II (SH-aLRT = 100 %, ML ultrafast bootstrap = 100 %, BPP = 1.0) (Fig. 2). The African collections are grouped into three (04) lineages (taxa marked in red in Fig. 2) well supported and well distinct from other lineages in the phylogenetic tree. Because of their distinct phylogenetic positions, the African species *A. ballerinooides* and *A. bulbulosa* are described here as new and are placed in the non-lethal subclade III (Fig. 2). They form respectively fully supported groups (SH-aLRT = 100 %, ML ultrafast bootstrap = 100 %, BPP = 1.0; SH-aLRT = 100 %, ML ultrafast bootstrap = 100 %, BPP = 1.0). *Amanita* sp. 0595 is not described in this study due to the lack of enough materials, and the sample collected here is immature but probably could also be a new species. In the non-lethal subclade II, the Asian species (*A. zangii*) forms a highly supported group (SH-aLRT = 82.9 %, ML ultrafast bootstrap = 98 %, BPP = 1.0) sister to the American species (*A. hesleri*) (Fig. 2).

**Taxonomy**

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

Mycobank No: MB 841497

Figs. 3a, 4

**Etymology.** The epithet refers to the similarity with *A. ballerina*, a species known from Thailand.

**Type.** IVORY COAST. KATIOLA PROVINCE: Katiola, 08°12’00.1”N, 04°54’21.7”W, gallery forest of Katiola, date: 15 July 2019, leg. and det. Jean Evans I. CODJIA, Holotype JEIC0611 (UNIPAR), Isotype KUN-HKAS 122159, GenBank Acc. No.: (RPB2 = OK510846, TEF1-α = OK510827, TUB2 = OK510834).

**Description.** Basidiomata small. Pileus 34–56 mm in diam, at first hemispherical and then becoming convex and broadly convex at the maturity, slightly depressed at the center, whitish (1A2), the surface is covered by small thick adherent squamules or patchy volval remnants that are whitish (1A2) to grayish (5B3); margin non-striate when young then slightly striate at maturity, non-appendiculate; whitish (1A2). Lamellae-free, whitish (1A2) to pale yellowish white (2A2), lamellulae truncate. Stipe 49–75 mm long × 6–12 mm diam., nearly cylindrical, stuffed, bulbous, whitish (1A2), covered with white (1A1) squamules. Annulus present, medial, below the apex of the stipe, broad, persistent, membranous, skirt-like, with thickened edge, white (1A1) to whitish (1A2). Context, thin, white (1A1) to whitish (1A2). Bulb compressible, almost subglobose at young stage, then elongate to
ventricose and tapering downward, marginate, occasionally cracked, whitish (1A2), upper edge with shortly limbate, whitish (1A2) volval remnants. Odor and taste not recorded.

Lamellar trama bilateral. Mediostratum 30–60 μm wide, composed of abundant subglobose to ellipsoid inflated cells (20–30 × 15–20 μm), and abundant filamentous hyphae, 3–4 μm wide; vascular hyphae scarce. Lateral stratum composed of subglobose to ellipsoid inflated cells (15–20 × 10–15 μm), diverging at an angle of 30° to 45° to mediostratum; filamentous hyphae abundant, 3–5 μm wide. Subhymenium 30–50 μm thick, with 2–3 layers of ovoid to subglobose or irregular cells, 9–15 × 9–10 μm. Basidia (Fig. 4A) (17.5–) 30–40 (–53) × (6.5–) 8.5–10.5 (–13) μm, clavate, 4-spored, basal septa lacking clamps. Basidiospores (Fig. 4B) (80/4/4) (8–) 8.5–9 (–10) × (6–) 6.5–7 (–7.5) μm, Q = 1.2–1.4, Qm = 1.3 ± 0.05, broadly ellipsoid to ellipsoid, smooth, colorless, weakly amyloid. Lamellar edge sterile, composed of subglobose to ellipsoid, inflated cells (25–35 × 15–25 μm), filamentous hyphae, 3–4 μm wide, irregularly arranged or running parallel to lamellar edge. Pileipellis (Fig. 4C) 100–180 μm thick, 2--
Fig. 3 Basidiomata of the studied *Amanita* species. 
- A. ballerionoides (holotype JEIC0611); 
- A. bulbulosa (holotype JEIC0762). Scale bars: 1 cm

Fig. 4 Microscopic features of *A. ballerionoides* (holotype JEIC0611). 
A. Hymenium and subhymenium. 
B. Basidiospores. 
C. Pileipellis. 
D. Crushed tissue of volval remnants on pileus. 
E. Longitudinal section of outer layer of volval limb on stipe base. 
Scale bars = 10 μm
layered; suprapellis up to 35–80 μm thick, slightly gelatinized, composed of arranged, thin-walled, colorless to nearly colorless, ellipsoid to clavate terminal cells 80–130 × 15–20 μm, mixed with filamentous hyphae 3–8 μm wide; subpellis up to 65–100 μm thick, composed of undifferentiated, filamentous hyphae 3–5 μm wide; vascular hyphae scarce. Volval remnants on pileus (Fig. 4D) composed of longitudinally arranged elements: filamentous hyphae abundant 3–8 μm wide, colorless, thin- to slightly thick-walled, branching; inflated cells abundant, subglobose to ellipsoid, 20–60 × 20–40 μm, colorless, thin- to slightly thick-walled, terminal, mixed with long clavate terminal cells 55–150 × 8–20 μm; vascular hyphae rare. Outer part of volval limb on the stipe base (Fig. 4E) composed of longitudinally arranged elements: filamentous hyphae abundant 3–9 μm wide, colorless, thin- to slightly thick-walled, branching; inflated cells abundant, subglobose to ellipsoid, 20–70 × 15–50 μm, colorless, thin- to slightly thick-walled, terminal, mixed with long clavate terminal cells 60–140 × 10–20 μm; vascular hyphae rare. Stipe trama composed of longitudinally arranged, long clavate terminal cells 55–125 × 10–15 μm; filamentous hyphae scattered to abundant, 3–5 μm wide; vascular hyphae scarce. Clamps absent in all parts of basidioma.

**Habitat.** Solitary or in small group on the ground in woodland and gallery forests, associated with *Berlinia grandiflora* or *Isoberlinia doka* (Fabaceae/Leguminosae) and *Uapaca guineensis* (Phyllanthaceae).

**Distribution.** Currently known from Benin, Guinea, Ivory Coast, and Mali

**Additional specimens examined.** BENIN. ATACORA PROVINCE: Kota, 10°12'39"N, 01°26'45.8"E, gallery forest of Kota, date: 21 June 2018, leg. and det. Jean Evans I. CODJIA, JEIC0422-2, date: 06 June 2020, leg. and det. Nourou S. YOROU, SYN3950. DONGA PROVINCE: Djougou, 9°31‘07”N, 01°55’30”E, gallery forest of Danougou, date: 09 June 2020, leg. and det. Nourou S. YOROU, SYN3961. MALI. SIKASSO REGION: Farako, 11°12’33”N, 05°27’00”W, Forest Reserve of Farako, date: 28 June 2018, leg. and det. Jean Evans I. CODJIA, JEIC0466. GUINEA. Kankan Prefecture: Bissandougou Sub-precincture, 10°12’15”N, 09°11’40”W, Forest Reserve of Kourani-Ouletediene, date: 01 July 2018, leg. and det. Jean Evans I. CODJIA, JEIC0479.

**Notes.** *Amanita ballerinoides* presents the following characteristics: small, white to whitish basidiomata, floccose pileus with small adherent squamules, or patchy universal veil that are whitish to gray orange, striate at maturity, and marginate basal bulb. Phylogenetically, the species clustered in the non-lethal subclade III, including *A. ballerina*, *A. chuformis*, *A. franzii*, *A. levistriata*, *A. pseudogemmata*, and forms a well-distinct lineage in the phylogenetic tree.

Among these species, *A. ballerinoides* is nearly identical morphologically to *A. ballerina*. However, *A. ballerina* can be separated from *A. ballerinoides* by its slightly viscid pileus when moist covered with dull white to pale yellowish white thin adherent areolate squamules or patchy volval remnants (Thongbai et al. 2017). Ecologically, *A. ballerinoides* occurs in woodland or gallery forests, dominated by *Berlinia grandiflora* or *Isoberlinia doka* (Fabaceae/Leguminosae) and *Uapaca guineensis* (Phyllanthaceae), whereas *A. ballerina* occurs in evergreen *Fagaceae* hill forest or mixed deciduous *Dipterocarpaceae/Fagaceae* forest (Thongbai et al. 2017).

On the other hand, the marginate basal bulb, and the nature of the volval remnants of *A. ballerinoides* are reminiscent of species of *A. sect. Lepidella* subsect. *Limbatulae*, including *A. limbatula* Bas, *A. parva* Murrill, *A. mutabilis* Beardslee, *A. praelongispora* (Murrill) Murrill. The latter taxa are characterized by sublimbate stipe bases and narrower basidiospores (Beardslee 1919; Murrill 1941, 1945; Bas 1969; Jenkins 1979, 1986; Tulloss 1984).

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

Mycobank No: MB 841498

**Figs. 3b, 5**

**Etymology.** the epithet refers to the shape of the basal bulb of the stipe.

**Type.** BENIN. ATACORA PROVINCE: Kota, 10°12’39”N, 01°26’45.8”E, gallery forest of Kota, date: 10 July 2020, leg. and det. Jean Evans I. CODJIA, Holotype JEIC0762 (UNIPAR), Isotype (KUN-HKAS 122160), GenBank Acc. No.: (nrLSU = OK510860, RP2 = OK510849, TEF1-α = OK510832).

**Description.** Basidiomata small-sized. Pileus 35–43 mm in diam, hemispherical at young stage and expanding to convex and broadly convex at the maturity, slightly depressed at the center, covered by white (1A1) to gray orange (6B4) small thick adherent squamules or patchy volval remnants; margin non-striate at first then slightly striate at maturity, non-appendiculate; white (1A1). Lamellae-free, white (1A1) to pale yellowish white (2A2), lamellulae truncate. Stipe 42–75 mm long × 7–12 mm diam., nearly cylindrical, bulbous, white (1A1) to whitish (1A2), covered with white squamules. Anulus present, superior, below the apex of the stipe, broad, skirt-like, persistent, white (1A1). Context stuffed, white (1A1). Context stuffed, white (1A1). Bulb compressible, subglobose when young, then elongate to ventricose and tapering downward, marginate, white (1A1) to whitish (1A2), upper edge with shortly limbate, white (1A1) to whitish (1A2) volval remnants. Odor and taste not recorded.

Lamellar trama bilateral. Medistromatum 35–60 μm wide, composed of abundant, ellipsoid to subglobose inflated cells (15–30 × 10–15 μm), and abundant filamentous hyphae, 3–5 μm wide; vascular hyphae scarce. Lateral stratum composed of ellipsoid to subglobose inflated cells (25–30 × 15–20 μm), diverging at an angle of ca. 30° to 45° to mediostratum; fila-
mentous hyphae abundant, 3–5 μm wide. Subhymenium 35–45 μm thick, with 2–3 layers of ovoid to subglobose or irregular cells, 9–12 × 6–8 μm. Basidia (Fig. 5A) (28.5–42–55 (68.5) × (7.5–) 11.5–16 (–20) μm, clavate, 4-spored, basal septa lacking clamps. Basidiospores (Fig. 5B) [80/4/4] 10–12 × 7–8 μm, Q = (1.3) 1.4–1.6 (1.8), Qm = 1.5 ± 0.05, ellipsoid, smooth, colorless, weakly amyloid. Lamellar edge sterile, composed of subglobose to ellipsoid, inflated cells (15–40 × 10–25 μm), filamentous hyphae, 3–5 μm wide, irregularly arranged or running parallel to lamellar edge. Pileipellis (Fig. 5C) 115–180 μm thick, 2-layered; suprapellis up to 35–60 μm thick, slightly gelatinized, composed of arranged, thin-walled, colorless to nearly colorless, ellipsoid to clavate terminal cells 70–210 × 20–45 μm, mixed with filamentous hyphae 4–7 μm wide; subpellis up to 80–120 μm thick, composed of undifferentiated, filamentous hyphae 3–5 μm wide; vascular hyphae scarce. Volval remnants on pileus (Fig. 5D) composed of longitudinally arranged elements: filamentous hyphae abundant 4–8 μm wide, colorless, thin- to slightly thick-walled, branching; inflated cells abundant, subglobose to ellipsoid, 35–55 × 25–45 μm, colorless, thin- to slightly thick-walled, terminal, mixed with long clavate terminal cells 50–130 × 10–15 μm; vascular hyphae rare. Outer part of volval limb on the stipe base (Fig. 5E) composed of longitudinally arranged elements: filamentous hyphae abundant 5–10 μm wide, colorless, thin- to slightly thick-
walled, branching; inflated cells abundant, subglobose to ellipsoidal, 40–85 × 20–60 μm, colorless, thin- to slightly thick-walled, terminal, mixed with long elavate terminal cells 40–105 × 10–25 μm; vascular hyphae rare. Stipe trama composed of longitudinally arranged, long elavate terminal cells 60–200 × 10–40 μm; filamentous hyphae scattered to abundant, 3–6 μm wide; vascular hyphae scarce. Clamps absent in all parts of basidioma.

**Habitat.** Solitary or in small group on the ground in woodland and gallery forests, associated with *Isoberlinia doka* (Fabaceae/Leguminosae) and *Uapaca guineensis* or *Uapaca togoensis* (Phyllanthaceae).

**Distribution.** Currently known from Benin and Guinea.

**Additional specimens examined.** BENIN. ATACORA PROVINCE: Kota, 10°12’39”N, 01°26’45.8”E, gallery forest of Kota, date: 21 June 2018, leg. and det. Jean Evans I. CODJIA, JEIC0426_2, date: 22 June 2018, leg. and det. Jean Evans I. CODJIA, JEIC0432_2. BORGOU PROVINCE: Ndali, 09°45’51.2”N, 02°18’39.1”E, Forest Reserve of Ouéme Supérieur, date: 22 August 2017, leg. and det. Jean Evans I. CODJIA, JEIC0436. GUINEA. UPPER GUINEA, FORÉCARIAH PREFECTURE: Moussaya Subprefecture, 10°41’06.3”N, 09°46’59.6”W, Forest Reserve of Moussaya, date: 03 July 2018, leg. and det. Jean Evans I. CODJIA, JEIC0512. UPPER GUINEA, FARANAH PREFECTURE: Faranah, 10°30’21.6”N, 09°58’20.4”W, National Park of Upper Niger, date: 04 July 2018, leg. and det. Jean Evans I. CODJIA, JEIC0522.

**Notes.** *Amanita bulbulosa* is characterized by its small, white basidiomata, floccose pileus with small adherent squamules or patchy universal veil that are white to gray orange, striate at maturity, and marginate basal bulb. The phylogenetic analyses clustered this species in the non-lethal subclades (ML ultrafast bootstrap = 95%; SH-aLRT = 1.0). The subclade I contains the lethal species, and the subclades II and III contain the non-lethal species. The non-lethal subclades comprise few species until now. Previously, seven (07) species (including two species in subclade II and five species in the subclade III) were reported mostly from Asia and America (Jenkins 1988; Thongbai et al. 2017; Cui et al. 2014; Cui et al. 2018, 2021). In contrast, no species from those non-lethal subclades have been reported from Africa previously. This study represents the first contribution of that group with two new species from tropical Africa. The African collections in our phylogenetic tree form well-distinct lineages. *Amanita ballerinoides* and *A. bulbulosa*, newly described from Africa, are clustered in the same subclade III with other non-lethal species, namely *A. ballerina*, *A. chuformis*, *A. franzii*, *A. levistriata*, *A. pseudogemmata*, and it forms a well-distinct lineage in the phylogenetic tree.

At first sight, this species has some morphological similarities with *A. ballerina*, *A. ballerinoides*, *A. chuformis*, *A. franzii*, *A. levistriata*, *A. pseudogemmata*. These species have a striate pileal margin and a marginate basal bulb like *A. bulbulosa*. Although *A. ballerinoides* is very close to *A. bulbulosa*, it differs from the latter by its medial, broad, skirt-like annulus and smaller basidiospores (7.5–9.5 × 6.5–8.0 μm) (Jenkins 1988; Tulloss and Yang 2021). *Amanita pseudogemmata* can be distinguished from *A. bulbulosa* by its dirty yellow to pale yellow-brown pileus with yellow to yellow-brown volval remnants, a yellow stipe with its basal bulb covered with collar-like, white to yellowish volval remnants, and smaller basidiospores (7–9.5 × 6–8.5 μm) (Hongo 1974; Yang and Doi 1999; Yang 2005, 2015; Cui et al. 2018).

**Analysis of toxins by LC-HRMS**

No corresponding monoisotopic masses were identified for α-amanitin, β-amanitin, phalloidiin, and phallacidin. Thus, neither amatoxins nor phallootoxins were found in *A. ballerinoides*, *A. bulbulosa*, and *A. sp. 0595*. This result confirms the placement of those species within the non-lethal subclade III of *A. sect. Phalloideae*.

**Discussion**

**Phylogeny of *Amanita sect. Phalloideae***

In the present study, the phylogenetic analyses strongly support Cui et al. (2018, 2021) that recognize three subclades within *A. sect. Phalloideae* (ML ultrafast bootstrap = 95%; SH-aLRT = 100%, ML ultrafast bootstrap = 100%, BPP = 1.0). The subclade I contains the lethal species, and the subclades II and III contain the non-lethal species. The non-lethal subclades comprise few species until now. Previously, seven (07) species (including two species in subclade II and five species in the subclade III) were reported mostly from Asia and America (Jenkins 1988; Thongbai et al. 2017; Cui et al. 2014; Cui et al. 2018, 2021). In contrast, no species from those non-lethal subclades have been reported from Africa previously. This study represents the first contribution of that group with two new species from tropical Africa. The African collections in our phylogenetic tree form well-distinct lineages. *Amanita ballerinoides* and *A. bulbulosa*, newly described from Africa, are clustered in the same subclade III with other non-lethal species, namely *A. ballerina*, *A. chuformis*, *A. franzii*, *A. levistriata*, *A. pseudogemmata* reported previously by Jenkins (1988), Thongbai et al. (2017), and Cui et al. (2018, 2021).

In general, what makes lethal amanitas easily distinguishable from other groups is the morphological characteristics and the presence of deadly poisonous substances. The screening for the most notorious toxins by LC-HRMS revealed the absence of α-amanitin, β-amanitin, phalloidiin, and phallacidin in *A. ballerinoides* and *A. bulbulosa*. In previous studies, the screening for both α-amanitin and phalloidiin were also
performed in *A. ballerina*, *A. franzii*, and *A. pseudogemmata* (Thongbai et al. 2017; Fraiture et al. 2019). The results from those studies showed that toxins were not present in those species. Thus, Thongbai et al. (2017) proposed the separation of non-lethal species of *A. sect. Phalloideae* into a new section. However, the phylogenetic result was not statistically supported, and the group comprises few species. Moreover, the non-lethal species (*A. ballerina*, *A. ballerinooides*, *A. bulbulosa*, *A. franzii*, *A. pseudogemmata*) are reminiscent of some species belonging to *A. sect. Lepidella* subsect. *Limbatulae*, such as *A. limbatula* Bas, *A. parva* Murrill, *A. mutabilis* Beardslee, and *A. praelongispora* (Murrill) Murrill (Beardslee 1919; Murrill 1941, 1945; Bas 1969; Jenkins 1979, 1986; Tulloss 1984). Phylogenetically, those species within *A. sect. Lepidella* might probably cluster in the non-lethal subclades within *A. sect. Phalloideae*. Still, there is not enough molecular evidence to confirm the phylogenetic relationships of those species within *A. sect. Lepidella* with the non-lethal amanitas of *A. sect. Phalloideae*.

On the other hand, the second non-lethal subclade II in the multigene phylogenetic tree comprises *A. hesleri* and *A. zangii*, which were also reported as non-lethal taxa (Cai et al. 2014; Fraiture et al. 2019). Both species have some similar morphological characteristics with the non-lethal species of subclade III. The elongate to ventricose basal bulb is characteristic for both non-lethal subclades. However, the main morphological characteristic that differentiates the species of subclade II from those of subclade III is their appendiculate pileal margins (Yang et al. 2001; Thongbai et al. 2016; Cui et al. 2018). All those morphological characteristics of the non-lethal species do not fit the circumscription of *A. sect. Phalloideae* (Bas 1969), which makes their placement uncertain (Thongbai et al. 2017). Thus, based on our results, the non-lethal species could be considered either as early diverging lineages in *A. sect. Phalloideae*, or as new sections (Thongbai et al. 2017).

### Distribution and ecology of new species

The new species reported here are only known from West Africa and were collected during the rainy season. *Amanita ballerinooides* was found in Benin, Guinea, Ivory Coast, and Mali. It occurs in woodlands and gallery forests, associated with *Berlincia grandiflora* or *Isoperlinia doka* (*Fabaceae/Leguminosae*) and *Uapaca guineensis* (*Phyllanthaceae*). *Amanita bulbulosa* was found in Benin and Guinea in woodlands and gallery forests where it is associated with *Uapaca guineensis* or *U. togensis* (*Phyllanthaceae*) and *Isoperlinia doka* (*Fabaceae/Leguminosae*). Both woodlands and gallery forests of West Africa are home to a high diversity of mycorrhizal fungi (Bâ et al. 2012).

The genus *Amanita* is one of the most ecologically and economically important groups within the fungi kingdom, even if some species are deadly poisonous and cause a lot of fatalities around the world (Cai et al. 2014; Li et al. 2015; Cui et al. 2018; Codjia et al. 2020). As ectomycorrhizal fungi, *Amanita* species represent key and indispensable actors for the good functioning of forest ecosystems (woodland, gallery forests) and strongly influence the diversity and productivity of tropical African forests (Bâ et al. 2011). Albeit, many taxa are not yet fully documented, many representatives within the genus *Amanita* are consumed and sold by local people in West Africa (Yorou et al. 2014; Boni and Yorou 2015; Kamou et al. 2017; Fadeyi et al. 2017, 2019; Soro et al. 2019). Further ethnomycological investigations could help to check whether both newly described taxa are choice edibles or not and thus support their phylogenetic placement within the non-lethal groups of the section *Phalloideae*. So, increasing the investigations in the genus *Amanita* is crucial, especially in tropical Africa, so far remaining underexplored.

### Key to the West African species of *Amanita* sect. *Phalloideae*

1. Pileus glabrous, smooth; lamellulae mostly attenuate; basal bulb globose surrounded by the white membranous volva; basidiospores distinctly amyloid..............*A. albolimbata*

   1* Pileus floccose; lamellulae mostly truncate; basal bulb marginate, covered with white squamules; basidiospores weakly amyloid..................................................................................2

2. Basidiospores 8.5–9 × 6.5–7 μm, broadly ellipsoid to ellipsoid (Q = 1.2–1.4, Qm = 1.3 ± 0.05); annulus medial...........................................*A. ballerinooides*

   2* Basidiospores 10–12 × 7–8 μm, ellipsoid (Q = 1.4–1.6, Qm = 1.5 ± 0.05); annulus superior............................*A. bulbulosa*

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01778-0.

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### Author contribution

ZLY, JEIC, and NSY developed the concept. JEIC, NSY, and MR collected the species. JEIC generated the DNA sequences. JEIC and PMW performed the molecular phylogenetic analyses. JEIC performed the taxonomic studies. JEIC wrote the first draft of the manuscript. PMW, MR, NSY, and ZLY critically revised and approved the final version of the manuscript.
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Data availability The sequences generated in this study are available in the NCBI GenBank (Table 1). The specimens studied in this study are deposited in the Mycological Herbarium of the University of Parakou (UNIPAR). Duplicate specimens are conserved at the Herbarium of Cryptograms, Kunnming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

Declarations

Conflict of interest The authors declare no competing interests.

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