SYSTEMATICS OF *AMANITA* (AMANITACEAE, AGARICALES, BASIDIOMYCOTA) FROM THE GUIANA SHIELD AND CENTRAL AFRICA

By

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

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May 2020

ABSTRACT

SYSTEMATICS OF *AMANITA* (AMANITACEAE, AGARICALES, BASIDIOMYCOTA) FROM THE GUIANA SHIELD AND CENTRAL AFRICA

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This study is a systematic assessment of ten species of ectomycorrhizal fungi of the genus *Amanita* (*Amanitaceae, Agaricales, Basidiomycota*) from the lowland tropical rainforests of the Guiana Shield and Central Africa. Fungi were collected in each of the above regions, analyzed morphologically, and compared to the literature on *Amanita* diversity worldwide. All species addressed herein are given full formal taxonomic descriptions including complete macro- and microscopic character profiles, habit, habitat, and distribution. Phylogenetic assessments using ribosomal DNA and protein coding genes are provided for Guiana Shield taxa, and sequence data for two non-protein coding regions, the internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nrLSU) are provided for Central African taxa. to support infrageneric placement of each taxon. Chapter one describes three new species from the Guiana Shield: *A. cyanochlorinosma, A. fulvoalba,* and *A. guyanensis*. Chapter two describes four epitypes of otherwise poorly known Central African species: *A. echinulata, A. fulvopulverulenta, A. robusta,* and *A. bingensis,* and three new species from Cameroon: *A. minima, A. leucolamellata,* and *A. goossens-fontanae*. Each chapter is a manuscript that has been published in, or submitted to, a peer-reviewed mycological journal.

ACKNOWLEDGEMENTS

Funding was provided by the National Science Foundation grants DEB-0918591, DEB-1556338 to Dr. Terry Henkel from the Department of Biological Sciences at Humboldt State University and DEB-0732968 to Dr. M. Catherine Aime from the Department of Botany and Plant Pathology at Purdue University. I would like to thank additional sources of funding from the Humboldt Bay Mycological Society, Jennings Botany Award, and Sonoma County Mycological Association. Research permits were granted by the Guyana Environmental Protection Agency and Cameroon's Ministry of Research and Scientific Innovation. Field assistance in Guyana was provided by Mei Lin Chin, Piakai Henkel, Christopher Andrew, Valentino Joseph, Peter Joseph, Francino Edmund, and Luciano Edmund. In Cameroon the Conservator of the Dja Biosphere Reserve, Mr. Ndinga Hilaire, and his staff greatly assisted the fieldwork in the Dja Biosphere Reserve. Congo Basin Institute staff provided much logistical support. Field assistance was provided by Mei Lin Chin, Todd Elliott, Camille Truong, Bryn Dentinger, Noah Siegel, Blaise Jumbam, Constantin Engoulou, Jessie Uehling, Alamane Gabriel (a.k.a. Sikiro), and Essambe Jean-Pierre (a.k.a. Papa Chef). I also thank Dr. Andre De Kesel and staff of the Herbarium at the Belgian Botanic Garden Meise for access to Mme. Goossens-Fontana type specimens and permission to reproduce her watercolor illustrations. Drs. Cathie Aime and Rachel Koch in the Aime Lab at Purdue University provided resources and guidance for DNA extraction, sequencing, and phylogenetics. Committee members Dr. Erik Jules, Dr. Mihai Tomescu, and Dr. M. Catherine Aime improved this work with constructive editorial critiques. I also thank Carolyn Delevich for formatting assistance and for being a steadfast friend throughout this process. Finally, thanks to Dr. Terry Henkel for his instruction, guidance, and many hours of editing.

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INTRODUCTION

Fungal Diversity, Ecology, and the Value of Taxonomy

Fungi are ubiquitous organisms whose saprotrophic, parasitic, and mutualistic lifestyles play critical roles in terrestrial nutrient cycling and ecosystem dynamics (Webster & Weber 2007). Fungi compose a large proportion of Earth's eukaryotic species, yet they remain poorly documented. Only ~5% of an estimated 1.5 million fungal species have been described, and entire regions remain mycologically unexplored, especially in the tropics (Mueller & Schmit 2007; Webster & Weber 2007; Stork & Habel 2014). Most tropical ecosystems contain high levels of biodiversity and endemism across organismal groups, and fungi are likely no exception (Mittermeier et al. 1998). Tropical biodiversity hotspots are often threatened, yet the threats to fungi are unknown, in part due to the paucity of tropical mycological surveys. As habitat and species loss accelerates, understanding of fungal biodiversity in undisturbed tropical forests is important in order to comprehend the community dynamics of these fragile ecosystems (Mittermeier et al. 1998; Gaston 2000; Stork & Habel 2014).

While only around 3% of woody phanerogam groups form ectomycorrhizal (ECM) symbioses, they disproportionately dominate higher latitude regions and include many ecologically important timber species in the *Pinaceae*, *Fagaceae*, and other groups (Smith & Read 2008). In boreal forests, the ECM fungal symbionts can account for up to 39% of the microbial biomass and 10–35% of soil respiration (Tedersoo et al. 2012).

There is increasing evidence that ECM fungi play critical roles in in dynamics of many ecosystems worldwide (Taylor 2002; Dickie & Moyersoen 2008; Rinaldi et al. 2008). They facilitate water and mineral nutrient uptake from soil organic matter, thereby enhancing host plant growth, fitness, and tolerance of environmental stressors (Smith & Read 2008). This allows ECM plants to competitively exclude non-ECM plants in nutrient or water stressed environments (Halling 2001). Ectomycorrhizal-dominated communities are well-documented in temperate ecosystems, but there is a paucity of equivalent studies in the tropics, especially regarding the fungal symbionts (Smith & Read 2008; Tedersoo et al. 2010). Though few in number, surveys suggest that ECM fungi may be more diverse and abundant in specific tropical regions than previously thought (e.g. Singer et al. 1983; Buyck et al. 1996; Smith et al. 2011, 2013; Henkel et al. 2012).

Where they occur in the lowland tropics, ECM symbionts exert a strong influence on community structure and dynamics. There are several groups of tropical ECM canopy trees that can form monodominant or co-dominant stands surrounded by diverse arbuscular mycorrhizal tree assemblages (Newbery et al. 1988; Connell & Lowman 1989; Henkel et al. 2002; Henkel 2003). Examples include trees in the ECM taxa *Dipterocarpaceae* in Southeast Asia, *Myrtaceae* subfam. *Leptospermoideae* in Australasia, and *Fabaceae* subfam. *Detarioideae* (“detarioids”) in the African and South American tropics (Smith & Read 2008). Ectomycorrhizal fungi may provide a competitive advantage to these trees, allowing for increased conspecific seedling recruitment and greater tolerance of shade and nutrient depleted soil (Connell & Lowman 1989; Henkel et al. 2005; Peh et al. 2011; Henkel & Mayor 2019; Delevich et al. 2020).

Ectotrophic Forests in Cameroon and Guyana

The ECM symbiosis is an important component of tropical African ecosystems, though most research has focused on the host trees (Verbeken & Buyck 2002). The majority of monodominant forests in Guineo-Congolian Africa are formed by members of the *Fabaceae* subfam. *Detarioideae* (Newbery et al. 1988; Peh et al. 2011). For example, ten ECM genera of this subfamily compose a patchwork of monodominance and codominance in Korup National Park of southwestern Cameroon (Newbery et al. 1988). This park, along with the Dja Biosphere Reserve (DBR) in southeastern Cameroon, contains some of the largest, best-protected lowland Afro-tropical rainforests (Sonke & Couvreur 2014). These sites were likely rainforest refugia during the last glacial maximum and may contain historical reservoirs of fungal biodiversity (Newbery et al. 1988; Buyck et al. 1996). *Gilbertiodendron dewevrei* (De Wild.) J. Léonard is the predominant ECM detarioid of the DBR, establishing over 90% canopy to understory dominance in a mosaic of multi-hectare stands (Peh et al. 2011; Castellano et al. 2016). These *G. dewevrei* forests contain an extraordinarily rich diversity of ECM fungi, with over 400 species recovered with repeated annual plot sampling from 2014–2019 (T.W. Henkel unpubl. data).

Structurally similar monodominant ECM stands are Guyana, a country centrally situated in South America’s Guiana Shield. The detarioid *Dicymbe corymbosa* Spruce ex

Benth. exhibits 80–100% basal area dominance over extensive areas in the Pakaraima Mountains (Henkel et al. 2002; Henkel 2003; Degagne et al. 2009). Above- and belowground studies conducted in *Dicymbe*-dominated forests in Guyana have recovered ECM fungal species numbers rivaling those of ECM-diverse Holarctic forests (100–200+ spp. ha^{-1}), and the majority are undescribed species (e.g. Smith et al. 2011; Henkel et al. 2012). While much progress has been made in documenting ECM fungi from Guyana (e.g. Largent et al. 2008; Fulgenzi et al. 2010; Uehling et al. 2012; Grupe et al. 2015; Henkel et al. 2016; Mighell et al. 2019) and some areas of the Paleotropics (e.g. Verbeken & Walleyn 2010; Buyck et al. 2016), many tropical ECM fungal species remain undescribed, and this lack of basic taxonomic work limits understanding of their ecology, evolution, and biogeography (Mueller & Schmit 2007).

Biogeographical Connections Between the Guiana Shield and Central Africa

The Guiana Shield and Central Africa share a Gondwanan geological origin and contain related clades of ECM trees in the *Detarioideae* and *Dipterocarpaceae* (Moyersoen 2006; Smith et al. 2011; Smith et al. 2013; de la Estrella et al. 2017). The shared monodominant forest community structure and geological history of the two regions has raised the hypothesis of phylogeographic affinity between their ECM mycotas (e.g. Castellano et al. 2016; Koch et al. 2017). While over 140 species and eight new genera from Guyana and Cameroon so far, collections of many more remain to be described. In monodominant forests on both continents, the most speciose groups of

ECM fungi are the families *Russulaceae*, *Amanitaceae*, *Boletaceae*, and Boletaceae (Verbeken & Buyck 2002; Henkel et al. 2012).

An NSF grant issued to T. W. Henkel and M. C. Aime has funded an analysis of the above- and belowground diversity of ECM fungi in Cameroon's Dja Biosphere Reserve. This project is producing the most complete specimen and diversity database of ECM fungi of Afro-tropical forests. Permanent plot-based above- and belowground sampling methods mirrored those employed in long term surveys of ECM fungi in Guyana (e.g. Henkel et al. 2012). Taxonomic work is a critical component of this project, as many ECM fungi from the Afro-tropics have not been analyzed with modern systematic methods. Traditionally informative DNA sequence loci along with data generated with high throughput sequencing (HTS) are being used to generate robust phylogenies and test biogeographic relationships of ECM fungi between Guineo-Congolian Africa and the Guiana Shield. Ectomycorrhizal target taxa for the biogeographic analyses include the *Amanitaceae*, *Cantharellaceae*, and *Clavulinaceae*. The project seeks to determine whether the ECM fungi of the similarly structured forests of closely related monodominant ECM detarioids in the Afro-tropics and Guiana Shield are vicariantly distributed, have co-dispersed with their ECM partners, or have been recruited by the host trees from regionally native ECM fungi (T.W. Henkel pers. comm.).

Taxonomic History of *Amanita*

One of the target taxa in the project outlined above is the *Amanitaceae*. This family is composed primarily of the genus *Amanita* Pers., which is estimated to include 900–1000 species worldwide, approximately half of which have been described (Tulloss 2005; Smith & Read 2008). The name *Amanita* has a rich mycological history, being first applied to stalked, gill-bearing fungi as early as the late 1600s (Jenkins 1977). Persoon (1797) applied *Amanita* as a genus name for stalked, gill-bearing fungi with a membranous or friable volva, a concept further refined by Hooker (1821) to include white basidiospores. Fries (1838) subsequently added free gill attachment as a diagnostic character. The generic concept of Fries has rarely been disputed since (Jenkins 1977; Singer 1986). This is a result of the abundance of generic diagnostic features present at the macro- and microscopic level. Macro- and micromorphological features that have proven useful in delimiting infrageneric taxa within *Amanita* include basidiospore amyloidity, presence or absence of a volva, annulus, bulbous base, and marginal appendiculae. The infrageneric classification system of Corner & Bas (1962) recognized two subgenera based on basidiospore amyloidity: subgen. *Amanita* (inamyloid) and subgen. *Lepidella* (amyloid). They divided subgen. *Amanita* into two sections based on presence or absence of a basal bulb: sect. *Amanita* (bulb present), and sect. *Vaginatae* (bulb absent). Subgenus *Lepidella* was divided into four sections: sect. *Amidellae* (marginal appendiculae and saccate volva present), sect. *Lepidella* (marginal appendiculae present, saccate volva absent), sect. *Phalloideae* (marginal appendiculae absent, saccate volva present), and sect. *Validae* (marginal appendiculae and saccate volva absent).

This infrageneric classification system was amended by Singer (1986) to include sections *Caesareae* and *Ovigerae* as sister taxa to section *Vaginatae*, section *Roanokensis* as a sister taxon to section *Amidella,* and section *Mappae* as a sister taxon to section *Validae*. Molecular phylogenetic studies have subsequently supported the monophyly of Singer's sections *Amanita, Vaginatae, Caesareae, Amidellae, Validae,* and *Mappae,* though some rank *Mappae* as a subsection within section *Validae* (Weiss et al. 1998; Kim et al. 2013; Tang et al. 2015). Conversely, sections *Lepidella* and *Phalloideae* appear to be polyphyletic, and their systematics are still in a state of flux (Weiss et al. 1998; Drehmel et al. 1999; Moncalvo et al. 2000; Kim et al. 2013). *Ovigerae* is still recognized by some as a subsection within sect. *Amanita,* and sect. *Roanokensis* is no longer recognized (Drehmel et al. 1999; Moncalvo et al. 2000).

Amanita has a nearly cosmopolitan range, existing on every continent but Antarctica (Tulloss 2005). *Amanita* species exhibit a wide range of ECM host associations and are prevalent in *Fagaceae* and *Pinaceae* dominated temperate and boreal forests (Tulloss 2005). Some *Amanita* species have a circumboreal distribution (e.g. Geml et al. 2006). Conversely, tropical species, where known, appear to have smaller geographic ranges (Beeli 1935; Bas 1978; Halling 2001; Simmons et al. 2002; Zhang et al. 2004; Tulloss 2005; Mighell et al. 2019). This work examines ten tropical *Amanita* species that have rarely or never been encountered and are currently known only from this study's field sites or nearby locales.

CHAPTER 1: NEW SPECIES OF *AMANITA* SUBGEN. *LEPIDELLA* FROM GUYANA

Published in Fungal Systematics and Evolution 3:1–12, June 2019

Abstract

New species of *Amanita* subgen. *Lepidella* are described from Guyana. *Amanita cyanochlorinosma* sp. nov., *Amanita fulvoalba* sp. nov., and *Amanita guyanensis* sp. nov. represent the latest additions to the growing body of newly described ectomycorrhizal fungi native to *Dicymbe*-dominated tropical rainforests. Macro- and micromorphological characters, habitat, and DNA sequence data for the ITS, nrLSU, *rpb2*, and *ef1-a* are provided for each taxon, and b-*tubulin* for most. Distinctive morphological features warrant the recognition of the three new species and a molecular phylogenetic analysis of taxa across *Amanita* subgen. *Lepidella* corroborates their infrageneric placements.

Introduction

Amanita (*Amanitaceae*, *Agaricomycetes*, *Basidiomycota*) is a monophyletic mushroom genus with cosmopolitan distribution (Drehmel *et al.* 1999, Tulloss 2005). It is estimated that 900–1000 *Amanita* species exist worldwide, with over 500 currently described (Tulloss 2005, Thongbai *et al.* 2016, Vargas *et al.* 2017). *Amanita* has traditionally been divided into two subgenera based on basidiospore amyloidity (e.g. Singer 1986). Subgenus *Amanita* includes those species with inamyloid basidiospores, and has traditionally been subdivided into sections *Amanita, Vaginatae,* and *Caesareae* based on macromorphology (e.g. Moser 1967). Molecular studies have corroborated these sections by demonstrating the monophyly of each (Weiss *et al.* 1998, Tang *et al.* 2015). Subgenus *Lepidella* includes those species with amyloid basidiospores and has been further subdivided into four sections based on velar morphology: sect. *Amidella* (marginal appendiculae and saccate volva present), sect. *Lepidella* (marginal appendiculae present, saccate volva absent), sect. *Phalloideae* (marginal appendiculae absent, saccate volva present), and sect. *Validae* (marginal appendiculae and saccate volva absent) (Corner & Bas 1962). Molecular studies have supported the monophyly of sect. *Amidella* and sect. *Validae*, whereas sect. *Lepidella* and sect. *Phalloideae* may be polyphyletic (Weiss *et al.* 1998, Drehmel *et al.* 1999, Moncalvo *et al.* 2000a, Kim *et al.* 2013, Tang *et al.* 2015, Cui *et al.* 2018).

While a few earliest diverging *Amanita* species are saprotrophic (Wolfe *et al.* 2012), the genus is otherwise considered ectomycorrhizal (ECM) and exhibits a wide range of ECM host associations (Tedersoo & Brundrett 2017). *Amanita* species are prevalent in *Fagaceae* and *Pinaceae* dominated higher latitude forests (Tulloss 2005, Truong *et al.* 2017), some with Holarctic distributions (Geml *et al.* 2006). Of the ~ 500 validly described species of *Amanita*, around 130 are known from the tropics (Thongbai *et al.* 2016). Tropical *Amanita* species frequently occur in spatially restricted mono- or co-dominant stands of ECM host trees, and thus appear to have smaller geographic ranges. They can, however, be a major component of the local ECM fungal assemblage (Watling & Lee 1995, Henkel *et al.* 2012, Ebenye *et al.* 2017). Overall, tropical regions remain mycologically undersampled and many *Amanita* species remain to be described

(Beeli 1935, Bas 1978, Mueller & Halling 1995, Halling 2001, Simmons *et al.* 2002, Zhang *et al.* 2004, Tulloss 2005, Henkel *et al.* 2012, Cui *et al.* 2018).

Around 35 species of *Amanita* have been described from lowland tropical South America. Bas (1978) mentioned several poorly documented Brazilian species described in 1937 by Johann Rick and described eight new central Amazonian species. More recently 25 species have described from the South American tropics including 11 from Colombia (Tulloss *et al.* 1992, Tulloss & Franco-Molano 2008), nine from Brazil (Bas & De Meijer 1993, Menolli *et al.* 2009, Wartchow *et al.* 2009, Wartchow *et al.* 2013, Wartchow 2015, Wartchow *et al.* 2015, Wartchow 2016, Wartchow & Cortez 2016), four from Guyana (Simmons *et al.* 2002), and one from Ecuador (Wartchow & Gamboa-Trujillo 2012). An additional six species previously described from other regions have been recorded in tropical South America (Tulloss & Halling 1997, Sobestiansky 2005, Wartchow & Tulloss 2007, Lechner & Alberto 2008, Palacio *et al.* 2015). This steady accrual of new taxa suggests that many South America *Amanita* are discovered. The ectotrophic *Dicymbe* forests of Guyana provide a case in point, where 28 mostly undescribed *Amanita* species are known to occur (Henkel *et al.* 2012).

Here three new species of *Amanita* subgen. *Lepidella* are described from Guyana. *Amanita cyanochlorinosma* sp. nov. has a greyish blue pileus, saccate volva, and strong odor of chlorine. *Amanita fulvoalba* sp. nov. produces robust basidiomata with tawny pilei, marginal appendiculae, and a saccate volva. *Amanita guyanensis* sp. nov. has a greyish brown pileus with floccose warts and a floccose volva. Each of the new species was compared to previously described *Amanita* species from the world literature, and their novelty demonstrated by comparison with morphologically similar described species. Molecular data support placement of *A. cyanochlorinosma* and *A. fulvoalba* in sect. *Lepidella*, and *A. guyanensis* in sect. *Validae*. Morphological data support these placements with the exception of *A. fulvoalba,* whose molecular-based placement in sect. *Lepidella* contrasts with its marginal appendiculae, which typically characterize sect. *Amidella*. Each of these species has been encountered repeatedly in Guyana's *Dicymbe* forests over the past 20 years (Henkel *et al.* 2012).

Materials and Methods

Collections and morphological analyses

Collections were made in Guyana during the May–Jul rainy seasons of 2000, 2003, 2005, 2007, 2009, 2011, 2012, and 2015 and the Dec–Jan rainy seasons of 2004, 2009, and 2016 from the Upper Potaro River Basin, within a 15 km radius of a permanent base camp at 5°18'04.8" N 59°54'40.4" W, 710 m a.s.l. Additional collections were made from the Upper Mazaruni River Basin during Dec 2010 and Jun 2012 within a 0.4 km radius of a base camp at 5°26'21.3" N and 60°04'43.1" W, 800 m a.s.l., and Jun 2011 from the Upper Demerara River Basin at Mabura Ecological Reserve, near a field station located at 5°09′19.0″N 58°41′58.9″W, 100 m a.s.l. At the Potaro sites, basidiomata were collected from monodominant forests of ECM *Dicymbe corymbosa* (Henkel *et al.* 2012) and other stands containing ECM *Dicymbe altsonii*, *Aldina insignis* and *D. corymbosa* (Smith *et al.* 2011). At the Mazaruni site, collections were made from forests co-dominated by ECM *Pakaraimaea dipterocarpacea* and *Dicymbe jenmanii* (Smith *et al.*

2013). At the Mabura site, collections were made in monodominant stands of *D. altsonii* (Zagt 1997). Macromorphological features of fresh basidiomata were described in the field. Colors were described subjectively and coded according to Kornerup & Wanscher (1978), with color plates noted in parentheses. Fresh collections were field-dried with silica gel.

Micromorphological features were assessed using an Olympus BX51 microscope with bright field and phase contrast optics. Rehydrated fungal tissues were mounted in H_2O, 3% potassium hydroxide (KOH), and Melzer's solution. Twenty-five basidiospores were measured from each specimen of each species, including the types. Twenty basidia and hyphal elements of the subhymenium, hymenophoral trama, pileipellis, pileus and stipe trama, and universal veil were measured from each type specimen, and 10 from each additional specimen examined. Length/width Q values for basidiospores are reported as Qr (range of Q values over "n" basidiospores measured) and Qm (mean of Q values ± SD). The notation "[a/b/c]" preceding sets of basidiospore data denotes "'a' basidiospores from 'b' basidiomata from 'c' collections." Outlying measurements observed in less than 5% of a given structure are placed in parentheses. Line drawings are freehand composites of microscopic observations. Specimens were deposited in the following herbaria: BRG, University of Guyana; HSC, Humboldt State University; PUL, Kriebel Herbarium, Purdue University.

DNA extraction, amplification, sequencing and phylogenetic analyses

DNA was extracted from dried basidioma tissue of types and additional specimens using the Wizard® Genomic DNA Purification kit (Promega Co., WI, USA).

Five DNA gene fragments were sequenced, including those coding for the second-largest subunit of RNA polymerase II (*rpb2*), translation elongation factor 1-alpha (*ef1-a)* and beta-tubulin (b-*tubulin*), along with two non-protein coding regions, the internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nrLSU). Primer pairs ITS1F/ITS4B (Gardes & Bruns 1993), LROR/LR6 (Vilgalys & Hester 1990, Moncalvo *et al.* 2000b), 983F/2218R (Rehner & Buckley 2005), Am-b-*tubulin* F/Am-b-*tubulin* R (Cai *et al.* 2014) and Am-6F/Am-7R (Cai *et al.* 2014) were used to amplify ITS, nrLSU, *ef1-a*, b-*tubulin* and *rpb2*, respectively. PCR reactions included 12.5 µL of Promega PCR Mastermix (Promega Co., WI, USA), 1.25 µL of each primer (at 10 µM) and approximately 100 ng of DNA. The final PCR reaction volume was 25 µL. The recommended cycling conditions for each primer pair were followed. PCR products were sequenced by GeneWiz® (South Plainfield, NJ, USA). To get readable ITS and nrLSU sequences for specimens MCA 3962 and TH 9172, these fragments were cloned using the pGem®-T Easy Vector System (Promega Co., WI, USA) following manufacturer's protocols. Ten colonies for each specimen for each locus were suspended in 30 µL of sterile water. PCR reactions were done as described above, using the original primers for each gene fragment and ten µL of clone/sterile water mixture. All amplicons were sequenced. Sequences were edited using Sequencher 5.2.3 software (Gene Codes Corporation, MI, USA) and deposited in GenBank.

Initial BLAST searches with the ITS and nrLSU sequences for each new species confirmed their affinity with *Amanita* subgen. *Lepidella.* Infrageneric relationships of the three new species was assessed with phylogenetic analyses using nrLSU, *ef1-a*, b-*tubulin* and *rpb2* (Cai *et al.* 2014). Sequences for the final dataset were downloaded from GenBank and included exemplars from 76 specimens from all four sections of *Amanita* subgen. *Lepidella*. Eight species from *Amanita* subgen. *Amanita* were used as outgroup taxa. Supplementary Table I gives all taxa, collection information, GenBank numbers, and references for specimens used in the phylogenetic analysis.

Sequences were aligned in Mega 7 (Kumar *et al.* 2016) using the MUSCLE algorithm (Edgar 2004) with refinements to the alignment done manually. Individual gene alignments were concatenated manually after inspection for intergene conflict. Phylogenies were reconstructed using maximum likelihood (ML) and Bayesian methods. PartitionFinder v1.1.0 (Lanfear *et al.* 2012) was used to determine the best partitioning strategy and model of molecular evolution for each partition for both the ML and Bayesian analyses. Maximum likelihood bootstrap analysis for phylogeny and assessment of branch support by bootstrap percentages (% BS) was performed using RAxML (Stamatakis 2014). One-thousand bootstrap replicates were produced. Bayesian analyses for the reporting of Bayesian posterior probability (BPP) support for branches was conducted using the program Mr. Bayes v.3.2.6 (Ronquist *et al.* 2012). Four simultaneous, independent runs, each with four Markov chain Monte Carlo (MCMC) chains, were initiated and run at a temperature of 0.20 for 50 million generations, sampling trees every 1000 generations until the standard deviation of the split frequencies reached a final stop value of 0.01. The initial 20% of trees were discarded as burn-in and a maximum clade credibility tree from the remaining trees was produced using

TreeAnnotator. The final alignment and phylogeny can be accessed in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S23533).

Results

Nine ITS (Genbank accessions: MK064186-MK064193, MK097470), 15 nrLSU, seven *ef1-a*, ten *rpb2* and seven b-*tubulin* sequences were generated in this study, ranging from 444–761, 535–1075, 804–1120, 482–1174 and 289–380 bp, respectively. For specimens MCA 3962 and TH 9172, one ITS amplicon of *Amanita* origin was recovered for both specimens, while two nrLSU amplicons of *Amanita* origin were recovered from each specimen, one with a 15 bp intron relative to the other; both nrLSU sequences were used in the phylogenetic analysis. After the ends of the individual alignments were trimmed, the size of the aligned dataset was as follows: nrLSU was 941 bp, *ef1-a* was 536, *rpb2* was 669 and b-*tubulin* was 235. A 598 bp intron in the *rpb2* sequence that was present in specimens MCA 6920, TH 8056, TH 8455, TH 9043 and TH 10395 was removed from the alignment.

Amanita subgen. *Lepidella* was resolved as monophyletic with strong statistical support (99% BS/0.98 BPP), along with each of the four sections in subgen. *Lepidella*: *Amidella* (100% BS/0.99 BPP), *Lepidella* (88% BS/0.91 BPP), *Phalloideae* (99% BS/0.98 BPP) and *Validae* (99% BS/0.98 BPP) (Fig. 1). Specimens MCA 3155, TH 9767 and TH 9772 were conspecific and formed a well-supported lineage (98% BS/1.00 BPP) resolved to sect. *Validae* and represent *A. guyanensis* (Fig. 1). Specimens MCA 3962 and TH 9172 were conspecific and formed a well-supported lineage (100% BS/0.99 BPP), representing *A. cyanochlorinosma*, and specimens MCA 6290, TH 8056, TH 8455, TH 9043 and TH 10395 were conspecific and formed another well-supported lineage (100% BS/1.00 BPP), representing *A. fulvoalba*. The two latter species were resolved to sect. *Lepidella* (Fig. 1).

Taxonomy

Amanita cyanochlorinosma Mighell & T.W. Henkel, ***sp. nov***. Mycobank MB 827394. Figs 2, 3.

Diagnosis: Similar to *Amanita modesta* but differs in its strong chlorine odor, larger basidiospores, and greyish blue pileus color.

Type: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains. Upper Potaro River Basin, ~20 km east of Mt. Ayanganna, Tadang Base Camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, elevation 710–750 m; ~ 0.3 km ESE of base camp, on root mat in *Dicymbe corymbosa* and *Dicymbe altsonii* co-dominant forest, 30 Dec. 2009, *Henkel 9172* (BRG 41298, holotype; HSC G1229, isotype); GenBank accessions: ITS MK064187; nrLSU MK105493, MK105494; *rpb2* MK092933; *ef1-a* MK092956; b-*tubulin* MK092939.

Etymology: *cyano*- (Gk. comp.) = blue; *chlorinosma*, referring to the pileus color and chlorine odor reminiscent of *Amanita chlorinosma*.

Pileus 55–78(–114) mm broad, 3–8 mm tall, planate with slightly downturned margin, with age slightly upturned with a broad, shallow central depression, overall greyish blue (22F4), lighter concolorous (22D3) toward margin, progressively darker brownish grey (10F3–10F4, 11F3–11F4) over disc; surface subviscid, finely appressed radially fibrillose over outer 2/3, appressed matted fibrillose over disc; margin entire, under hand lens finely crenulate; volval elements absent; trama 1 mm thick at margin, 1 mm over lamellae, 3 mm at stipe, white, solid, unchanging. *Lamellae* finely and abruptly adnexed, thin, crowded, off-white to faintly pinkish cream (5A1–5A2), unchanging; edges concolorous, under hand lens very finely roughened-eroded; lamellulae numerous, usually 2–3, 2–21 mm long. *Stipe* 55–89(–136) × 6–13 mm, equal to slightly tapering upward from basal bulb, white to faint grey (10A1–10B1), subglabrous macroscopically, under hand lens finely matted fibrillose-floccose, darkening slightly with pressure; bulb 22–30 × 16–31 mm, subglobose, subabrupt apically, narrowing toward base and subradicate; trama white, solid, unchanging. *Volva* membranous, tightly adhering to bulb with 1–2 ascending limbs loosely appressed to stipe, off-white (2A1–2A2), discolored brown from adhering soil, with white hyphal cords descending from base. *Annulus* initially superior, subsuperior with age, white to off-white (2A1–2A2 KW) throughout, thin-membranous, appressed to stipe at apex, lower margin outflaring and pendant, occasionally finely perforate. *Odor* strongly of chlorine; *taste* not obtained.

Basidiospores white in medium deposit, [200/8/8] (6.0–)7.0–9.0(–10.0) × (4.0–) 4.5–6.0(–8.0) μm, Qr = (1.0–)1.16–1.6(–1.66), Qm = 1.32, broadly ellipsoid, smooth, hyaline, amyloid; wall slightly thickened; hilar appendix truncate, up to 1 μm long; contents usually granular-guttulate. *Basidia* 23–41 × 5.5–8.0 μm, clavate, 4-sterigmate, rarely 3-sterigmate; sterigmata 1.0–5.0 μm long. *Subhymenium* up to 35 μm thick, composed of globose to ovoid, angular elements up to 44 μm wide. *Marginal tissue of* *lamellae* composed of abundant, easily dislodged subglobose to pyriform elements, these 16–27 × 10–19 µm. *Lamellar trama* bilateral; mediostratum 15–35 µm wide, composed of branched, interwoven, occasionally inflated hyphae, 2–17 µm wide; lateral stratum diverging obtusely from the mediostratum, composed of uninflated hyphae terminating in 1–3 inflated cylindrical to ovoid elements, these 10–49 × 8–18 µm. *Pileipellis* an ixomixtocutis with two distinct layers; suprapellis 20–115 µm thick; hyphae 2–11 µm wide, partially gelatinized, hyaline, thin-walled, loosely interwoven; subpellis 40–75 µm thick; hyphae 2–8 µm wide, non-gelatinized, hyaline, thin-walled, densely interwoven. *Pileus trama* with abundant acrophysalides, these 23–122 × 10–44 µm, ellipsoidal to clavate, usually with an abruptly tapered base; contents occasionally granular-guttulate; uninflated tramal hyphae 2–10 µm wide, frequently branching. *Stipe trama* composed of longitudinally arranged, ovoid, ellipsoid, or clavate acrophysalides, these 25–203 × 12–60 µm, occasionally with granular contents; uninflated, non-conductive tramal hyphae 2–15 µm; conductive hyphae absent to moderately frequent in localized clusters, up to 23 µm wide. *Volva at stipe base* composed of densely interwoven, often branching, uninflated hyphae 36–174 × 2–10.5 µm; terminal cells cylindrical or clavate, 25–158 × 6–43 µm. *Partial veil* composed of uninflated hyphae 1–6.5 µm wide, highly branched, thin-walled, densely interwoven; terminal cells 18–50 × 6–16 µm, mostly clavate, occasionally cylindrical or subfusiform; all elements occasionally containing diffuse or clustered granules. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat and distribution: Solitary, or rarely in pairs, on humic mats of forest floor under *D. corymbosa*; also found in stands containing *D. corymbosa, D.*

altsonii and *A. insignis* or *P. dipterocarpaceae* and *D. jenmanii*; known from the type locality in the Upper Potaro River Basin and ~25 km to the west in the Upper Mazaruni Basin.

Additional specimens examined: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, Potaro base camp at 5°18′04.8″N 59°54′40.4″W, 710–750 m a.s.l.; under *D. corymbosa*, 3 km E of base camp, 7 May 2001, *Henkel 8057* (BRG 41299, HSC G1230); 3.5 km SE of base camp, 19 May 2001, *Henkel 8182* (BRG 41300, HSC G1230); 1 km SW of base camp, 25 June 2001, *Henkel 8375* (BRG 41301, HSC G1231); 3 km SE of base camp, 11 June 2004, *Henkel 8669* (BRG 41302, HSC G1232); 0.4 km SW of base camp near Blackwater Creek, 1 July 2006, *Aime 3147* (BRG 41303, PUL F24395); 14 June 2015, *Henkel* 10083 (BRG 41304, HSC G1233); vicinity of base camp, 18 May 2010, *Aime 3962* (BRG 41305, PUL F24396); ~20 km east of Mt. Ayanganna, Tadang base camp at 5°16′14.5″N 59°50′39.1″W, 710–750 m a.s.l.; 100 m E of base camp, under *D. corymbosa* and *D. altsonii*, 4 June 2013, *Henkel 9737* (BRG 41306, HSC G1234).

Commentary: *Amanita cyanochlorinosma* is a distinctive species recognized in the field by its medium-sized, solitary basidiomata, glabrous, viscid, greyish blue pileus contrasting with the white hymenophore, stipe, and veils, strong odor of chlorine, superior annulus, and saccate-limbate volva. The species is best placed in sect. *Lepidella* due to its amyloid, broadly ellipsoid basidiospores, saccate volva, membranous annulus, chlorine odor, and gills that do not darken with desiccation (Corner & Bas 1962, Bas 1969). Although the absence of pileal appendiculae suggests placement in sect.

Phalloideae, the strong chlorine odor, while known from a handful of sect. *Phalloideae* species, was emphasized by Bas (1969) as an important character of sect. *Lepidella*, occurring in about half of the known species. Additionally, phylogenetic analysis indicated that *A. cyanochlorinosma* is nested within sect. *Lepidella* (Fig. 1).

Few other *Amanita* species resemble *A. cyanochlorinosma*. *Amanita modesta* from lowland tropical rainforests of Malaysia is similar to *A. cyanochlorinosma* in its small to medium size, membranous annulus, saccate volva, and bluish pileus lacking volval remnants (Corner & Bas 1962). *Amanita cyanochlorinosma* differs from *A. modesta* by its chlorine odor, longer basidiospores (7.0–9.0 µm versus 5.9–7.8 µm), and greyish blue as opposed to mouse grey to purplish umber pileus. *Amanita cyanochlorinosma* resembles the Japanese *A. griseoturcosa* in its similarly-sized basidiomata, greyish blue pileus, and velar structures, but differs in its chlorine odor and shorter basidiospores (7.0–9.0 µm versus 8.4–12.0 µm) (Tulloss & Yang 2018).

Amanita fulvoalba Mighell & T.W. Henkel, ***sp. nov***. Mycobank MB 827395. Figs 4, 5.

Diagnosis: Similar to *A. aurantiobrunnea* but differs in its yellowish brown pileus and gelatinized inner volva layer.

Type: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, Potaro base camp located at 5°18′04.8″N 59°54′40.4″W, 710–750 m, 1 km SW of base camp, on root mat in *Dicymbe corymbosa* monodominant forest, 29 Dec. 2016, *Henkel 10395* (BRG 41307, holotype; HSC G1235, isotype); GenBank accessions: ITS MK064190; nrLSU MK105497; *rpb2* MK092926; *ef1-a* MK092942.

Etymology: *fulvo*- (L. comp.) = yellowish brown; *alba*- (L. adj. A) = white, referring to the pileus and stipe colors, respectively.

Pileus 85–110 mm in broad, 11–22 mm tall, broadly convex to planate with low, broad umbo, yellowish brown (5C8) over disc, lighter concolorous (5B5) toward margin, tacky to moist, shiny and glabrous macroscopically, under hand lens finely appressed radially fibrillose, toward margin minutely rivulose; margin entire, bearing fugacious, fibrillose, irregular white appendiculae, these occasionally triangular, 1–5 mm long; volval remnants lacking; trama 0.5–2 mm thick at margin, 3 mm over lamellae, 6–8 mm over stipe, solid, white, unchanging. *Lamellae* finely adnexed to adnate, thin, subcrowded, off-white, unchanging; edges concolorous, under hand lens minutely eroded; lamellulae one, 1–40 mm long. *Stipe* 55–120 × 14–28 mm, sub-cylindrical, white, bruising light orange, longitudinally striate and floccose basally, downy-woolly centrally, finely pulverulent with short striations at apex; bulb 37–47 × 25–40 mm, subglobose to ellipsoid, subabrupt to abrupt, radicating slightly; trama white, solid, unchanging. *Volva* two layered; inner layer a white, erect to outcurved flange extending 2–3 mm above bulb apex; outer layer enclosing basal bulb, densely membranous, with 1–3 non-clasping limbs ascending 10–19 mm above bulb; exterior off-white, dry, glabrous; interior off-white, moist, shiny, glabrous. *Annulus* superior, descending with age to central, pendant, fugacious-membranous; exterior off-white, floccose; interior concolorous, striate, floccose. *Odor* mildly fruity-fungoid. *Taste* fungoid, indistinct.

Basidiospores white in medium deposit, [250/8/8] (6.0–)7.0–9.0(–11) × (5.0–)5.5–8.0 µm, Qr = (1.1–)1.14–1.43(–1.5), Qm = 1.29, subglobose to broadly ellipsoid, thin-walled, smooth, hyaline, opaque, amyloid; hilar appendix cylindric to conic, truncate, up to 1 mm long; contents granular-guttulate. *Basidia* 32–53.5 × 7.75–10 µm, clavate, 4-sterigmate; sterigmata 1.5–4.5 µm long; contents granular-guttulate. *Subhymenium* 18–35 µm thick, composed of 3–4 layers of globose to elliptical, irregularly polygonal elements, these 8–42 µm wide. *Marginal tissue of lamellae* sterile, composed of easily dislodged globose to pyriform elements, these 16–25 µm wide. *Lamellar trama* bilateral; mediostratum 20–45 µm wide, composed of interwoven, frequently branched uninflated hyphae with scattered ovoid to ellipsoid inflated elements, these 26–110 × 2–15 µm; lateral stratum diverging obtusely from the mediostratum, composed of branched uninflated hyphae with clavate to ovoid, inflated elements, these 26–106 × 7–28 µm. *Pileipellis* an ixtomixtocutis with two distinct layers; suprapellis 150–260 µm thick; hyphae 1.5–8 µm wide, strongly gelatinized, periclinal, hyaline, thin-walled, loosely interwoven; subpellis 170–340 µm thick; hyphae 1.5–4 µm wide, slightly gelatinized, mostly periclinal, hyaline, thin-walled, densely interwoven. *Pileus trama* with abundant acrophysalides, these 25–244 × 11–49 µm, cylindrical, clavate, ellipsoid, or ovoid, often with irregular swollen protrusions; contents frequently granular; uninflated tramal hyphae 2–14 µm wide, branching, often swollen at branch nodes and near acrophysalides; conductive hyphae rare to moderately abundant. *Stipe trama* with abundant acrophysalides, these 19–97(–223) × 9–44 µm, longitudinally oriented, cylindrical, clavate, ovoid, or ellipsoid; uninflated, non-conductive tramal hyphae 3–12 µm wide; conductive hyphae rare, up to 25 µm wide. *Volva at stipe base* composed of densely interwoven, uninflated, highly branched hyphae 2–10 µm wide, and abundant inflated cells, these 24–154 × 10–75 µm, limoniform, clavate, globose, or ellipsoid; contents often with granular clusters; inner layer partly gelatinized. *Partial veil* composed of spherical or rarely limoniform, ellipsoid, or subclavate elements, these 8–45 µm wide, with one or rarely two protruding lateral bulges that occasionally extend into short filaments; uninflated hyphae 2–8 µm wide, thin-walled, attached to inflated cells or in short, detached fragments; all elements diffuse granular or with granular clusters. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary or rarely in pairs on humic mat of forest floor under *D. corymbosa*; known only from the type locality in the Upper Potaro River Basin.

Additional specimens examined: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, Potaro base camp at 5°18′04.8″N 59°54′40.4″W, 710–750 m a.s.l., under *D. corymbosa*; vicinity of base camp, 7 May 2001, *Henkel 8056* (BRG 41308, HSC G1236); 3 km SE of base camp, 10 June 2002, *Henkel 8455* (BRG 41309, HSC G1237); 28 June 2004, *Henkel 8720* (BRG 41310, HSC G1238); 1.5 km SE of base camp, 30 June 2006, *Henkel 8863* (BRG 43311 , HSC G1239); 2 km SE of base camp, 13 July 2009, *Henkel 9043* (BRG 41312, HSC G1240); 1 km SW of base camp, 25 June 2016, *Aime 6290* (BRG 41313, PUL F24397); 28 Dec. 2016, *Henkel 10394* (BRG 41314, HSC G1241).

Commentary: *Amanita fulvoalba* is recognized in the field by its medium to large, solitary or paired basidiomata, glabrous yellowish brown pileus often bearing fugacious marginal appendiculae, white hymenophore, stipe, and veils, fugacious-membranous annulus, and robust, saccate-limbate volva. *Amanita fulvoalba* is best placed in sect. *Lepidella* due to its amyloid, subglobose to broadly ellipsoid basidiospores, basal bulb, limbate volva, pileal appendiculae, and lamellae that do not darken with desiccation (Corner & Bas 1962, Bas 1969). Several features of *A. fulvoalba*, however, suggest an affinity for sect. *Amidella*, including the saccate volva, friable partial veil, and marginal appendiculae. However, given the gelatinized inner volva layer of *A. fulvoalba*, its marginal appendiculae are likely remains of the friable partial veil, not of the universal veil as in members of sect. *Amidella*. Additionally, phylogenetic analysis shows that *A. fulvoalba* is nested within sect. *Lepidella* (Fig. 1).

Among the very few described *Amanita* species worldwide that resemble *A. fulvoalba*, the sympatric *A. aurantiobrunnea* is most similar in pileus color, its white stipe, delicate membranous annulus, saccate, two-layered volva, and similar basidiospore dimensions (Simmons *et al.* 2002). *Amanita aurantiobrunnea* can be separated from *A. fulvoalba* by its deeper orange (vs. yellowish brown) pileus and orange, friable (vs. gelatinized) inner volva layer.

The European *Amanita proxima* has a saccate volva, basal bulb, and similar basidioma dimensions as *A. fulvoalba.* However, the former has an ochraceous to reddish brown volva, whitish to ivory pileus, and a more persistent annulus than *A. fulvoalba*. *Amanita gayana*, a species known only from the description from Chile, loosely resembles *A. fulvoalba* in its orange pileus, limbate, membranous volva, and white stipe. The species is, however, much smaller than *A. fulvoalba*, with a pileus < 54 mm wide and a stipe < 13.5 mm tall, and has pale yellow as opposed to white lamellae (Tulloss & Yang 2018).

Amanita guyanensis Mighell & T.W. Henkel, ***sp. nov***. Mycobank MB 827396. Figs 6, 7.

Diagnosis: Similar to *A. brunnescens* but differs in its smaller, unclefted basal bulb.

Type: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, ~20 km east of Mt. Ayanganna, Tadang base camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, elevation 710–750 m; 0.3 km NE of base camp in *Dicymbe corymbosa* and *Dicymbe altsonii* co-dominant forest, 7 June 2013, *Henkel 9767* (BRG 41315, holotype; HSC, isotype); GenBank accessions: ITS MK064192; nrLSU MK105502; *rpb2* MK092929; *ef1-a* MK092948; b-*tubulin* MK092937.

Etymology: Guyana, and -*ensis* (L. adj. B), referring to the known distribution of the species across central Guyana.

Pileus 10–94 mm broad, 4–34 mm tall, broadly convex, with age upturned, dark grey-brown or greyish brown (6F2, 7F4) throughout or darker over disc; surface dry to subviscid, glabrous macroscopically, under hand lens minutely appressed radially fibrillose, finely felted over disc; marginal fibrils separating with age revealing pale brown ground; margin entire, splitting slightly with age; volval warts with uniform, occasionally concentric arrangement, flattened to pyramidal, 1–10 mm wide, up to 1 mm tall, light grey-brown (6E3–6F3) with lighter concolorous to nearly off-white (4A1–4A2) apices, detersile; trama 0.5 mm at margin, 2 mm over lamellae, 3 mm over stipe, solid, white, unchanging. *Lamellae* finely adnexed to subfree, thin, close to crowded, white, becoming greyish or slightly orange-tinted (5C3–5C4) with age; edges concolorous, finely roughened, unchanging or browning slightly with pressure; lamellulae 1–2, 2–5 mm long. *Stipe* 35–105 × 5–14 mm, equal to slightly tapering upward from basal bulb, flaring slightly at extreme apex to 8–22 mm; apical portion above annulus usually white, occasionally grey (6D3–6D4), subscabrous; lower portion with greyish brown (6F4) appressed fibrils over white ground, these more concentrated toward base; bulb 8–20 × 11–30 mm, subglobose, subabrupt and occasionally flattened at apex, angled slightly from stipe axis, greyish over apex, lower portion progressively lighter concolorous and finely tomentose; volva of light grey-brown matted fibrillose to floccose scales adhering to bulb apex; trama white, subsolid, unchanging. *Annulus* subsuperior, 20–30 mm below stipe apex, membranous, pendant; interior white to off-white; exterior whitish marginally, grey near stipe; extreme margin an eroded band of grey, fine, floccose scales. *Odor* minimal, fungoid. *Taste* slightly sweet, occasionally with bitter overtones.

Basidiospores white in medium deposit, [225/9/9] 5.0–9.0(–11.0) × (4.0–)5.0–9.0(–10.0) µm, Qr = (0.94–)1.0–1.27(–1.33), Qm = 1.09, subglobose to globose, thin-walled, smooth, hyaline, amyloid; hilar appendix subpyramidal, truncate, up to 1 mm long; contents usually one dark brown guttule, rarely of smaller guttules or granules. *Basidia* 22–38 × 5.5–13 µm, clavate, 4-sterigmate; contents granular or guttulate, sometimes both; sterigmata 2–5 µm long, lanceolate to slightly incurved. *Subhymenium* 16–30 µm thick, composed of 3–4 layers of subglobose to irregularly polygonal elements, these 3–19 µm wide. *Marginal tissue of lamellae* partially gelatinized, composed of easily dislodged, mostly globose, occasionally ellipsoid, fusiform or clavate elements, these 9–65 × 8–22 µm. *Lamellar trama* bilateral; mediostratum 20–40 µm wide, of interwoven, branching hyphae; inflated elements up to 17 µm wide, cylindrical or clavate; lateral stratum contiguous with mediostratum and diverging at an obtuse angle; hyphae 4–19 µm wide; conductive hyphae rare. *Volval pileal warts* composed of inflated, brownish globose cells often with a small, protruding filament. *Pileipellis* an ixtomixtocutis, two-layered with indistinct boundary; suprapellis 140–240 µm thick, hyphae heavily gelatinized, thin-walled, densely interwoven; subpellis 140–250 µm thick, hyphae brownish, densely interwoven, periclinal. *Pileus trama* with frequent acrophysalides, these cylindric, fusiform, ellipsoid, or ovoid, 22–190 × 8–50 µm; uninflated tramal hyphae densely interwoven, highly branching, 2–6 µm; conductive hyphae not observed; contents of most elements of small (≤ 1 µm), globose, hyaline guttules and/or opaque granules, often in clusters. *Stipe trama* longitudinally oriented, with clavate or occasionally subglobose to fusiform acrophysalides, these 32–315 × 17–58 µm; uninflated hyphae highly branched, 2–6 µm wide; conductive hyphae not observed. *Volva at stipe base* composed mostly of inflated, globose or rarely ellipsoid to limoniform elements, 22–49 µm wide; filamentous hyphae sparse, present as branched fragments or attached to inflated elements. *Partial veil* composed of densely interwoven, branching, highly serpentine uninflated hyphae 1–7 µm wide, most with opaque granular contents. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary to scattered on humic mat of forest floor under *D. corymbosa*; also found in stands containing *D. corymbosa* and *D. altsonii* or

Pakaraimea dipterocarpaceae and *Dicymbe jenmanii* on a variety of soil types*;* known from the type locality in the Upper Potaro River Basin, ~25 km to the west in the Upper Mazaruni Basin, and ~100 km east in the Mabura Ecological Reserve.

Additional specimens examined: **Guyana**, REGION 8 POTARO-SIPARUNI: Pakaraima Mountains, Upper Potaro River Basin, ~15 km east of Mt. Ayanganna, Potaro base camp at 5°18′04.8″N 59°54′40.4″W, 710–750 m a.s.l., under *D. corymbosa*; 4 km SW of base camp, 5 May 2001, *Henkel 8034* (BRG 41316, HSC G1244); 3 km SE of base camp, 11 May 2001, *Henkel 8083* (BRG 41317, HSC G1245); 28 June 2004, *Henkel 8712* (BRG 41318, HSC G1246); 0.4 km SW of base camp near Blackwater Creek, 1 July 2006, *Aime 3155* (BRG 41319, PUL F24398); vicinity of base camp, 11 July 2008, *Henkel 8931* (BRG 41320, HSC G1243); 4 km SW of base camp, 20 May 2010, *Aime 3991* (BRG 41321, PUL F24399); 0.5 km E of base camp, 15 June 2015, *Henkel 10081* (BRG 41322, HSC G1247). ~20 km east of Mt. Ayanganna, Tadang Base Camp 2 km south of Potaro River at 5°16′14.5″N 59°50′39.1″W, 710–750 m a.s.l., under *D. corymbosa* and *D. altsonii*; 2 km SW of base camp, 8 June 2013, *Henkel 9772* (BRG 41323, HSC G1248). REGION 10 UPPER DEMERARA-BERBICE: Mabura Ecological Reserve, field station at 5°09′19.0″N 58°41′58.9″W, ~100 m a.s.l.; 0.4 km WNW of field station in *Dicymbe altsonii* plot #1, 1 June 2011, *Henkel 9624* (BRG 41324, HSC G1249). REGION 7 CUYUNI-MAZARUNI: Pakaraima Mountains. Mazaruni River Basin, ~20 km NW of Mt. Ayanganna summit, base camp located at 5°26′21.3″N 60°04′43.1″W, 760 m a.s.l., in savanna fringing forest dominated by *P. dipterocarpaceae* and *D. jenmanii*; ~0.4 km NW of base camp, 28 Dec. 2010, *Henkel 9563* (BRG 41325, HSC G1250); 0.2 km ESE of base camp, 4 June 2012, *Henkel 9674* (BRG 41326, HSC G1251).

Commentary: *Amanita guyanensis* is recognized in the field by its dark grey-brown pileus bearing off-white to greyish volval patches, white stipe, subglobose, angled basal bulb with a apical ring of grey floccose scales, and white, pendant annulus with a grey margin. *Amanita guyanensis* is best placed in sect. *Validae* due its amyloid, subglobose to globose basidiospores, deeply pigmented pileus, pulverulent volva, and the absence of marginal appendiculae (Corner & Bas 1962, Bas 1969, Cui *et al.* 2018). Additionally, phylogenetic analysis shows that *A. guyanensis* is allied with other species in a monophyletic sect. *Validae*, including the type species of the section, *A. excelsa* (Fig. 1).

Wordwide there are numerous species of sect. *Validae* which resemble *A. guyanensis* in stature and color. In the Neotropics, *A. campinaranae* from the central Amazon closely resembles *A. guyanensis* in stature, pigmentation, and velar characteristics. However, *A. campinaranae* differs from *A. guyanensis* in its pallid, white to greyish pileus, forked lamellae, and smaller basidiospores (5.6–6.7 × 5.5–6.5 µm vs. 5–9 × 5–9 µm) (Bas 1978). The sympatric and loosely similar *A. perphaea* can be separated by its more fragile partial veil, sulcate-striate pileus margin, distinct encrusting pigments in the pileipellis, and overall darker grey color of the pileus, volva, and stipe (Simmons *et al.* 2002).

Among tropical African *Amanita* species *A. echinulata* is similar to *A. guyanensis* in stature and both may have a bitter taste, but *A. echinulata* can be separated by its grey annulus, dark sooty brown pileus and stipe, and smaller basidiospores (Beeli 1935; Mighell & Henkel pers. obs.). An as yet undescribed species (*Amanita* morphospecies #19) collected in Cameroon's Dja Biosphere Reserve is macroscopically very similar to *A. guyanensis* (Mighell & Henkel unpub. data). This species exhibits similar basidioma size ranges, pigmentation, and velar characteristics as those of *A. guyanensis*, but differs by its less defined volval remnants on the stipe and odor of raw potato.

Amanita innatifibrilla from subtropical China has a dark grey-brown pileus bearing concolorous to grey warts and floccose volval remnants at its bulb apex, but differs from *A. guyanensis* in its smaller stature, innate pileal fibrils, regularly central annulus, fusiform bulb, and smaller basidiospores (Cui *et al.* 2018). Species from Singapore described by Corner & Bas (1962) include *A. squamosa* which resembles *A. guyanensis* in its sepia-fuscous pileus, pale brownish warts, and white annulus, but differs in its spindle-shaped bulb with recurved scales and smaller basidiospores. *Amanita tristis* is similar in basidioma size, colors, and volval remnants to *A. guyanensis*, but its ellipsoid basidiospores are smaller (4.9–6.1 × 4.3–4.6 µm vs. 5–9 × 5–9 µm) than the subglobose to globose spores of *A. guyanensis*.

Among north temperate species similar to *A. guyanensis*, *A. brunnescens* and *A. sepiacea* each have similarly sized basidiomata, a dark grey to brown pileus, white, superior annulus, basal bulb with apically adhering volval remnants, and basidiospores of similar size. *Amanita brunnescens* is separated by its large, marginate, clefted basal bulb, as opposed to the smaller, unclefted bulb of *A. guyanensis*. *Amanita sepiacea* has grey squamules on the stipe base, which contrast with the longitudinal fibrils of *A. guyanensis*

(Tulloss & Yang 2018). *Amanita porphyria* and *A. submaculata* loosely resemble *A. guyanensis*, but the former is differentiated by its violaceous pileus and volva, and the latter by its reddening of bruised lamella and exposed stipe trama.

Finally, *A. xanthomargaros, A. pausiaca*, *A. walpolei*, *A. luteolovelata,* and *A. luteofusca* all have a grey to brown pileus and similar basidioma size but can be differentiated from *A. guyanensis* by their yellow pigments in either the universal veil, annulus, or stipe tissues.

CHAPTER 2: *AMANITA* IN THE GUINEO-CONGOLIAN RAINFOREST: NEW SPECIES AND EPITYPES FROM THE DJA BIOSPHERE RESERVE

Formatted for publication in Mycologia, submitted May 2020

Abstract

Four epitypes and three new species of *Amanita* are described from Cameroon. *Amanita echinulata*, *A. fulvopulverulenta, A. robusta,* and *A. bingensis* are epitypified based on multiple recently collected specimens. Morphological features of the epitypes are described and enumerated to update their heretofore poorly detailed species profiles. *Amanita minima* sp. nov., *Amanita luteolamellata* sp. nov. and *A. goossensfontanae* sp. nov. are added to the known mycota of tropical Africa. Habit, habitat, and known distribution are provided for each species. Sequence data for the ITS locus is provided for all taxa, and a phylogenetic analysis of nuc 28S rDNA across the genus *Amanita* corroborates morphology-based infrageneric placement for each.

Introduction

The ectomycorrhizal (ECM) habit has evolved in several plant families across myriad habitat types worldwide (Tedersoo et al. 2012). Temperate latitudes feature continental-scale forests of gymnosperm and/or angiosperm ECM host trees, whereas tropical ectotrophic forests usually occur as disjunct mono- or co-dominant stands of angiosperm hosts within larger matrices of non-ECM plants (Corrales, Henkel and Smith 2018). This disparity may partially explain why ECM fungi have been hypothesized to be more diverse at temperate latitudes (Tedersoo et al. 2014). Recent studies have found that ECM fungal diversity within spatially isolated stands of host trees in both the Paleo- and Neotropics rivals that of the most diverse temperate forests, and includes many endemic and undescribed species (e.g. Verbeken and Buyck 2002; Smith et al. 2011; Brearley 2012; Henkel et al. 2012; Smith et al. 2013). African tropical forests harbor the greatest diversity of ECM host plants and remain mycologically underexplored (Corrales et al. 2018).

Amanita Pers. is one of the most speciose and widespread agaric genera in tropical ectotrophic forests (Verbeken and Buyck 2002; Brearley 2012; Corrales et al. 2018). However, only around 60 of the estimated ~1000 *Amanita* species are currently known from the Afro-tropics (Tulloss 2005; Thongbai et al. 2016). From 1919 to 1953 Mme. Martha Goossens-Fontana collected, described, and produced watercolors of macrofungi, including *Amanita*, from Guineo-Congolian rainforests dominated by the ECM tree *Gilbertiodendron dewevrei* (De Wild.) J. Léonard (Fabaceae subfam. Detarioideae). Her collections and illustrations were sent to Maurice Beeli, scientific collaborator at the Botanical Garden of the State at Brussels, who used this material to initiate the iconographic flora of Congo series that became the Illustrated Flora of Mushrooms of Central Africa (Fraiture 2006). Subsequently Beeli (1927, 1931, 1935) described 29 *Amanita* species from the Guineo-Congolian rainforest. Gilbert (1940, 1941) described six more Afrotropical species and re-assessed some of the species erected by Beeli. Many of these were, however, based on the single type collection with minimal morphological description and no molecular data. Since the mid-20th century new African *Amanita* species have been described from habitats outside of the Guineo-Congolian rainforest (Pegler and Piearce 1980; Bas 1982; Westhuizen and Eicker 1987; Thoen and Ba 1989; Reid and Eicker 1991; Eicker et al. 1993; Harkonen and Mwasumbi 1994; Walleyn 1996; Pegler and Shah-Smith 1997; Walleyn and Verbeken 1998; Tang et al. 2015).

Collecting expeditions from 2014–2019 to the Dja Biosphere Reserve (DBR) in southern Cameroon have yielded an extraordinary diversity of *Amanita*, with ~ 70 morphospecies occurring in a small area of forest dominated by *G. dewevrei* (Henkel, unpublished). This work constitutes our first effort to document the Cameroonian *Amanita* mycota. Here we epitypify *A. echinulata* Beeli, *A. fulvopulverulenta* Beeli, *A. robusta* Beeli, and *A. bingensis* (Beeli) R. Heim, previously known only from the Congolian type collections. This work resolves sectional placement and morphological uncertainties of these species that have lingered since their original treatment nearly 100 years ago. Additionally, we describe *A. minima, A. luteolamellata* and *A. goossensfontanae* as new to science**.** Complete morphological profiles, illustrations, and molecular data are provided for each species. Over the past 6 y each of these species was repeatedly encountered in the DBR's *G. dewevrei* forests.

Materials and Methods

Collections and morphological analyses

Collections were made annually in Cameroon during the Aug–Sep early rainy seasons of 2014, 2017, 2018, and 2019, and the Nov–Dec late rainy season of 2016 from the Dja Biosphere Reserve, Northwest Sector, near the village of Somalomo, Upper Dja

River Basin, within a five km radius of a base camp at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m. Basidiomata were collected from lowland forests dominated by ECM *G. dewevrei* and other stands containing ECM *Uapaca* (Phyllanthaceae) species (Corrales, Henkel, and Smith 2018). Colors were described subjectively and coded according to Kornerup and Wanscher (1978), with color plates noted in parentheses. Fresh collections were field-dried with silica gel.

Micromorphological features were assessed using an Olympus BX51 microscope with bright field and phase contrast optics. Rehydrated fungal tissues were mounted in water, 3% potassium hydroxide (KOH), and Melzer's solution. Twenty-five basidiospores were measured from each collection of each species, including the types and epitypes. Twenty basidia and hyphal elements of the subhymenium, hymenophoral, pileus, and stipe tramas, pileipellis, and universal veil were measured from each type specimen, and 10 from each additional specimen examined. Length/width Q values for basidiospores are reported as Qr (range of Q values across all basidiospores measured) and Qm (mean of Q values). The notation "[a/b/c]" preceding sets of basidiospore data denotes "'a' basidiospores from 'b' basidiomata from 'c' collections." Outlying measurements observed in less than 5% of a given structure are placed in parentheses. Epitype and type descriptions include ranges observed across all conspecific collections examined. Line drawings are freehand composites of microscopic observations. Type specimens of *A. fulvopulverulenta* and *A. robusta* were obtained from Meise Botanic Garden Herbarium (BR), Belgium, and analyzed as above. The types for *A. echinulata* and *A. bingensis* were not found at BR. Holotypes and duplicate specimens were deposited YA, Cameroon National Herbarium; isotypes and other duplicates were deposited in HSC, Humboldt State University, BR, and PUL, Purdue University.

DNA extraction, amplification, sequencing and phylogenetic analyses

DNA was extracted from dried basidioma tissue of types and additional specimens using the Wizard® Genomic DNA Purification kit (Promega Co., WI, USA). PCR reactions included 12.5 µL of Promega PCR Mastermix (Promega Co., WI, USA), 1.25 µL of each primer (at 10 µM) and approximately 50 ng of DNA. The final PCR reaction volume was 25 µL. The recommended cycling conditions for each primer pair were followed. Uncleaned PCR products were sequenced by GeneWiz® (South Plainfield, NJ, USA). Sequences were edited using Sequencher 5.2.3 software (Gene Codes Corporation, MI, USA) and deposited in GenBank.

To corroborate morphology-based conspecificity among specimens of the individual species, PCR on the nuc rDNA ITS1-5.8S-ITS2 (ITS barcode) was performed, using the primer pair ITS1F/ITS4B (Gardes and Bruns 1993). The resulting sequences from a morphospecies were blasted against one another other to verify that they share at least 97% nucleotide identity.

To determine the sectional placement of each of our target taxa using a phylogenetic approach, PCR was performed on one or several representative specimens of each target taxon at the nuc 28S rDNA D1-D2 domains (28S) using the primer pair LROR/LR6 (Vilgalys and Hester 1990; Moncalvo et al. 2000). Next, we compiled a dataset of 104 nrLSU sequences inclusive of our target taxa, other *Amanita* species from Cameroon and representative species from all seven sections across the subgenera *Amanita* and *Lepidella*. *Amanita subcaligata*, *A. nauseosa*, *Limacella illinita*, *L. glischra*, *Pluteus cervinus* and *P. petasatus* served as outgroup taxa fide Wolfe et al. (2012). Sequences were aligned in MAFFT v7 (Katoh and Standley 2013), with refinements to the alignment done manually. The phylogeny was reconstructed using maximum likelihood (ML). The GTR + GAMMA + I model of molecular evolution was used as determined by PartitionFinder 2 (Lanfear et al. 2017). Maximum likelihood bootstrap analysis for phylogeny and assessment of the branch support by bootstrap percentages (% BS) was performed using RAxML v8 (Stamatakis 2014). One thousand bootstrap replicates were produced. The final alignment and phylogeny can be accessed in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S26277).

Results

Fifteen ITS (GenBank accessions: MT446250–MT446264) and 33 28S (GenBank accessions: MT446265–MT446297) were generated in this study, ranging from 627–1021 and 824–1075 bp, respectively. ITS sequences from conspecific collections differed by no more than two percent nucleotide identity. The length of the alignment after the ends were trimmed was 992 bp.

Each of the four sections in *Amanita* subgen. *Lepidella* were resolved as monophyletic with strong statistical support: *Amidella* (99%), *Lepidella* (77%), *Phalloideae* (98%) and *Validae* (98%) (Fig. 8). *Amanita echinulata* was resolved to section *Validae*, while *A. fulvopulverulenta* and *A. goossensfontanae* were resolved to section *Lepidella*. *Amanita* subgen. *Amanita* was resolved as monophyletic with strong statistical support (98% BS), along with each of the three sections in subgen. *Amanita*: *Amanita* (95% BS), *Caesareae* (100% BS) and *Vaginatae* (100%). *Amanita bingensis*, *A. minima* and *A. robusta* were all resolved to sect. *Amanita*, while *A. luteolamellata* was resolved to sect. *Vaginatae*. Blastn searches of these ITS sequences yielded no hits greater than 90.5% nucleotide identity, indicating a lack of conspecificity of all species treated here with other taxa accessioned on GenBank.

Taxonomy

Amanita echinulata Beeli, *Bull. Soc. Roy. Bot. Belg.* **59**: 102 (1927) FIGS. 9, 10

MycoBank MBT392027

*Original diagnosis***:** "*Pileo tenui, e convexo plano, brunneo-fuliginoso, verrucis pyramidalis atris tecto,* 5–7 *cm. lato; stipite basi bulboso, solido, nudo, brunneo,* 9 × 0.5–0.9 *cm; annulo supero, membranaceo, albido demum fuigineo; volva leniter distincta; lamellis subliberis, albidis; sporis hyalinis globulosis, levis,* 4—5.5 µ; *carne alba.*"

Holotype: DEMOCRATIC REPUBLIC OF THE CONGO. ÉQUATEUR PROVINCE: Eala, isolated or grouped on the ground in dry forests, June-July 1923, *Goossens-Fontana 214* (BR5020030948054)

Iconography: Beeli (1927, Pl. 1, Fig 3; 1935 Pl. II, Fig. 9)

Description (Beeli 1927, 1935): (freely translated from French) "*Pileus* fleshy, plano-convex, margin smooth, sooty brown, with dark pyramidal warts, 50–70 mm diameter. *Stipe* cylindrical, straight, with bulbous base, glabrous, concolorous with pileus, 90 × 5–9 mm; *annulus* superior, membranous, thin, pendant, sooty gray; *volva* ephemeral. *Lamellae* free, tapered at both ends, white, 6 mm tall. *Trama* firm, white; taste bitter. *Basidiospores* hyaline, globose, smooth, 4–4.5 µm, amyloid. *Spore print* white. *Pileipellis* hyphae filamentous, interwoven, with numerous erect filaments of darker homogenous content and others with vesicular endings.

Epitypification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8"

N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on humic mat, 28 Sep 2017, *Mighell* 87(**epitypus hic designatus** YA; isoepitypes HSC G1295, BR), GenBank accessions: ITS = MT446259; 28S = MT446291.

Description of the epitype: *Pileus* 20–44 mm broad, 5–17 mm tall, convex to planate, dry, appressed radially fibrillose, grayish brown (7F5–8F5); surface fibrils medium brown (6F8) over off-white ground, occasionally radiating outwards from velar warts, becoming slightly rivulose near margin; margin entire to crenulate, occasionally slightly splitting; trama 0.5 mm thick at margin, 1 mm over lamellae, 3–5 mm at stipe, solid, white. *Volva on pileus* of warts, these 0.5–2 mm tall, 0.5–1.5 mm wide, detersile, conic to pyramidal, diminishing toward margin, usually concentrically arranged and evenly spaced over entire pileus, 1–5 mm apart, occasionally more densely arranged toward margin, dark gray-brown (5F5) with white apices. *Lamellae* finely adnexed to minutely notched, thin, crowded, white to off-white, discoloring reddish brown around mycophagy channels; edges white to gray, minutely roughened; lamellulae 1–4, 0.5–28 mm long, attenuate to subtruncate. *Stipe* 40–54 × 3–6 mm, cylindric, equal or tapering slightly upwards, flaring basally into bulb, with minute appressed to erect floccose fibrils over entire surface, these dark gray-brown (5D3–5D4), darkening downwards, over lighter concolorous ground; fibrils forming slightly larger tufts above bulb; bulb globose, subabrupt, rarely subclavate, 7–12 × 9–12 mm, usually oblique, occasionally rounded-acuminate at base, usually fused with dense white hyphal tomentum descending into substratum; trama solid, fibrous, white, occasionally light gray within bulb, unchanging. *Volva at stipe base* friable, inconspicuous, of concentric rings of minute, dark brown (5F6–5F7) squamules, these roughly rectangular, flocculent, occasionally extending over apical bulb. *Partial veil* a friable membranous annulus often retained only in fragments, superior; exterior white to light gray and striate; interior dark gray (6E2), flocculent. *Odor* mild, indistinct to faintly of plastic. *Taste* none.

Basidiospores [125/7/5] (5–)5.5–7(–9) × (4–)4.5–6(–7) µm, Qr = (1–)1.2–1.45(–1.5) Qm = 1.26, inamyloid, subglobose to broadly ellipsoid, slightly flattened adaxially, smooth, hyaline, thin-walled; hilar appendix sublateral, up to 1 µm long; contents uni- to multiguttulate, often with granules. *Basidia* 19–34 × 6.5–9 µm, clavate, (3–)4-sterigmate; contents not evident or granular to multiguttulate; sterigmata 2–5 µm long. *Subhymenium* 23–45 µm thick, of angular spheroid elements, these 8–15 µm wide. *Marginal tissue of lamellae* sterile, of globose, ellipsoid, reniform, or limoniform, elements 12–30 µm wide. *Lamellar trama* bilateral; mediostratum 20–60 µm wide, of branched, interwoven, occasionally inflated hyphae, with moderately abundant refractive hyphal fragments, these tuberculate, irregularly branching, absent in some collections; lateral stratum 20–78 µm wide, diverging obtusely from mediostratum, of mostly allantoid inflated elements, these 16–55 µm × 8–22 µm. *Pileipellis* single-layered, 205–320 µm thick; elements interwoven and mostly erect; uninflated hyphae few, 2–6 µm wide; inflated elements dominant, cylindric, ellipsoid, or rarely ovoid or fusiform, 38–204 × 8–35 µm, ~half of these with dark brown pigments in walls; pigmented cells nearly always terminal; refractive hyphae abundant, irregularly serpentine with protruding lobes. *Pileus trama* heavily acrophysalidic in matrix of abundant interwoven uninflated hyphae; acrophysalides ovoid to ellipsoid, 55–250 × 25–70 µm; uninflated hyphae non-refractive, 2–6 µm wide. *Volva on pileus* as many layers of cells, these mostly globose, occasionally ovoid to ellipsoid, 10–60 µm wide, occasionally in parallel, erect chains of increasing size; pigments brown, cytoplasmic. *Stipe trama* of longitudinally oriented acrophysalides 10–45 µm wide interspersed with interweaving uninflated and refractive hyphae, these 2–6 µm wide and occasionally with swollen apices; stipitipellis cells with brown pigments. *Volva at stipe base* same as on pileus. *Partial veil* of globose, ellipsoid, or limoniform terminal elements 18–100 × 10–40 µm, these occasionally in short chains, most with light brown pigments, and uninflated, mostly non-refractive hyphae 2–10 µm wide. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary to scattered on humic mat or rarely on heavily decayed wood in forests dominated by *G. dewevrei.* Known from the Democratic Republic of Congo (DRC) type locality near Binga and in the vicinity of Eala, and from the epitype locality in the Dja Biosphere Reserve, Cameroon. Basidiomata were infrequent in a 5 y *G. dewevrei* plot study, occurring in 1.3% of 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished)

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *Gilbertiodendron dewevrei* forests, on humic mat, 1 Sep 2014, *Henkel 9920* (YA; HSC G1296), GenBank accession: ITS = MT446256; 13 Nov 2016, *Henkel 10253* (YA; HSC G1297), GenBank accession: ITS = MT446257; 9 Sep 2017, *Mighell 36* (YA; HSC G1298), GenBank accession: 28S = MT446290; 17 Sep 2018, *Mighell 141* (YA; HSC G1299), GenBank accessions: ITS = MT446255.

Commentary: Amanita echinulata was originally described by Beeli (1927) from a single Goossens-Fontana collection made in 1923. It is characterized by small to medium-sized basidiomata, a dark brown pileus adorned with darker, pyramidal warts, a concolorous stipe with a membranous annulus, and minimal veil remnants at the stipe base. Notable from the watercolor plates in Beeli (1927) is the presence of a slightly oblique basal bulb (Fig. 9). The epitype specimen selected here perfectly agrees with the original description and plates of *A. echinulata*. It also agrees microscopically with the protologue description in its small basidiospores and interwoven pileipellis hyphae with inflated, occasionally darkly pigmented terminal cells and abundant refractive hyphae

The type specimen was re-examined by Gilbert (1940) and placed in the now illegitimate genus *Aspidella*. Bas (1969) rejected this placement, asserting that *A. echinulata* belongs in *Amanita* sect. *Validae* due to its amyloid basidiospores, non-appendiculate pileus, deeply pigmented pileipellis, membranous annulus, and friable volva barely retained at the stipe base. This sectional determination was supported by observations of our new collections, as well as phylogenetic analysis (Fig. 8). Bas (1969) also examined the type, recording basidiospores of 5.5–6.5 × 4.5–5.5 µm, contrasting with Beeli's (1927) measurement of 7–8 µm (presumably referring to length, but not specified) and Gilbert's (1940) measurement of 4–4.5 µm diameter. The measurements given by Bas represent the best dataset known until recently and are taken here to be the most accurate measurements of the type basidiospores. While we were not able to examine the type of *A. echinulata* (unavailable at BR), the basidiospores of the epitype match Bas' measurements well, although the upper size ranges are slightly higher (7 µm long, and 6 µm wide, respectively).

Amanita echinulata resembles the poorly known Central African *A. fuliginosa* (Beeli 1927). Beeli initially (1931: 107), and then later Gilbert (1940) suggested that the two species concepts should be united, though Gilbert acknowledged that *A. fuliginosa* can be differentiated by the absence of velar tissue on the stipe, flattened pileus warts, striate pileus margin, and larger basidiospores. Bas (1969) rejected this conspecificity, noting that the volval remnants in *A. fuliginosa* consist of abundant hyphae bearing terminal inflated cells, contrasting with the parallel-erect rows of inflated cells in *A. echinulata*.

Amanita echinulata resembles the Guyanese *Amanita perphaea* Simmons, Henkel and Bas but differs in its smaller basidiomata, brown pigments, gray partial veil, and scattered (as opposed to solitary) fruiting habit (Simmons, Henkel and Bas 2002).

The recently described *A. ahmadii* Jabeen, Ahmad, Kiran, Khan and Khalid from Pakistan resembles *A. echinulata* in its small to medium basidiomata, gray-brown pigments, regularly radially arranged, dark pileal warts, grayish annulus, bulbous base, and globose to ellipsoid basidiospores (Jabeen et al. 2019). The flat pileal warts, lighter stipe pigments, absence of velar remnants on the stipe, and larger basidiospores (7–8.5 × 6.5–7.5 vs. 5.5–7 × 4.5–6 µm) distinguish *A. ahmadii* from *A. echinulata*.

Amanita fritillaria (Berk.) Sacc. is known from India, China, and Singapore and resembles *A. echinulata* in its gray-brown, innately fibrillose pileus with dark gray warts, grayish annulus, and apically verrucose bulbous base (Saccardo 1891). *Amanita fritillaria* can be distinguished from *A. echinulata* by its larger basidiomata and larger, more ellipsoid basidiospores (7.0–9.0 × 5.5–7.0 vs. 5.5–7 × 4.5–6 µm).

Amanita echinulata is listed by Kamalebo et al. (2018) as an edible mushroom of relatively little cultural importance to villagers near the Yangambi Man-and-Biosphere Reserve, DRC.

Amanita fulvopulverulenta Beeli, *Bull. Soc. Roy. Bot. Belg.* **63**: 104 (1931) FIGS. 11, 12
MycoBank MBT392028

Original diagnosis: “*Pileo carnoso, convexo-plano, carneo brunneo-rubro pulverulento, margine striato,* 9–10 *cm lato; stipite cylindrico solido, fibroso, bulboso, glabro, albo vel rubiginoso,* 12–17 × 1–2 *cm; annulo tenui, membranaceo, albo; volva tenui, membranacea, subpermanens, brunneo-rubra; lamellis liberis sublutescentibus, sporis ellipsoideis, hyalinis, levis,* 7–8 × 4 µm; *carne fibr oso-carnoso, albida, in stipite rosea; sapore amara; odore acris.*”

Holotype: DEMOCRATIC REPUBLIC OF THE CONGO. MONGALA PROVINCE: Binga, scattered on the ground in dry forest, 1928, *Goossens-Fontana 775* (BR5020030954116).

Iconography: Beeli (1931, Pl. VII, Fig. 3; 1935 Pl. III, Fig. 1)

Description (Beeli 1931, 1935)*:* (freely translated from French) "*Pileus* fleshy, broadly convex, with short umbo, pale ground, covered with a reddish brown pulverulence, 90–100 mm diameter; margin substriate. *Stipe* cylindrical with slightly bulbous base, fibrous, stuffed, glabrous, white or with light reddish tint, 120–170 × 10–20 mm, easily detachable from pileus*; annulus* membranous, white, pendant; *volva* membranous, thin, brown-red. *Lamellae* free, subrounded near stipe, slightly yellowish, 8–9 mm tall. *Trama* fleshy fibrous, white, pinkish in stipe; *taste* bitter*; odor* strongly acrid. *Basidiospores* hyaline, ellipsoid, smooth, 7–8 × 4 µm, *Spore print* white. *Pileipellis* hyphae filamentous, ending in vesicular cells.

Epitypification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forests, on upper mineral soil, 26 Aug 2014, *Henkel 9887* (**epitypus hic designatus** YA; isoepitypes HSC G1300, BR), GenBank accessions: ITS = MT446250; 28S = MT446285.

Description of the epitype: *Pileus* 45–75 mm broad, 10–34 mm tall, broadly convex with decurved to evenly uplifted margin, dry to moist; ground glabrous, pale tan-brown (4A3–4B3) with pinkish tint to brick red (8D8); margin entire, bruising dark brick red, appendiculate; appendiculae 1–5 mm long, red-brown (7C5), occasionally white-tipped, in pendant palisade, occasionally coalescing into triangular or rectangular flaps, these 3–8 mm wide; trama 0.5 mm thick at margin, 4.5 mm over lamellae, 5 mm at stipe, solid, pale pinkish white to red. *Volva on pileus* pulverulent, detersile, in light brown (5B3–5C3 KW) to dark orange-brown (5E8–6E8 KW) patches over ground, these 0.5–2 mm wide, rarely erect and blunt over disc, becoming nearly contiguous and pulvurulent near margin. *Lamellae* adnexed, thin to subthin, crowded, pale yellow (4A2), occasionally with pinkish red (9B8–9C8) spots, darkening with dessication; edges concolorous or white, minutely roughened; lamellulae 1–31 mm long, attenuate. *Stipe* 66–210 × 6.5–10 mm, cylindric, equal above basal bulb, off-white to occasionally pinkish (7A2–8A2) over apical ½, pink (8A5) to brick red (8C8–8D8) basally, longitudinally appressed fibrillose; vestiture more dense and floccose basally, bruising medium brick red (8C7); bulb 35–60 × 15–25 mm, subglobose to ovoid to fusiform, subradicate with a rounded tip, yellowish brown, occasionally with subconcentric rings of recurved floccose squamules apically, these deep pink-red (9B7), glabrous and subtended by hyphal cords at base; trama solid, fibrous, pale yellow toward apex, pink-red (9C6) in basal half and bulb, staining dark red (9C8–10C8) around larval channels. *Volva at stipe base* arising from bulb apex as a loosely attached membranous band, this adhering to one side or circumscissile, extending 7–15 mm above bulb apex, occasionally attached to stipe above bulb or rarely appressed to bulb; exterior pale orangish cream, fibrillose; interior light orange-brown (6B6), pulverulent. *Partial veil* a subsuperior or superior, fugacious, membranous annulus clasping at stipe apex and flaring below, 10–20 mm long; exterior striate, pulverulent, white to pinkish gray (9A2–9B2); interior white with concentric rings of flocculent squamules, these becoming pale orange-brown (6B4) near margin; margin serrulate. *Odor* strongly of chlorine, nauseating. *Taste* of chlorine, unpleasant, lingering.

Basidiospores [250/13/10] 6–8(–9) × (3–)3.5–5 µm, Qr = 1.4–2.29, Qm = 1.78, amyloid, ellipsoid to subovoid, pronouncedly flattened adaxially, smooth, hyaline, thin-walled; hilar appendix 1 µm long. *Basidia* 27–42 × 6–9 µm, clavate, (3–)4-sterigmate; sterigmata 3–4.5(–5.5) µm long. *Subhymenium* 17–35 µm thick, of roughly spherical to ellipsoid, rounded-angular elements, these 5–20 µm wide, occasionally with pyriform cells giving rise to basidia. *Marginal tissue of lamellae* sterile, with tufts of globose, clavate or pyriform elements, these 10–25 × 8–20 µm. *Lamellar trama* bilateral; mediostratum 14–29 µm wide, of mostly ellipsoid, occasionally subfusiform or subglobose elements, these 25–130 × 8–39 µm, interspersed with highly branched uninflated hyphae; lateral stratum diverging obtusely, elements in chains, mostly ellipsoid, occasionally subclavate or subglobose, 31–145 × 8–36 µm; refractive hyphae rare in medio- and lateral stratum. *Pileipellis* a single-layered mixtocutis 175–300 µm thick, of interwoven uninflated hyphae, these 2–8 µm wide; terminal cells with light brown pigment. *Pileus trama* with abundant acrophysalides, these ellipsoid, rarely ovoid or subfusiform, 34–210 × 12–45 µm; uninflated hyphae 2–5 µm wide; refractive hyphae 3–11 µm wide. *Volva on pileus* of frequently branched, interwoven uninflated hyphae, these 2–7 µm wide with globose, ovoid or ellipsoid terminal elements often in chains of increasing size, with brown pigments, 12–65 × 10–42 µm; refractive hyphae frequent, serpentine with irregular protrusions, 3–15 µm wide. *Stipe trama* longitudinally acrophysalidic; acrophysalides elongate-clavate, 15–30 µm wide; uninflated hyphae highly branched, up to 7 µm wide; refractive hyphae abundant, serpentine or rarely linear, with irregular protrusions, 5–10 µm wide. *Volva at stipe base* of globose to ellipsoid or narrowly clavate inflated elements, these 24–75 × 20–48 µm, uninflated hyphae 3–13 µm wide, and few to abundant fragmented refractive hyphae up to 5 µm wide. *Partial veil* of interwoven, highly branched, uninflated hyphae, these 2–5 µm wide, with abundant globose, ovoid, clavate, or ellipsoid terminal elements, these 15–81 × 9–23 µm; refractive hyphae common, often bundled, serpentine, 2–12 µm wide, with irregular protrusions. *Clamp connections* infrequent on hyphae of lamellar trama.

Habit, habitat, and distribution: Solitary to scattered on upper mineral soil in forests dominated by *G. dewevrei*. Known from the type locality near Binga, DRC, and from the epitype locality in the Dja Biosphere Reserve, Cameroon. Basidiomata were relatively frequent in a 5 y *G. dewevrei* plot study, occurring in 2.3% of 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished)

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *Gilbertiodendron dewevrei* forests, on upper mineral soil, 29 Aug 2014, *Henkel 9899* (YA; HSC G1301); 1 Dec 2016, *Henkel 10323* (YA; HSC G1302), GenBank accession: ITS = MT446251; 8 Aug 2017, *Mighell 16* (YA; HSC G1303); 29 Aug 2017, *Mighell 26* (YA; HSC G1304); 9 Sep 2017, *Mighell 79* (YA; HSC 1305), GenBank accession: 28S = MT446286; 28 Sep 2017, *Mighell 90* (YA; HSC G1306), GenBank accession: 28S =

MT446287; 27 Sep 2018, *Mighell 171* (YA; HSC G1307); 11 Sep 2019, *Henkel 10875* (YA; HSC G1308); 20 Sep 2019, *Henkel 10924* (YA; HSC G1309). DEMOCRATIC REPUBLIC OF THE CONGO. MONGALA PROVINCE: Binga, scattered on the ground in dry forests, 1928, *Goossens-Fontana 775*, *Amanita fulvopulverulenta* Beeli! (**holotype**, BR5020030954116).

Commentary: Amanita fulvopulverulenta was originally described by Beeli (1931) based on a single 1928 Goossens-Fontana collection. This unusual species is characterized by its pinkish tissues that darken when bruised, fugacious annulus, double-layered volva that leaves a membranous, occasionally limbate band at the bulb apex and a brown powder on the pileus, yellowish lamellae, and strong chlorine odor. Our examination of the type (*Goossens-Fontana 775*) confirmed that the epitype is conspecific. In addition to identical macromorphology (Fig. 11), key micromorphological features observed in the type consistent with the epitype include the mostly uninflated hyphae of the volva with few refractive hyphae, globose elements up to 45 μm wide on the pileus, darkly pigmented suprapellis, few clamp connections, and amyloid, ellipsoid to subovoid basidiospores measuring [25/1/1] (6.5–)7–8 × 3.5–4.5 μm, Qr = 1.75–2.0, Qm = 1.89. The original description (Beeli 1931) omits amyloidity of basidiospores, whereas Beeli's (1935) redescription of the type mistakenly describes them as "non amyloid".

The combination of elongate basidiospores, blushing trama, membranous volva with a powdery inner layer, fugacious marginal appendiculae of the pileus, and lamellae that darken with desiccation suggests placement of *A. pulverulenta* in sect. *Amidella* (Bas

1969). Contrastingly, the presence of a well-developed bulb, membranous volva, yellowish lamellae, persistent partial veil, and odor of chlorine indicate sect. *Lepidella* subsect. *Limbatulae*, a placement also supported by phylogenetic analysis (Fig. 8).

The strong chlorine odor of *A. fulvopulverulenta* is detectable in the field from a distance of several meters. A few other *Amanita* species with chlorine odor are known, including *A. brunnescens* G. F. Atk., *A. chlorinosma* (Peck) Lloyd, *A. cinereoconia* G. F. Atk., *A. cinereopannosa* Bas, *A. cyanopus* Simmons, T.W. Henkel & Bas, *A. cylindrispora* Beardslee, *A. goossensfontanae* (described below), *A. griseibrunnea* O.K. Mill., *A. herrerae* Aroche, *A. ochroterrea* Gentilli ex Bas, *A. ocreata* Peck, *A. odorata* Beeli, *A. ravenelii* Berk. & M. A. Curtis, *A. submaculata* Peck, *A. tephrea* Bas, *A. foetens* Singer, *A. albocloccosa* A. V. Sathe and S. D. Deshp., and *A. cyanochlorinosma* Mighell & T.W. Henkel. These species are tropical or extratropical and scattered across multiple sections, suggesting that chlorine odor carries little taxonomic signal in *Amanita*. Aside from the chlorine odor, none of the aforementioned species are phenotypically similar to *A. fulvopulverulenta*.

Amanita fulvopulverulenta bears resemblance to two Congolian species that also occur at the Dja site. *Amanita fulvosquamulosa* Beeli and *A. goossensiae* Beeli both have flesh that stains pinkish when cut and a volva with a membranous outer layer and a friable inner layer that leaves remnants on a brownish pileus (Beeli 1935). *Amanita fulvopulverulenta* differs from each in its partially elongating stipe, innately pink pigments, more coherent annulus, less robust volva, and strong chlorine odor. Further, the pileus of *A. goossensiae* has innate squamules and no volval remnants (K. S. Mighell pers. obs.).

The Singaporean *A. duplex* Corner & Bas resembles *A. fulvopulverulenta* in its double layered volva leaving some remnants on the pileus and partially attached membrane at the stipe base. The pileus with membranous volval remnants, pinkish red colors, lack of a partial veil, and lack of chlorine odor differentiate *A. duplex* from *A. fulvopulverulenta.*

Amanita robusta Beeli, *Bull. Soc. R. Bot. Belg.* **63**(2): 105 (1931) FIGS. 13, 14

MycoBank MBT392029

Original diagnosis: "*Pileo carnoso, ovoideo-plano, glabro, viscoso, margine substriato, testaceo, centro violaceoatro,* 12 *cm. lato; stipite cylindrico, bulboso, cavo, glabro fibroso,* 13 × 1–2 *cm; annulo fibrilloso fugaci; volva membranacea ampla, brunnea verrucosa; lamellis liberis, luteolis; sporis ellipsoideis, hyalinis, levis,* 7–8 × 5–6 µm; *carne alba; sapore dulcis.*"

Holotype: DEMOCRATIC REPUBLIC OF THE CONGO. MONGALA PROVINCE: Binga, grouped on the ground in *G. dewevrei* forest. May 1928, *Goossens-Fontana 732* (BR5020030973308).

Iconography: Beeli (1931, Pl. VIII, Fig. 8; 1935 Pl. II, Fig. 1)

Description (Beeli 1931, 1935)*:* (freely translated from French) "*Pileus* fleshy, firm, globose then broadly convex, glabrous, viscid, then dry, margin substriate, testaceous red, the center becoming dark purplish. 120 mm diameter. *Stipe* cylindrical with bulbous base, hollow, fibrous, glabrous, 130 × 10–25 mm; *annulus* fibrillose, very ephemeral, leaving cortiniform debris on lamellar edge; *volva* membranous, wise, very thick, verrucose, brownish ochre outside, violaceous inside. *Lamellae* free, tapered at both ends, yellowish, 15 mm tall. *Trama* firm, white. *Taste* sweet. *Basidiospores* hyaline (spore print white), ellipsoid, smooth, 7–8 × 5–6 µm, inamyloid. *Pileipellis hyphae* filamentous, thin, interwoven."

Epitypification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on senescent termite mound, 6 Sep 2017, *Mighell 41* (**epitypus hic designatus** YA; isoepitypes HSC G1310, BR) GenBank accessions: ITS = MT446252; 28S = MT446278.

Description of the epitype: *Pileus* 55–108 mm broad, 8–21 mm tall, broadly convex to planate to uplifted with a shallow, 10–50 mm broad central depression, striate-tuberculate toward margin, initially subglutinous and shiny, later tacky to viscid, glabrous, pink-orange (8A2–8A8) and progressively lighter concolorous toward margin; disc white to lighter concolorous over central 6–11 mm and surrounded by a dark violet-gray (15F3–15F5) ring, this 20–35 mm wide; margin entire to crenulate, splitting slightly with age; trama < 0.25 mm thick at margin, 1 mm over lamellae, 4–7 mm at stipe, solid, off-white, browning where bruised, pink-orange near pileipellis. *Lamellae* free to finely adnexed, thin, crowded, cream to white; edges concolorous, finely roughened; lamellulae 1–35 mm long, truncate. *Stipe* 42–137 mm × 7–14 mm, cylindric, equal, flaring slightly at apex, minutely longitudinally appressed-fibrillose, off-white, darkening slightly where handled, exannulate; bulb 16–39 × 19–38 mm, abrupt, globose, firm; trama fibrous, off-white, with a 4–7 mm wide central hollow stuffed with spongy, fibrillose-floccose pith, occasionally with cavities. *Volva at stipe base* saccate-membranous, 45–70 mm tall, 2.5–7 mm thick at base, 1–1.5 mm thick at apex, ascending from bulb apex, free from stipe; exterior verrucose, off-white between medium brown (5D6) warts, these circular to rectangular, flocculent, 1–4 mm wide patches upon slightly raised tubercles 2–3 mm apart, smaller basally, occasionally fused together into larger, areolate patches; ground below bulb apex orangish brown (5C7), glabrous; interior tacky to viscid, glabrous, white to off-white, with faint concave impressions of outer tubercles. *Odor* none. *Taste* mildly fungoid or slightly metallic.

Basidiospores [250/16/12] 7–11 × (5–)5.5–7 µm, Qr = (1.23–)1.38–1.5(–1.66), Qm = 1.41, inamyloid, broadly ellipsoid, minimally or not flattened adaxially, smooth, hyaline, thin-walled; hilar appendix 1 µm long. *Basidia* 31–51 × 7–11 µm, slender-clavate, 4-sterigmate, occasionally clamped at base; sterigmata 2–5 µm long. *Subhymenium* 20–55 µm thick with 3–5 layers of slightly angular, subglobose to obpyriform elements 8–35 µm wide. *Marginal tissue of lamellae* sterile, 50–75 µm thick, composed of 4–6 layers of globose, clavate, or rarely ellipsoid elements, these 11–30 × 5–15 µm. *Lamellar trama* bilateral, with many fragments of refractive hyphae, these 2–12 µm wide, serpentine with irregular tubercles; mediostratum 20–80 µm wide, of ellipsoid to allantoid elements, these 45–225 × 20–30 µm, with scattered, uninflated, infrequently branching hyphae up to 5 µm wide; lateral strata 60–120 µm wide, of inflated, ellipsoid to allantoid elements, these 40–115 × 15–35 µm, with infrequent, branching, uninflated hyphae up to 5 µm wide. *Pileipellis* an ixomixtocutis with two distinct layers; suprapellis 45–175 µm thick, of heavily gelatinized, hyphal fragments up to 5 µm wide, some extending outwards forming a loose matrix above pellis with infrequent clamp connections; subpellis 75–260 µm thick, of moderately gelatinized, interwoven, uninflated hyphae 2–5 µm wide; upper half of subpellis with abundant refractive, light brown hyphae. *Pileus trama* acrophysalides globose, ovoid, or ellipsoid, 27–200 × 18–74 µm; uninflated hyphae 2–8 µm wide; refractive hyphae few, 4–8 µm wide. *Stipe trama* elements oriented longitudinally; acrophysalides mostly clavate, occasionally subglobose to ellipsoid, 38–120 × 15–65 µm; uninflated hyphae frequently branching, 2–8 µm wide; refractive hyphae scattered, serpentine, 5–14 µm wide; central core and bulb of irregularly-oriented, uninflated hyphae with ellipsoid, limoniform, or globose acrophysalides. *Volva at stipe base* of densely interwoven, frequently branching uninflated hyphae 2–10 µm wide, often with small protruding nubs and slight swelling around septa; inflated elements scattered, these globose, ovoid or ellipsoid, 21–94 × 15–80 µm; refractive hyphae infrequent to abundant, irregularly tuberculate, 2–10 µm wide; wart tissue with light brown pigments.

Habit, habitat, and distribution: Scattered to gregarious on or near senescent termitaria at the base of trees in forests dominated by *G. dewevrei*. Known from the DRC type locality near Binga and the Tshopo Region, and from the epitype locality in the Dja Biosphere Reserve, Cameroon. Basidiomata were rare in a 5 y *G. dewevrei* plot study, occurring in 0.7% of 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished).

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on senescent termite mound, 18 Aug 2014, *HenkelDja 5* (YA; HSC G1311); 26 Aug 2014, *Henkel 9892* (YA; HSC G1312); 8 Sep 2014, *Henkel 9945* (YA; HSC G1313), GenBank accession: ITS = MT446254; 16 Nov 2016, *Aime 6966* (YA; PUL); 26 Nov 2016, *Henkel 10301* (YA; HSC G1314); 8 Sep 2017, *Mighell 47* (YA; HSC G1315); 1 Sep 2018, *Henkel 10590* (YA; HSC G1316); 19 Sep 2018, *Mighell 143* (YA; HSC G1317), GenBank accession: ITS = MT446253; 6 Sep 2019, *Siegel 4368* (YA; HSC G1318); 29 Sep 2019, *Henkel 11003* (YA; HSC G1319). DEMOCRATIC REPUBLIC OF CONGO. MONGALA PROVINCE: Binga, grouped on the ground in *G. dewevrei* forest, May 1928, *Goossens-Fontana 732*, *Amanita robusta* Beeli! (**holotype**, BR5020030973308). TSHOPO REGION: Lomako-Yokokala Faunal Reserve, substratum unknown, 2016, *De Kesel 5236, Amanita robusta* Beeli! (BR), HAUT-KATANGA REGION: vicinity of Lubumbashi, terrestrial, Dec 1931, *de Loose 8*, *Amanita pudica* Beeli! (**holotype**, BR5020047505721).

Commentary. Amanita robusta was originally described by Beeli (1931) based on a single 1928 Goossens-Fontana collection. It is characterized by its bright pink-orange pileus with a violaceous ring around the disc, large basal bulb with a thick, membranous, verrucose volva, and scattered to gregarious termitariophilic habit in *G. dewevrei* forests. The inamyloid basidiospores and basal bulb indicate placement of *A. robusta* in subgen. *Amanita* sect. *Amanita*, also supported by the phylogenetic analysis (Fig. 8)

The type specimen of *A. robusta* (*Goossens-Fontana 732*) agrees both macro- and micromorphologically with the epitype and other collections analyzed here. All have basidiospores of a similar size and shape, clamped basidia, a partially gelatinized suprapellis, a slightly gelatinized subpellis with abundant refractive hyphae, and volva tissue with abundant uninflated hyphae and mostly globose inflated cells. The volva of the type also has abundant, highly branched refractive hyphae.

A Malagasy specimen was identified as *A. robusta* by Bouriquet (1941) and described as having a pale yellow to reddish brown non-striate pileus bearing large volval warts and a stipe with fugacious veil remnants. These features diverge from *A. robusta* and the recorded association with *Eucalyptus* suggest a different identity for the Bouriquet specimen.

Amanita pudica (Beeli) Walleyn is the only species that could be confused with *A. robusta*. The two species are quite similar, sharing the bright pinkish pileus, large basal bulb, and robust, saccate, verrucose volva. *Amanita pudica* was originally described as fruiting terrestrially near Lubumbashi, DRC (Beeli 1936). Further treatment described it as “a typical and fairly common ectomycorrhizal fungus associated with leguminous trees of miombo woodland vegetations in Burundi, Zaire and Zambia” (Walleyn 1996), and also that in Burundi *A. pudica* was found on termitaria. Our collections of *A. robusta* were also found exclusively on termitaria. However, the occurrence of *A. pudica* in miombo woodland vegetation distinguishes it from *A. robusta*, the latter being described from Binga, DRC in closed-canopy rainforest dominated by *G. dewevrei* (Beeli 1935). We maintain that the Cameroon material is conspecific with *A. robusta* type given the similar ecology, presence of a violaceous ring around the pileus disc, and otherwise exact morphological match. Additionally, our examination of the *A. pudica* type (*deLoose 8*) revealed notably larger basidiospores (see below). These species were originally differentiated by Beeli by a partial veil, putatively present in *A. robusta* and absent in *A. pudica*, that was described as “fibrillose, very ephemeral, leaving cortiniform debris on the lamellar edge” (Beeli 1935, 1936). This observation, presumably made on fresh *A. robusta* material by Goossens-Fontana, was likely a misinterpretation of the roughened lamellar edges. Indeed, none of the watercolor images depicting *A. robusta* in fresh condition show a partial veil, which is also lacking across all development stages from our Cameroon collections (Fig. 13).

Basidiospore dimensions of *A. robusta* were reported by Beeli (1935) as 7–8 × 5–6 µm. Gilbert (1941) re-examined the type material and produced several basidiospore illustrations, from which Tulloss and Yang (2020) estimated to measure 9–10 × 6.5–7.5 µm. Our re-examination of the type yielded the following basidiospore measurements: [25/1/1] 8.5–10 × 6–7 µm, Qr = 1.31–1.50, Qm = 1.38. The latter dataset agrees closely with the estimates from Gilbert’s drawings and falls within the range measured from our Cameroon collections. While dissimilar from Beeli’s original measurements, these are known to be unreliable (Tulloss and Yang 2020). We also examined the basidiospores of a collection (*De Kesel 5236*) identified as *A. robusta* in Kamalebo et al. (2018) and recorded dimensions of [25/1/1] 7.5–9 × 5.5–7 µm, Qr = 1.23–1.45, Qm = 1.34. These measurements fall within the ranges of our Cameroon collections and are notably smaller than those we recorded from the type of *A. pudica* (*deLoose 8*) as [25/1/1] 9–11.5 × 6.5–8.5 µm, Qr = 1.23–1.53, Qm 1.42.

Amanita bingensis (Beeli) R. Heim, *Revue Mycol.*, Paris **5**: 22 (1940) FIGS. 15, 16

≡ *Amanitopsis bingensis* Beeli, *Bull. Soc. R. Bot. Belg* **63**(2): 108 (1931)

MycoBank MBT392030

Original diagnosis: "*Pileo carnoso tenui, convexo-plano, luteo pulverulento aurantiaco, margine striato,* 4–5 *cm lato; stipite cylindrico, basi bulboso, subcavo, fibroso glabro; luteo-aurantiaco,* 9 × 0.5–0.7 *cm; volva friabilis luteo-aurantiaca; lamellis subliberis, albidis, luteo marginatis; sporis globulosis, hyalinis, levis;* 5–6 × 3–4.5 µ; *carne alba; sapor e dulcis.*"

Holotype: DEMOCRATIC REPUBLIC OF THE CONGO. MONGALA PROVINCE: Binga, isolated on the ground in dry forest. Sep 1929, *Goossens-Fontana 890* (BR).

Iconography: Beeli (1931, Pl. IX, Fig. 15; 1935 Pl. IV, Fig. 4)

Description (Beeli 1931, 1935)*:* (freely translated from French) "*Pileus* fleshy, thin, broadly convex, pale yellow, covered with pulverulent volval debris, 40–50 mm diameter; margin striate. *Stipe* cylindrical with bulbous base, straight, 90 × 5–7 mm, hollow, fibrous, smooth, orangish yellow; *annulus* absent; *volva* friable, orangish yellow, forming (a few 1931) slight rings on the stipe bulb. *Lamellae* subfree, tapered at both ends, white with yellow edges, 4 mm tall. *Trama* firm,

white. *Taste* sweet. *Basidiospores* hyaline, globose to ovoid, smooth, 5–6 × 3–4.5 µm, inamyloid. *Spore print* white. *Pileipellis* hyphae thin, some terminated by vesicular cells."

Epityification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, at base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on humic mat, 3 Sep 2018, *Mighell 104* (**epitypus hic designatus** YA; isoepitypes HSC G1320, BR), GenBank accessions: ITS = MT446264; 28S = MT446281.

Description of the epitype: *Pileus* 45–71 mm broad, 10–16 mm tall, broadly convex to slightly uplifted, occasionally with a broad, shallow central depression, sulcate over marginal half, dry, deep orange or orange-red over disc (5A8; 8A8), lighter toward margin, white in sulcation troughs; margin entire to crenulate or splitting; trama < 0.25 mm thick at margin, 1 mm over lamellae, 3 mm at stipe, off-white to yellow-orange, solid. *Volva on pileus* friable; granules detersile, iridescent, bright yellow (3A8), scattered over sulcate zone, concolorous to bright orange (5A8) over disc, and there coalescing into erect, subpyramidal warts, these 0.5–3 mm wide × 0.5–3 mm tall, some with white apices. *Lamellae* adnexed, thin, close to subcrowded, white; edges smooth, yellowish to deep orange; lamellulae 1–22 mm long, truncate. *Stipe* 85–100 × 6–10 mm centrally, cylindric or tapering upwards, flaring at apex, minutely pulverulent to flocculent-fibrillose over finely longitudinally striate ground, pale yellow to bright orange (2A8–4A8) with pink tones (7A2–3 KW) near base, exannulate; bulb 10–15 × 11–14 mm, subglobose, subacuminate at base; trama off-white to yellowish, with stuffed hollow central core 1.5–3 mm wide. *Volva at stipe base* friable; granules same as on pileus, in a

1–3 mm tall band of suberect, fibrillose-flocculent tufts on apical part of bulb, occasionally leaving fragments on soil near base. *Odor* none to mildly fungoid. *Taste* mildly fungoid.

Basidiospores [125/5/5] (6–)6.5–7(–8) × 4.5–5.5(–6) µm, Qr = (1.17–)1.20–1.56(–1.6), Qm = 1.34, inamyloid, subglobose to broadly ellipsoid, slightly flattened adaxially, smooth, hyaline, thin-walled; hilar appendix sublateral, truncate, up to 1 µm long. *Basidia* 25–40 × 8–10 µm, narrowly clavate to clavate, 4-sterigmate; sterigmata (1–)2–4(–4.5) µm long. *Subhymenium* 20–40 µm thick, inflated-ramose, with 3–7 layers of angular to rounded, globose, broadly ellipsoid or pyriform elements, these 5–25 µm wide. *Marginal tissue of lamellae* sterile, 50–70 µm thick, heavily gelatinized, yellow. *Lamellar trama* parallel, subdivergent near pileus trama; elements ellipsoid, globose, rarely ovoid, 11–100 × 7–45 µm, with interwoven uninflated hyphae; refractive hyphae rare. *Pileipellis* a single layered ixomixtocutis, 75–100 µm thick, highly gelatinized, individual elements indistinguishable. *Pileus trama* predominantly of acrophysalides, these ellipsoid to broadly ellipsoid, 18–100 × 15–59 µm; uninflated and refractive hyphae 2–6 µm wide. *Volva on pileus* with 1–5 layers of inflated, subglobose to globose, thin-walled, pale yellow-orange elements, these 11–51 µm wide and often attached to short filaments, rarely in terminally-broadening chains. *Stipe trama* of longitudinally oriented, ellipsoid acrophysalides, these 46–265 × 16–35 µm, and highly branched uninflated hyphae 2–7 µm wide; refractive hyphae of variable abundance, with dark contents. *Volva at stipe base* same as on pileus. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary to paired on humic mat in forests dominated by *G. dewevrei*, rarely in mixed forests near *Uapaca* species. Known from the DRC type locality near Binga and from the epitype locality in the Dja Biosphere Reserve, Cameroon. Rare; in a 5 y *G. dewevrei* plot study basidiomata were absent from the 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished).

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in mixed forests near *Uapaca* species, 1 Dec 2016, *Henkel 10325* (YA; HSC G1321); in monodominant *G. dewevrei* forest on humic mat, 12 Sep 2017, *Mighell 54* (YA; HSC G1322); 24 Sep 2019, *Henkel 10954* (YA; HSC G1323); 26 Sep 2019, *Henkel 10977* (YA; HSC G1324).

Commentary: Amanita bingensis was originally described by Beeli (1935) based on a single 1929 Goossens-Fontana collection. It is characterized by a small to medium-sized, sulcate pileus with a bright yellow-orange friable volva. Microscopically this species is unique in *Amanita* due to its cellular lamellar trama, single-layered pileipellis, and broadly ellipsoid acrophysalides. The inamyloid basidiospores and basal bulb indicate placement in subgen. *Amanita* sect. *Amanita*, also supported by phylogenetic analysis (Fig. 8).

Amanita bingensis resembles several known species of Gondwanan distribution that lack a partial veil and have small basidiomata with sulcate pilei and bright orange or yellow volva particles on the pileus and bulb apex. Among these species the Guyanese *Amanita calochroa* C.M. Simmons, T.W. Henkel & Bas is most similar to *A. bingensis* but has even smaller basidiomata and fruits gregariously. *Amanita calochroa* also has larger basidiospores (6.3–7.8 × 5.5–7.8 µm vs. 5–7 × 4–5 µm), a thinner pileipellis, and bilateral lamellar trama (Simmons et al. 2002).

Amanita chrysoleuca Pegler from semi-deciduous forest in Martinique is also similar to *A. bingensis* but differs in its smaller in stature, more yellow volva, fertile lamellar edge, and bilateral lamellar trama (Pegler 1983).

Amanita aurantiovelata Schalkw. & G.M. Jansen from the *Nothofagus* forests of Chile is macroscopically similar to *A. bingensis* and shares some micromorphological features (Schalkwijk and Jansen 1982). It was described as having “approximately bilateral” lamellar trama and a seemingly single-layered ixocutis, both unusual features for *Amanita* shared with *A. bingensis*. The larger basidiospores (8–11.5 × 6.5–8) and clamped basidia of *A. aurantiovelata* allow its separation from *A. bingensis*.

Amanita minima Mighell & T.W. Henkel, sp. nov. FIGS. 17A–B, 18

MycoBank MB835370

Diagnosis: Similar to *A. bingensis* but differs in its smaller size and association with *Uapaca* species on muddy, periodically inundated riverbanks.

Typification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, at base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in mixed rainforest near a grove of *Uapaca guineensis*, on muddy, periodically inundated bank of Sim Creek, 5 Sep 2017, *Mighell 38* (**holotype** YA; isotypes HSC G1325, BR), GenBank accessions: ITS = MT446261; 28S = MT446280.

Pileus 4.5–20 mm broad, 1.5–3 mm tall, broadly convex to planate, dry, sulcate-striate over marginal 1/2–2/3; bright orange (6A8) to orangish red (8A8), bright yellow (2A8) near margin, white in sulcation troughs; margin entire to crenulate or eroding; trama < 0.25 mm thick at margin, 0.5 mm over lamellae, 0.5 mm at stipe, off-white, solid. *Volva on pileus* friable; granules yellow (2A8) to bright orange (6A8), iridescent, coating pileus surface. *Lamellae* free, thin, close, white to off-white; edges roughened, off-white to concolorous with volva; lamellulae 0.5–5 mm long, subtruncate to truncate. *Stipe* 13–26 × 1.5–2.5 mm, cylindric, equal, minutely fibrillose to pulverulent, white to pale yellow (4A4), exannulate; bulb 3–5 × 3–5 mm, subabrupt, globose; trama off-white, with hollow core 1 mm wide. *Volva at stipe base* friable, of granules as on pileus, these evenly coating the upper bulb. *Odor* none. *Taste* none.

Basidiospores [175/8/7] 5–7(–8) × (3.5–)4–5(–6) µm, Qr = (1.1–)1.25–1.5(–1.6), Qm = 1.37, inamyloid, subglobose to broadly ellipsoid or ellipsoid, slightly flattened adaxially, smooth, hyaline, thin-walled; hilar appendix truncate, up to 1 µm long. *Basidia* 19–35(–40) × 6–9(–10) µm, broadly clavate, often curved, highly variable, 4-sterigmate; sterigmata (1–)2–4 µm long. *Subhymenium* 20–40 µm thick, with 3–7 layers of angular, globose, or broadly ellipsoid elements, these 5–25 µm wide; interlamellar spaces fertile. *Marginal tissue of lamellae* sterile, with a palisade of ~8–15 ellipsoid to pyriform cells, these 9–20 × 5–15 µm. *Lamellar trama* parallel, of globose, ellipsoid, to rarely ovoid elements, these 11–61 × 7–45 µm, with scattered and interwoven uninflated and refractive hyphae; mediostratum inevident. *Pileipellis* undifferentiated from trama or slightly gelatinized with repent elements, 30–75 µm thick, thinner over trama of each lamella, with clavate, ellipsoid, or globose elements 9–25 µm wide and abundant uninflated hyphae 2–7 µm wide. *Pileus trama* of acrophysalides, these globose to broadly, 18–78 × 16–62 µm, and uninflated and refractive hyphae, these 2–6 µm wide. *Volva on pileus* with 1–5 layers of inflated, subglobose to globose, thin-walled elements, these pale yellow-orange, 10–48 µm wide, often attached to short filaments, rarely in chains with elements becoming larger terminally. *Stipe trama* of longitudinally oriented, ellipsoid acrophysalides, these 46–255 × 17–38 µm, and highly branched, interweaving uninflated hyphae, these 2–7 µm wide; refractive hyphae abundant, with dark contents. *Volva at stipe base* same as on pileus. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary to gregarious on exposed mineral soil in frequently inundated portions of riparian forests, near *U. guineensis* or *U. acuminata* or rarely under *G. dewevrei.* Known only from the type locality in the Dja Biosphere Reserve, Cameroon. In a 5 y *G. dewevrei* plot study, basidiomata were absent from 750 quadrats sampled during the Sep–Nov rainy seasons over 5 y (Henkel, unpublished).

Etymology: minimus (L. adj A) = extremely small, referring to the diminutive basidiomata.

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, in vicinity of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest on humic-mineral soil interface, 1 Sep 2014, *HenkelDja 70* (YA; HSC G1326), GenBank accession: ITS = MT446260; in mixed forest on mineral soil, near *U. guineensis* 25 Aug 2017, *Mighell 7* (YA; HSC G1327); 3 Sep 2017, *Mighell 33* (YA;

HSC G1328), GenBank accession: 28S = MT446279; in mixed forest on mineral soil, near *G. dewevrei*, 13 Sep 2017, *Mighell 57* (YA; HSC G1329)*;* 27 Sep 2017, *Mighell 82* (YA; HSC G1330); near *U. acuminata* on bank of Sim Creek, 1 Sep 2019, *Henkel 10829* (YA; HSC G1331).

Commentary: Amanita minima is characterized by its extremely small basidiomata and gregarious fruiting on mineral soil in riparian zones, usually near ECM *Uapaca* species. Like the sympatric *A. bingensis,* it can be identified by its abundant bright yellow-orange volval powder on the pileus and apex of the basal bulb. Unlike *A. bingensis*, *A. minima* is consistently very small and lacks pinkish tones on the stipe. Microscopically *A. minima* differs slightly from *A. bingensis* in its thinner single-layered pileipellis, more globose pileus trama elements, cellular as opposed to inflated-ramose subhymenium, non-diverging cellular lamellar trama, and short, broad basidia. Like *A. bingensis,* the species is best placed in subgen. *Amanita* sect. *Amanita*, also supported by phylogenetic analysis (Fig. 8). The unique combination of features shared by *A. bingensis* and *A. minima* allow differentiation of the latter species from similar known taxa as indicated above.

Amanita luteolamellata Mighell & T.W. Henkel, sp. nov. FIGS.17C–D, 19

MycoBank MB835371

Diagnosis: Similar to *A. strobilaceovolvata* but differs in its larger basidiomata, smooth volva, and yellow lamellae.

Typification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8"

N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest on mineral soil, 23 Sep 2017, *Mighell 75* (**holotype** YA; isotypes HSC G1332, BR), GenBank accessions: ITS = MT446258; 28S = MT446266.

Pileus 98–175 mm broad, 18–60 mm tall, planate to strongly uplifted with rounded-conic umbo, tacky, glabrous and somewhat shiny over umbo, radially appressed-fibrillose surrounding umbo, deeply sulcate-striate over marginal half, medium brown (5E8–5F8) with pale yellow (2A2) ground between separating fibrils, darker yellow (2A4–2A5) in sulcation troughs; sulcations 1 mm wide × 1 mm deep, forking occasionally toward margin, occasionally tuberculate, with very fine, shiny fibrils in troughs; margin serrate to splitting; trama < 0.25 mm thick at margin, 1.5 mm over lamellae, 10 mm at stipe, solid, off-white, in umbo yellow (2A6) immediately below the pileipellis. *Lamellae* free, steeply ascending, subthin, crowded, yellow (2A5–2A6); edges light yellow, minutely roughened; lamellulae 1–2 between each lamella, 2.5–82 mm long, truncate. *Stipe* 154–320 × 10–17 mm, deeply inserted in soil, cylindrical or slightly compressed, slightly tapering upwards, flaring at apex; ground light brown (4B1–4B2) to yellow-brown (3B5–3B5) overlain with longitudinally appressed fibrils, these light gray (4B1–4B2) to tan-brown (4C5–5D5), squamulose near base, darkening where handled; trama fibrous, off-white to pale brown, with a hollow core 2.5–7.5 mm wide. *Volva at stipe base* saccate-membranous with free to clasping limbs, 45–65 mm tall; tissue 6 mm thick at base, 0.5 mm apically; exterior with minute, orange (5A8–5B8), gelatinized fibrils in patches over white ground; interior dry to viscid, white to light gray-brown. *Partial veil* a superior, fugacious, membranous annulus; upper portion of appressed white flocculent scales descending 17–44 mm below the stipe apex, lower portion pendant, 8–25 mm long, occasionally fragmented; exterior striate, white; interior with minute, recurved, brown-tipped scales over white ground, yellow (2A6) near margin. *Odor* none. *Taste* none.

Basidiospores [150/6/6] (7.5–)8–10.5(–11.5) × (5.5–)6.5–8.5(–9) µm, Qr = (1.0–)1.13–1.42(–1.5), Qm = 1.31, inamyloid, ellipsoid to broadly ellipsoid, rarely subglobose, barely flattened adaxially, smooth, hyaline, thin-walled; hilar appendix cylindric, truncate, up to 1 µm long. *Basidia* 29–41 × 9–12(–12.5) µm, clavate to broadly clavate, (2–)4-sterigmate; sterigmata 2–4(–5) µm long. *Subhymenium* 25–50 µm thick, with 3–5 layers of globose, ellipsoid, or obpyriform elements, these 12–27 µm wide. *Marginal tissue of lamellae* sterile, of globose cells 15–25 µm wide. *Lamellar trama* bilateral; mediostratum 30–90 µm wide, with bundled, slightly inflated hyphae 5–10(–20) µm wide; lateral strata 80–150 µm wide, with allantoid, ovoid, or subglobose elements, these 30–173 × 15–37 µm; refractive hyphae scattered, interwoven, uninflated. *Pileipellis* a single-layered ixoparallelocutis, 30–70 µm thick, with scattered inflated elements up to 15 µm wide; uninflated hyphae 2–8 µm wide; refractive hyphae abundant; distal cells with orangish brown pigment. *Pileus trama* predominantly of acrophysalides, these ellipsoid to cylindrical, 50–190 × 12–32 µm; uninflated hyphae highly branched, interwoven, 4–8 µm wide; refractive hyphae rare, serpentine, fragmented, 5–20 µm wide.

Stipe trama with longitudinally oriented acrophysalides, these clavate to cylindric, 13–39 µm wide; uninflated hyphae interwoven, 2–6 µm wide; refractive hyphae abundant, long, infrequently branching, slightly tuberculate, 5–12 µm wide; stipitipellis with periclinal bundles of gelatinized, dark brown hyphae. *Volva at stipe base* with globose to ellipsoid inflated elements, these 22–105 × 50–155 µm, and interwoven uninflated and refractive hyphae 2–8 µm wide; interior surface sometimes gelatinized. *Partial veil* of abundant interwoven uninflated hyphae 2–8 µm wide; terminal cells inflated, ellipsoid or clavate, rarely globose, 10–35 × 22–70 µm. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary, rarely in pairs, emerging from mineral soil at a depth of 50–100 mm below humic layer in forests dominated by *G. dewevrei.* Known only from the type locality in the Dja Biosphere Reserve, Cameroon. Basidiomata were rare in a 5 y *G. dewevrei* plot study, occurring in 0.8% of 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished).

Etymology: Luteus (L. adj. A) = yellow and -*lamellatus* (L. adj. A) = lamellate, referring to the yellow lamellae.

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 2 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m, a.s.l. in monodominant *G. dewevrei* forest, from mineral soil, 14 Nov 2016, *Henkel 10256* (YA; HSC G1333); 8 Sep 2017, *Mighell 44* (YA; HSC G1334); 13 Sep 2017, *Mighell 56* (YA; HSC G1335); 28 Sep 2018, *Mighell 174* (YA; HSC G1336); 27 Sep 2019, *Henkel 10981* (YA; HSC G1337).

Commentary. The combination of inamyloid basidiospores, membranous volva, and lack of clamp connections indicate placement of *A. luteolamellata* in subgen. *Amanita* sect. *Vaginatae,* also supported by phylogenetic analysis (Fig. 8). *Amanita luteolamellata* is unusual among sect. *Vaginatae* in its very large, annulate-vaginatoid basidiomata with yellow lamellae and development from deep within the mineral soil. Two morphologically similar sympatric taxa are *A. strobilaceovolvata* Beeli and the undescribed *Amanita* morphospecies #11. These taxa share a yellow-brown, striate pileus and annulate-vaginatoid stature. *Amanita strobilaceovolvata* is known elsewhere from the Guineo-Congolian region in association with *G. dewevrei* but has a strongly tuberculate membranous volva (Beeli 1931; Ndolo and Yorou 2017). *Amanita* morphospecies #11 has a smooth volva like *A. luteolamellata* but has smaller basidiomata with pale yellow as opposed to yellow lamellae and associates with ECM *Uapaca* species (K.S. Mighell pers. obs.).

The annulate-vaginatoid stature and habitat type suggest an affinity for *A. annulatovaginata* Beeli, a poorly known species also described from *G. dewevrei* forests of the DRC. *Amanita luteolamellata* can be separated from *A. annulatovaginata* by its much larger basidiomata, yellow lamellae, and smaller basidiospores (Beeli 1935; Tang et al. 2015).

Within sect. *Vaginatae* the above-listed taxa may belong to the provisional series *Penetratrices* as recognized by Tulloss and Yang (2020). Members of this group share an unusual nrLSU sequence motif which is yet to be found in *A. luteolamellata*. The brown, umbonate, striate pileus and annulate-vaginatoid stature may characterize species in

Penetratrices including *A. strobilaceovolvata, A. insinuans* nom. prov., *A. pseudoinsinuans* nom. prov., *Amanita* sp-IN02*, Amanita* sp-N58*, Amanita* sp-T24, and *Amanita* sp-Arora_01-555 (Tulloss and Yang 2020). Some species in this series may also have a deeply buried volva (e.g. *A. penetratrix* nom. prov.*, Amanita* sp-WI03*).* This group is insufficiently known and most taxa were recently discovered and remain undescribed. It may be especially diverse in the southeastern United States (Tulloss and Yang 2020).

Amanita luteolamellata is the most distinctive and striking annulate member of sect. *Vaginatae* encountered in the Dja study site, but the species limits within this group remain unclear and merit much further study. Pileus color, presence or absence of a partial veil, stipe color and texture, and volva texture are all highly variable among sect. *Vaginatae*. These character states combine in numerous ways to allow a preliminary recognition of 20 distinct morphospecies in sect. *Vaginatae*, among the ~ 70 local species of *Amanita* writ large (Henkel and Mighell, unpublished).

Amanita goossensfontanae Mighell & T.W. Henkel, sp. nov. FIGS. 17E–F, 20

MycoBank MB835372

Diagnosis: Similar to *A. smithiana* but differs in its smaller basidiospores and lack of clamp connections on the basidia.

Typification: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 5 km of base camp located at 3° 21' 29.8"

N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on upper mineral soil, 22 Sep 2019, *Henkel 10938* (**holotype** YA; isotypes HSC G1338, BR), GenBank accessions: 28S = MT446283.

Pileus 48–80 mm broad, 9–16 mm tall, broadly convex to planate, dry, matted-fibrillose with irregular, floccose, innate scales, these areolate near margin, yellowish white (1A2–1A3), white near margin; margin entire, with a contiguous, fugacious, downy-fibrillose, white to pale yellow tissue band descending 2–12 mm from pileus margin, this eventually splitting into triangular appendiculae; trama 1 mm thick at margin, 4 mm over lamellae, 6–8 mm at stipe, solid, pale yellow. *Volva on pileus* friable, of scattered particulate elements concolorous with ground, these iridescent, occasionally forming rounded tufts. *Lamellae* finely adnexed to minutely sinuate, thin, crowded, off-white with slight pink or yellow tint; edges concolorous, pruinose; lamellulae 3, 1–27 mm long, attenuate. *Stipe* 132–147 × 10–14 mm above bulb, cylindric, equal or tapering slightly upwards, flaring at apex, densely downy-fibrillose, squamulose near bulb, white, darkening slightly with handling; bulb 40–59 × 20–30 mm, clavate, ellipsoid, or turbinate with rounded to subacuminate base, matted fibrillose, compressible, white; trama solid, fibrous, off-white. *Volva at stipe base* inevident. *Partial veil* superior, submembranous, fugacious, clasping or pendant, 6–15 mm long, downy-fibrillose, white to pale yellow. *Odor* fragrant, pleasant, faintly of chlorine. *Taste* pleasant, nutty, with overtones of chlorine.

Basidiospores [175/8/7] 6–7(–8) × 4–5.5(–6) µm, Qr = (1.09–)1.2–1.5(–1.6), Qm = 1.35, amyloid, subglobose to broadly ellipsoid, flattened adaxially, smooth, hyaline, thin-walled; hilar appendix cylindric to subconic, up to 1 µm long. *Basidia* (32–)35–45(–48) × 7–9(–10) µm, narrowly clavate, 4-sterigmate; sterigmata (2–)3–4.5 µm long. *Subhymenium* 20–40 µm thick, coralloid, with 3–5 layers of globose, ovoid, ellipsoid, or obpyriform elements, these 5–22 µm wide, often with irregular protrusions; outermost cells usually obpyriform. *Marginal tissue of lamellae* sterile, of clavate or rarely globose elements, these 15–30 × 8–20 µm. *Lamellar trama* bilateral; mediostratum 12–25 µm wide, of bundled uninflated hyphae, these 2–8 µm wide, and infrequent inflated elements; lateral stratum 8–35 µm wide, mostly of chains of broadly ellipsoid elements diminishing in size outwards; individual elements 30–80 × 10–26 µm; uninflated hyphae interwoven; refractive hyphae rare. *Pileipellis* a single-layered ixomixtocutis 55–255 µm thick; uninflated hyphae 2–6 µm wide, with scattered globose to clavate elements, these 8–25 µm wide; refractive hyphae rare. *Pileus trama* with abundant periclinally-oriented acrophysalides, these ellipsoid or allantoid, 30–210 × 15–40 µm, and interwoven uninflated hyphae, these 2–8 µm wide; refractive hyphae scattered, serpentine, 2–5 µm wide. *Stipe trama* poorly rehydrating; acrophysalides longitudinally oriented, clavate, rarely spathulate, 10–70 µm wide; uninflated hyphae interwoven, 2–8 µm wide; refractive hyphae infrequent, 5–12 µm wide; stipitipellis hyphae periclinal, terminating in small swollen projections or globose inflated elements similar to those of the volva. *Volva on pileus* 4–15 cells thick, of subglobose to globose, rarely ellipsoid, thin-walled elements, these 12–60 µm wide, with sparsely interwoven uninflated hyphae, these up to 8 µm wide. *Volva at stipe base* of globose to broadly ellipsoid elements, these 15–70 µm wide and arising from highly branched, serpentine, uninflated hyphae, these 2–8 µm wide and often with short, terminally inflated branches. *Clamp connections* absent on hyphae of all tissues.

Habit, habitat, and distribution: Solitary, rarely scattered on humic mat or upper mineral soil in forests dominated by *G. dewevrei*. Known only from the type locality in the Dja Biosphere Reserve, Cameroon. In Cameroon, basidiomata were rare in a 5 y *G. dewevrei* plot study, occurring in 0.3% of 750 quadrats sampled during the Sep–Nov rainy seasons (Henkel, unpublished).

Etymology: In honor of Mme. Martha Goossens-Fontana, collector and illustrator of numerous Guineo-Congolian macrofungi.

Other specimens examined: CAMEROON. EAST REGION: Dja Biosphere Reserve, Northwest Sector near Somalomo, Upper Dja River Basin, within 5 km of base camp located at 3° 21' 29.8" N; 12° 43' 46.9" W, 650 m a.s.l., in monodominant *G. dewevrei* forest, on humic mat, 9 Sep 2014, *HenkelDja 101* (YA; HSC G1339); 4 Sep 2018, *Mighell 107* (YA; HSC G1340); 5 Sep, 2018, *Mighell 110* (YA; HSC G1341); 13 Sep 2018, *Mighell 134* (YA; HSC G1342), GenBank accessions: ITS = MT446263; 28S = MT446284; 14 Sep 2019, *Henkel 10889* (YA; HSC G1343); from upper mineral soil, 23 Sep 2019 *Henkel 10947* (YA; HSC G1344).

Commentary: Amanita goossensfontanae is characterized by its medium, white to yellowish pileus with friable, iridescent volval tissue, flocculent partial veil clinging to the stipe and pileus margin, basal bulb, and small basidiospores. *Amanita goossensfontanae* is best placed in subgen. *Lepidella* sect. *Lepidella* subsect. *Solitariae* as defined by Bas (1969) because it lacks features defining the other subsections of sect.

Lepidella, such as volva tissue composed of cylindrical, elongate-fusiform, and slenderly clavate cells as found in subsect. *Vittadiniae,* and the membranous volva of subsect. *Limbatulae* (Bas 1969).

Some white, bulbous-based, floccose-veiled species of sect. *Lepidella* species are similar to *A. goossensfontanae*. These include *A. smithiana* Bas, which also has a robust stature and chlorine odor, differs from *A. goossensfontanae* in its larger basidiospores (8.8–12.0 × 5.8–8.0 µm vs 6–7 × 4–5.5 µm) and clamped basidia. The otherwise similar *A. pulverulenta* Beeli, *A. polypyramis* Berk. & M. A. Curtis, *A. cokeri* E.-J. Gilbert, and *A. rhopalopus* Bas all differ from *A. goossensfontanae* in their pyramidal volva warts, more membranous partial veils, and larger basidiospores (10.0–12.3 × 5.6–7.0, 9.1–12.9 × 5.9–7.6, 11.0–13.5 × 7.0–9.0, 8.0–10.9 × 5.4–6.8 µm, respectively, vs. 6–7 × 4–5.5 µm). *Amanita gracilior* Bas, *A. virgineoides* Bas, and *A. heishidingensis* Li and Cai are separated from *A. goossensfontanae* by their clamped basidia.

Some members of sect. *Lepidella* stirps *Thiersii* Bas resemble *A. goossensfontanae*. *Amanita foetens* Singer and *A. albofloccosa* A. V. Sathe & S. D. Deshp. are white to pale yellow, have floccose veils, bulbous bases, and chlorine odor (Tulloss and Yang 2020). Both species differ from the putatively ECM *A. goossensfontanae* in their larger basidiospores (8.0–9.5 × 7.5–9.0 and 7.3–9.6 × 6.4–7.7 µm respectively, vs. 6–7 × 4–5.5 µm) and saprotrophic habit in grassland habitats lacking ECM host plants (Wolfe et al. 2012).

FIGURES

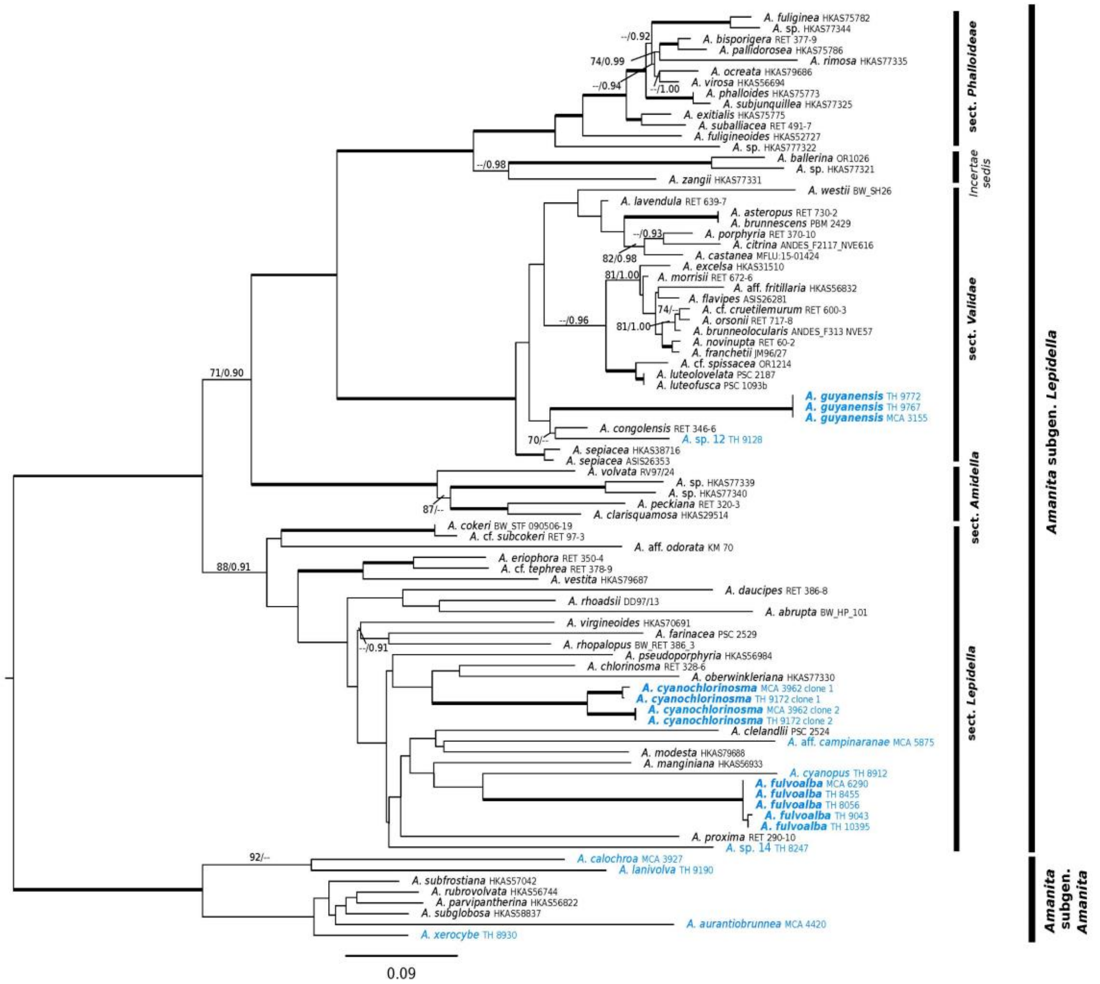

Figure 1. Maximum-likelihood phylogeny generated from the analysis of four gene fragments (nrLSU, *ef1-α*, β-*tubulin* and *rpb2*) from 76 specimens in *Amanita* subgen. *Lepidella* and eight outgroup specimens in *Amanita* subgen. The new species *A. cyanochlorinosma* and *A. albofulva* are resolved in sect. *Lepidella* and *A. guyanensis* in sect. *Validae*. Thickened black bars represent nodes with greater than 90% BS and 0.95

BPP; support values shown above branches represent % BS/BPP; -- represents no support. New species are in bold, and all species from Guyana are in blue.

Figure 2. *Amanita cyanochlorinosma* (*Henkel 9172*, type). Bar = 1 cm.

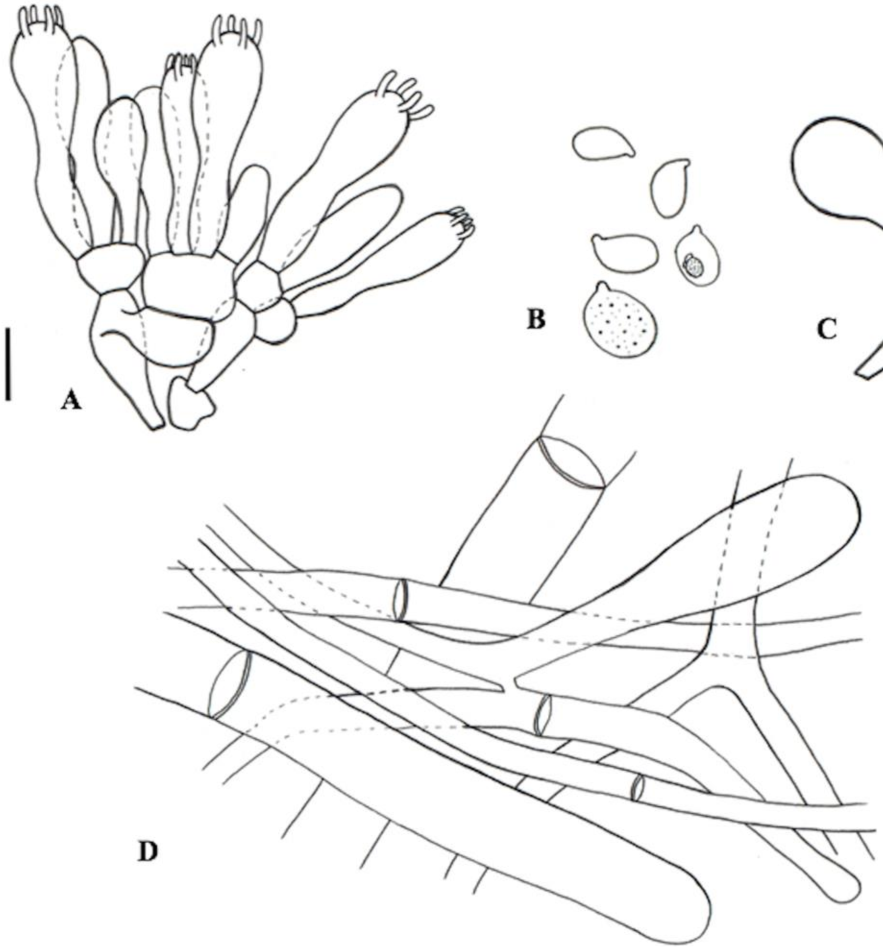

Figure 3. *Amanita cyanochlorinosma*. **A.** Basidia and subhymenium. **B.** Basidiospores. **C.** Marginal tissue of lamellae. **D.** Slightly crushed tissue from volval limb. Bar =10 µm.

Figure 4. *Amanita fulvoalba* (*Henkel 10395*, type): Bar = 1 cm.

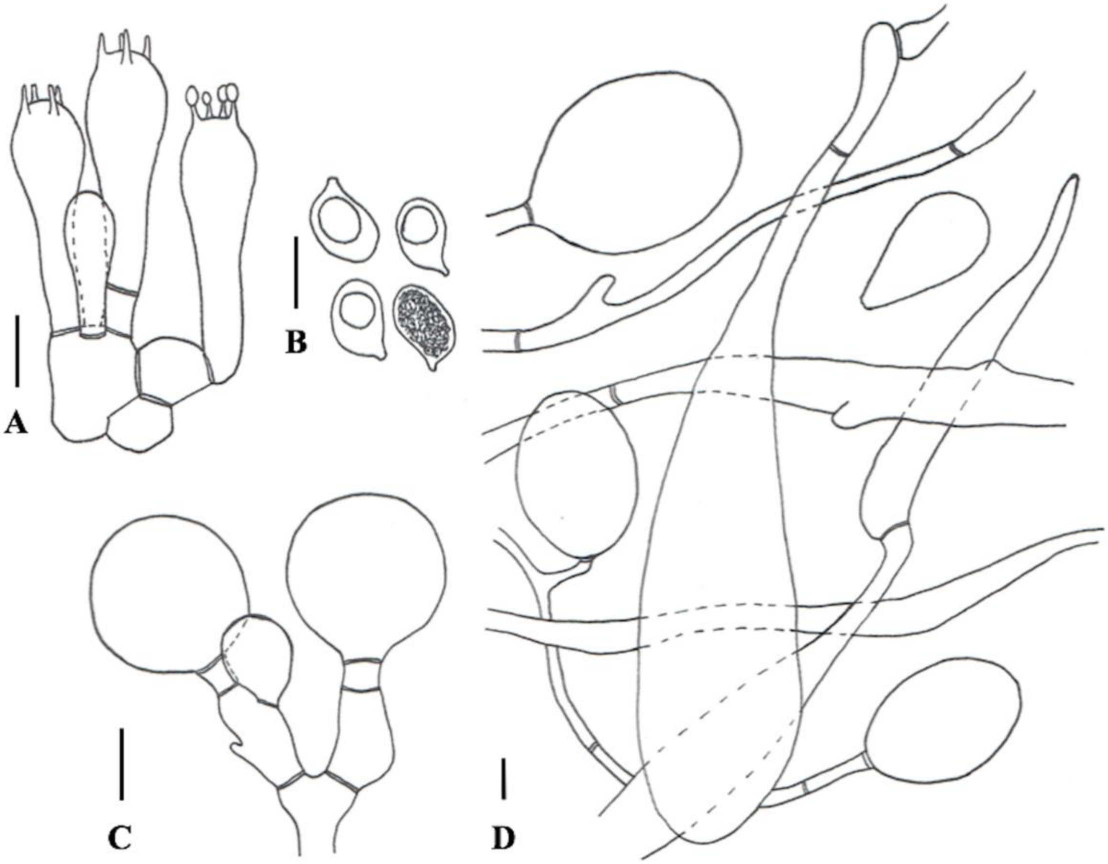

Figure 5. *Amanita fulvoalba.* **A.** Basidia and subhymenium. **B.** Basidiospores. **C.** Marginal tissue of lamellae. Scale bars = 10 µm. **D.** Slightly crushed tissue from volval limb. Bars = 10 µm.

Figure 6. *Amanita guyanensis* (*Henkel 9767*, type). Bar = 1 cm.

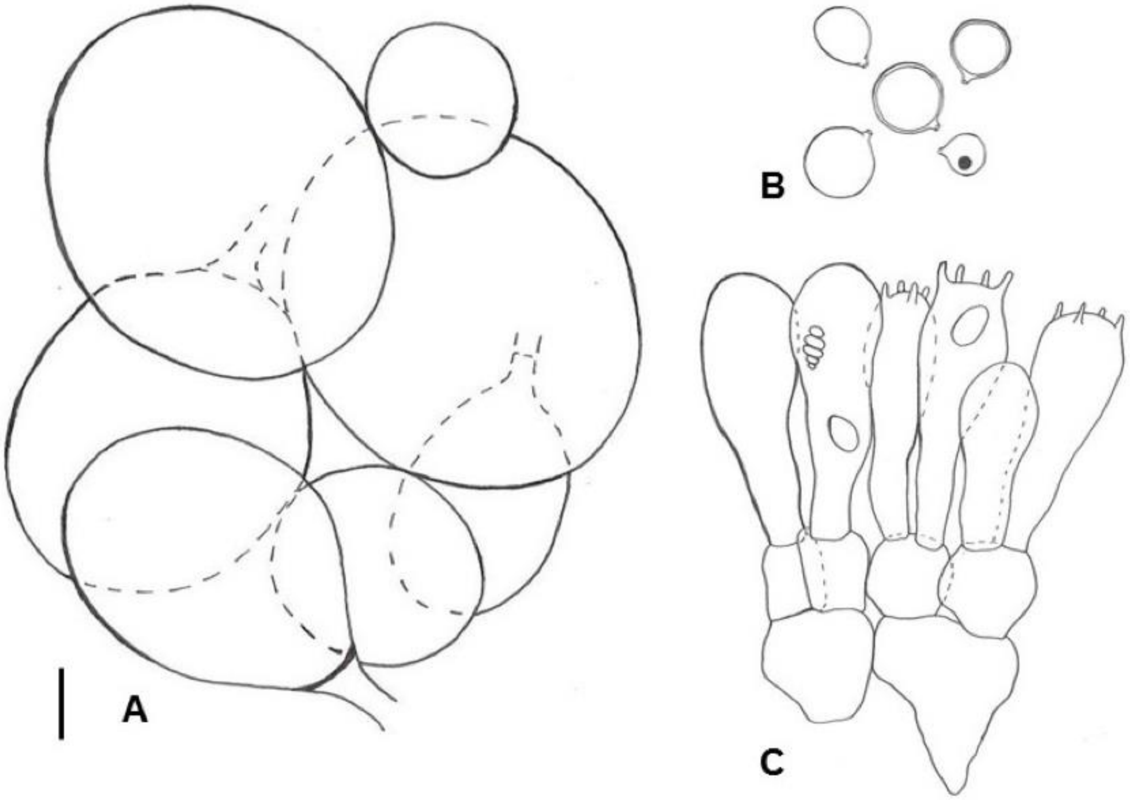

Figure 7. *Amanita guyanensis.* **A**. Basidia and subhymenium. **B.** Basidiospores. **C.** Slightly crushed tissue of volval wart on pileus. Bar = 10 µm.

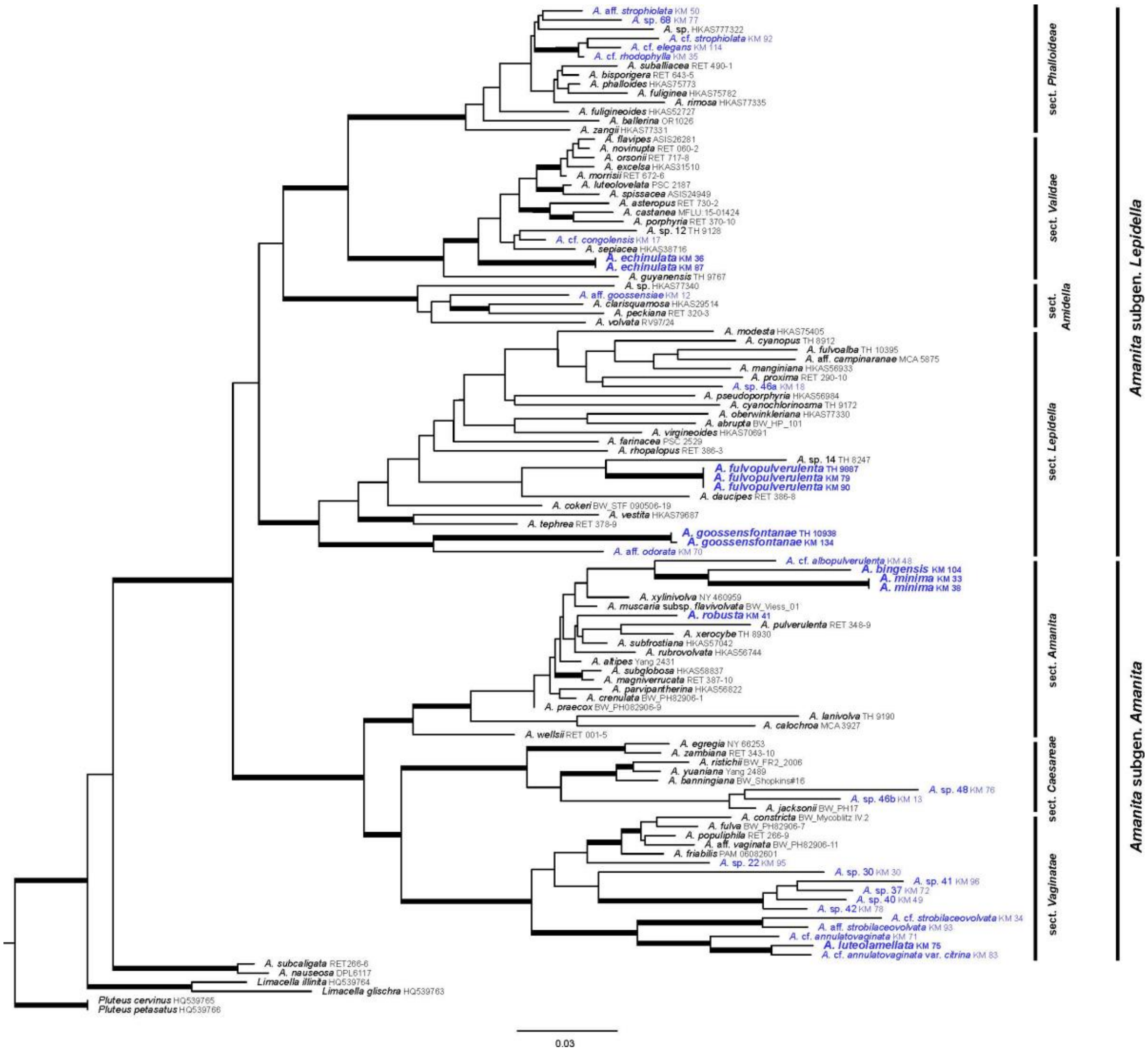

Figure 8. Maximum-likelihood phylogeny generated from the analysis of 28S gene fragments from 104 specimens in *Amanita* subgen. *Amanita* and *Lepidella*. Two free-living *Amanita* species, two *Limacella* species and two *Pluteus* species served as the outgroup. The new species *A. goossensfontanae*, *A. luteomellata* and *A. minima* are resolved to sects. *Lepidella*, *Vaginatae* and *Amanita*, respectively. The epityped species *A. bingensis*, *A. echinulata*, *A. fulvopulverulenta* and *A. robusta*, are resolved to sect. *Amanita*, *Validae*, *Lepidella* and *Amanita*, respectively. Thickened black bars represent

nodes with greater than 70% BS; -- represents no support. New and epityped species are in bold, and all species from Cameroon are in blue.


A
B
Spores blanches
Lolanga
C
D

Figure 9. *Amanita echinulata.* **A.** Field habit (*Mighell 36*) showing the dark brown pileus with pyramidal volval warts, membranous partial veil, and oblique basal bulb. Photo: Noah Siegel. **B.** Original Goossens-Fontana watercolor of the holotype from Beeli (1927). **C.** Original watercolor of collection *Goossens-Fontana 843*. **D.** Epitype (*Mighell 87*) showing mature pileus, trama, and squamulose dark brown volva on basal bulb. Scale bars = 10 mm.

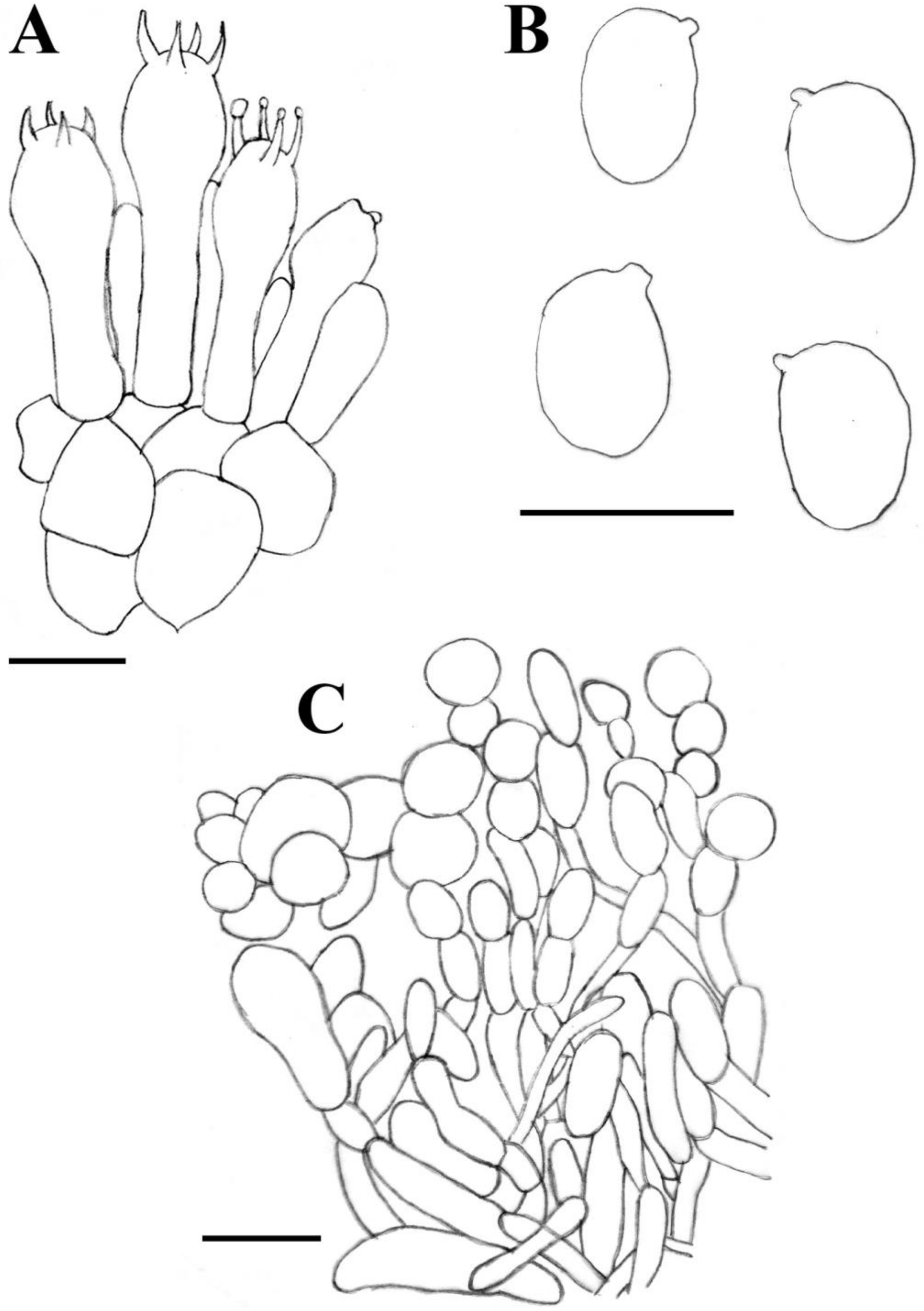

Figure 10. *Amanita echinulata.* **A.** Basidia and subhymenium. Scale bar = 10 µm. **B.** Basidiospores. Scale bar = 10 µm. **C.** Slightly crushed tissue from universal veil. Scale bar = 50 µm.

Figure 11. *Amanita fulvopulverulenta.* **A.** Epitype (*Henkel 9887*) illustrating subsaccate volva ascending stipe, partial veil, red color, basal bulb, and pulverulent volva on pileus. Scale bar = 20 mm. **B.** Field habit (*Henkel 10875*) showing volval limbs still attached to apex of bulb. Scale bar = 10 mm. Photo: Noah Siegel. **C.** Original Goossens-Fontana watercolor of the holotype (*Goossens-Fontana 775*) from Beeli (1931).

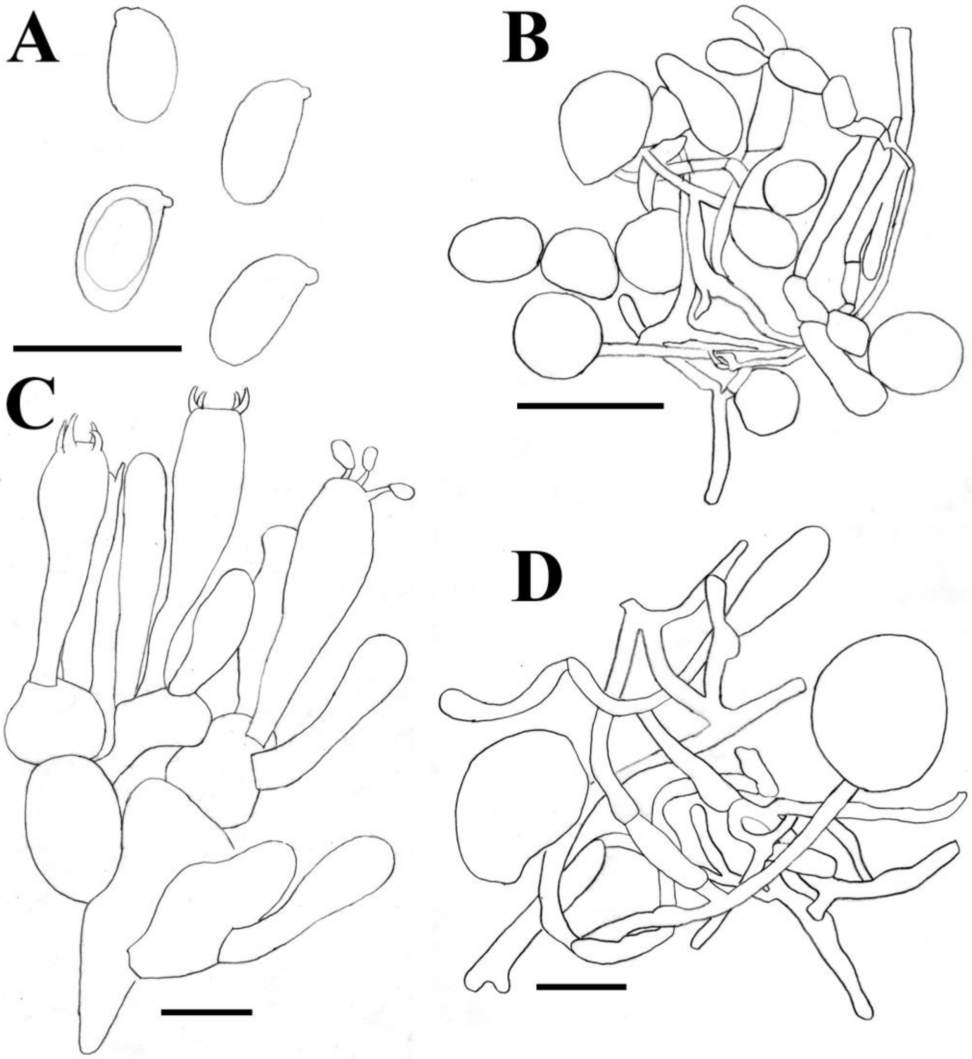

Figure 12. *Amanita fulvopulverulenta.* **A.** Basidiospores. Scale bar = 10 µm. **B.** Slightly crushed tissue from the universal veil from pileus. Scale bar = 50 µm. **C.** Basidia and subhymenium. Scale bar = 10 µm. **D.** Slightly crushed tissue from membranous part of the universal veil on the stipe. Scale bar = 20 µm.

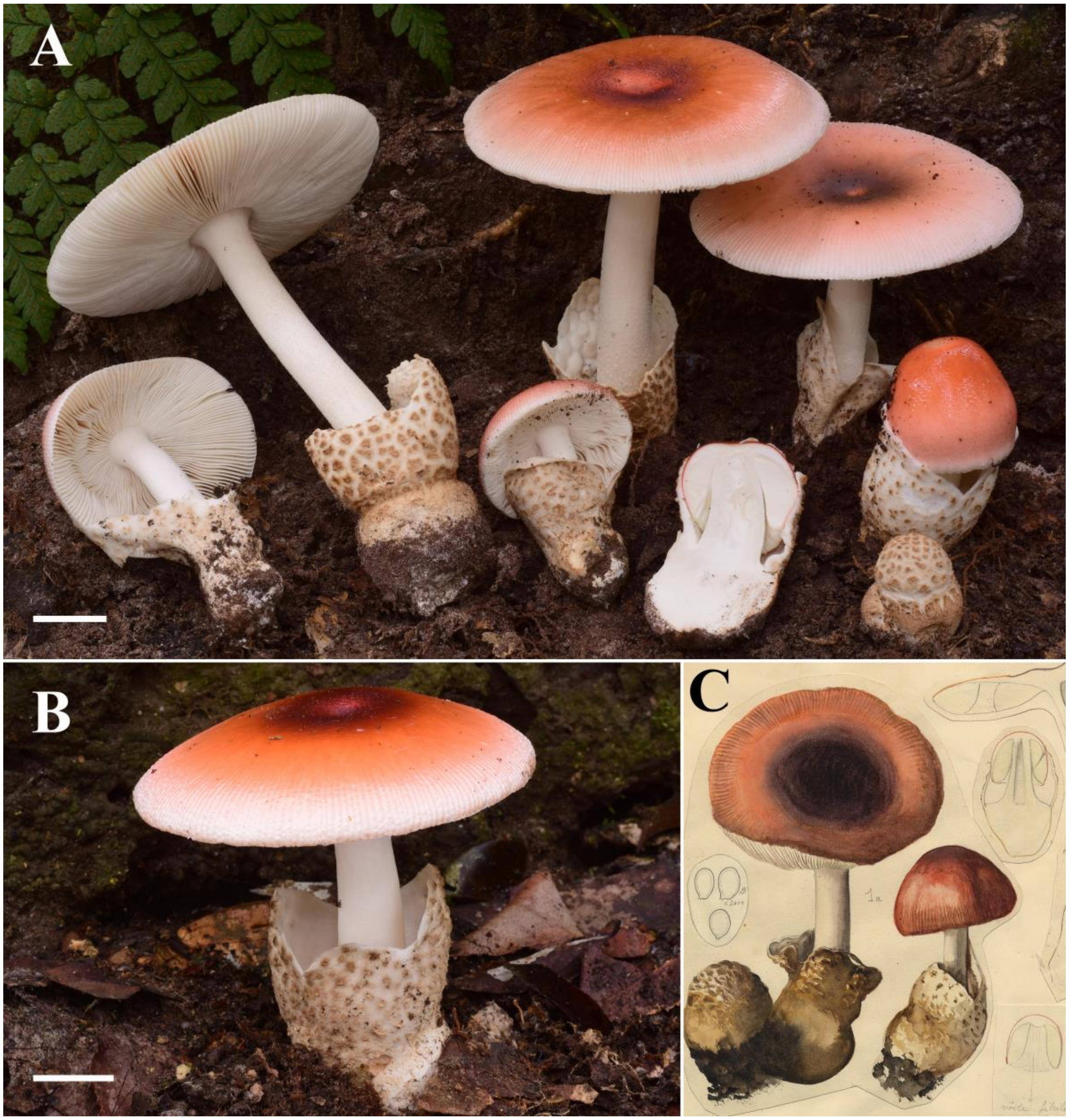

Figure 13. *Amanita robusta.* **A.** Epitype (*Mighell 41*) showing the bright pink-orange pileus with violaceous ring over disc, large basal bulb, and thick, verrucose membranous volva. Photo: Noah Siegel. **B.** Field habit (*Mighell* 47). Photo: Noah Siegel. **C.** Original Goossens-Fontana watercolor of the holotype (*Goossens-Fontana 732*) from Beeli (1931). Scale bars = 20 mm

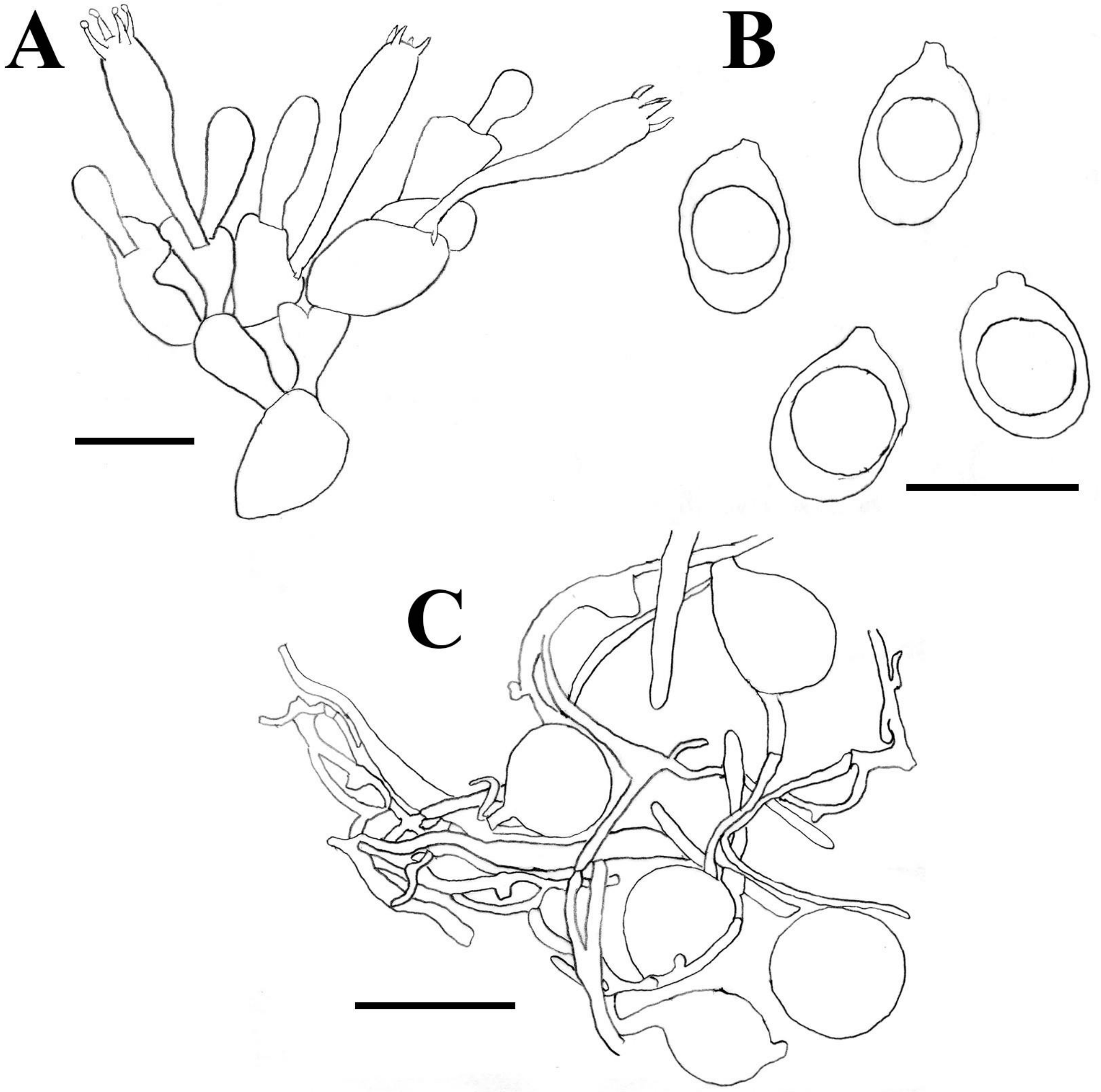

Figure 14. *Amanita robusta.* **A.** Basidia and subhymenium. Scale bar = 10 μm **B.** Basidiospores. Scale bar = 10 μm. **C.** Slightly crushed tissue from universal veil at stipe base. Scale bar = 50 μm.

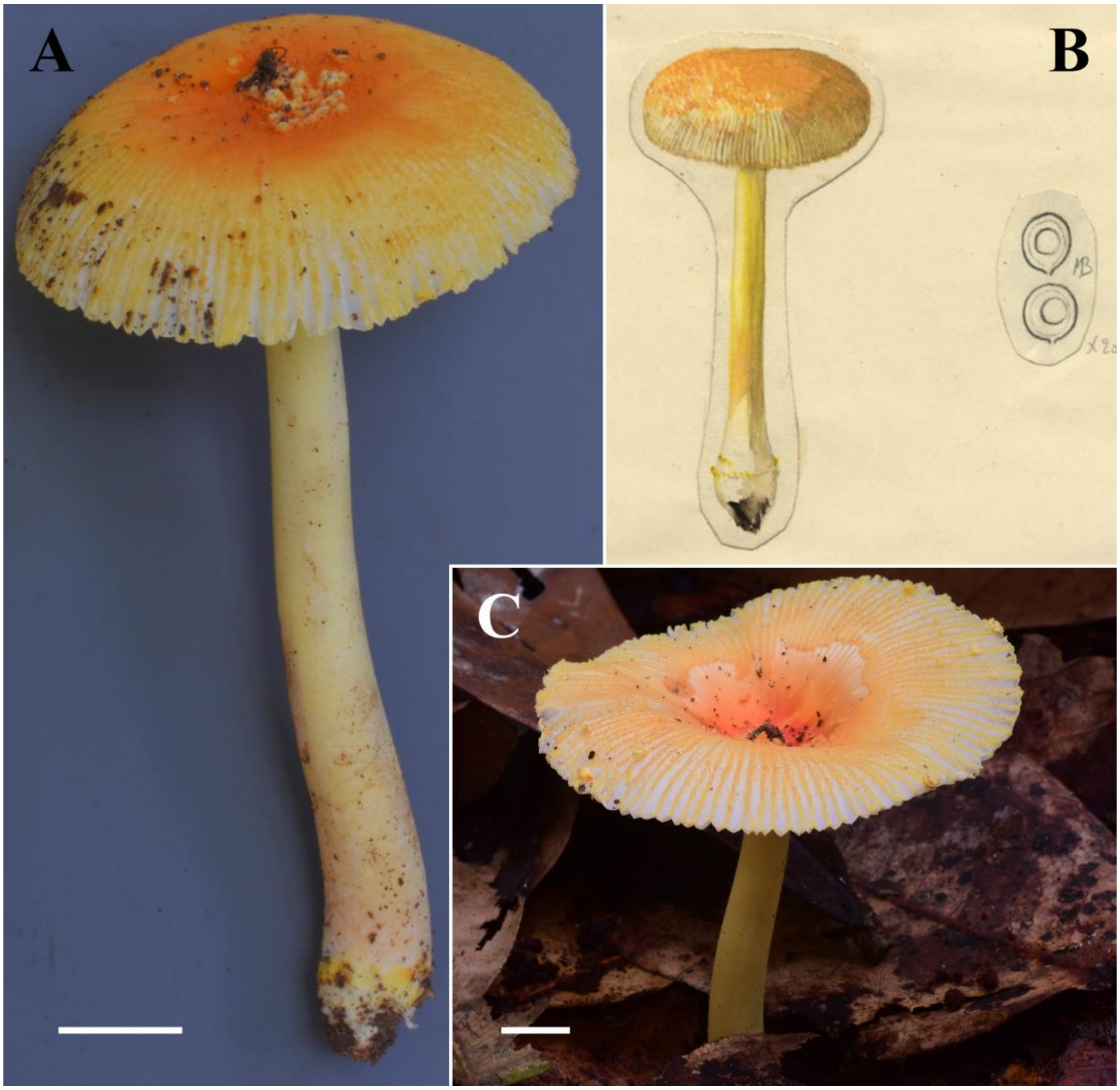

Figure 15. *Amanita bingensis.* **A.** Epitype (*Mighell 104*) showing the bright yellow to orange volva as powder on the pileus, coalescing into warts over the disc, and as a scant ring near the base of the stipe. **B.** Original Goossens-Fontana watercolor of the holotype (*Goossens-Fontana 890*) from Beeli (1931). **C.** Field habit (*Mighell 54*). Photo: Noah Siegel. Scale bars = 10 mm.

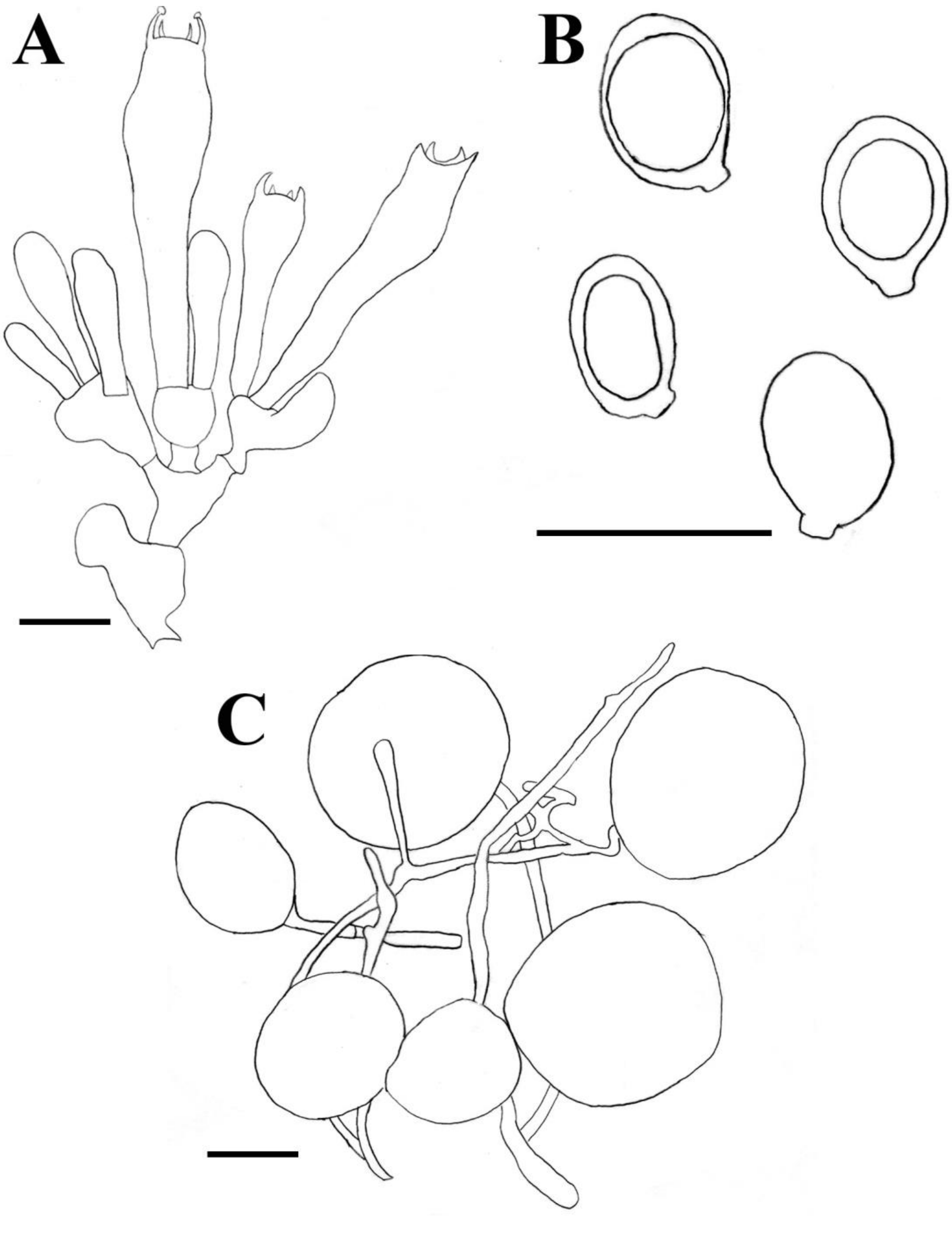

Figure 16. *Amanita bingensis.* **A.** Basidia and subhymenium. Scale bar = 10 μm. **B.** Basidiospores. Scale bar = 10 μm. **C.** Slightly crushed tissue from the universal veil. Scale bar = 20 μm.

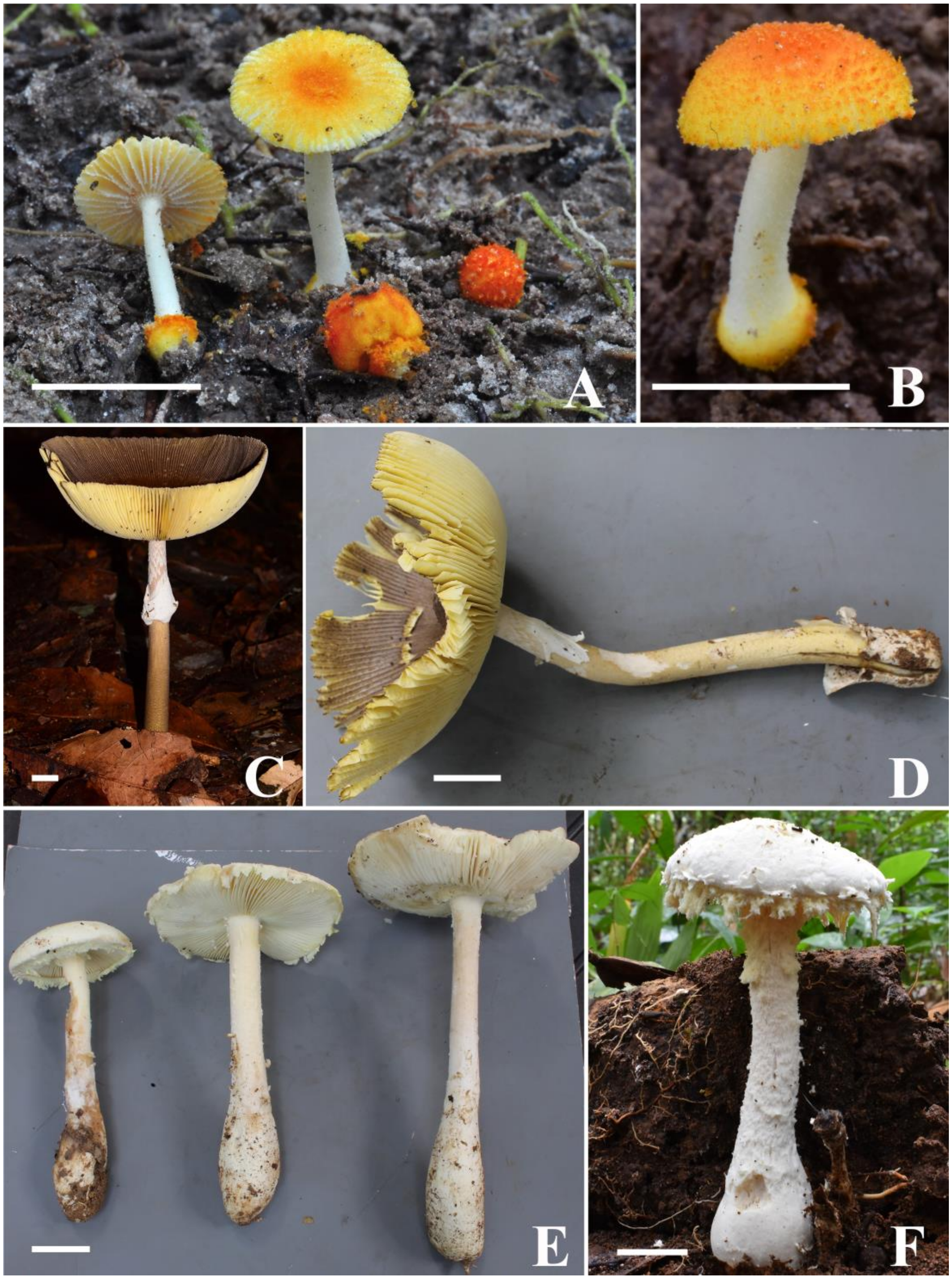

Figure 17. Basidiomata of new species of *Amanita* from Cameroon. *Amanita minima*. **A.** *Henkel 10829*. Photo: Noah Siegel. **B.** Holotype, *Mighell 38*. Scale bars = 10 mm.

Amanita leucolamellata. **C.** Holotype, *Mighell 75*. Photo: Noah Siegel. **D.** *Mighell 56*. Scale bars = 20 mm. *Amanita goossens-fontanae.* **E.** Holotype, *Henkel 10938*. **F.** *Mighell 110*. Photo: Noah Siegel. Scale bars = 20 mm.

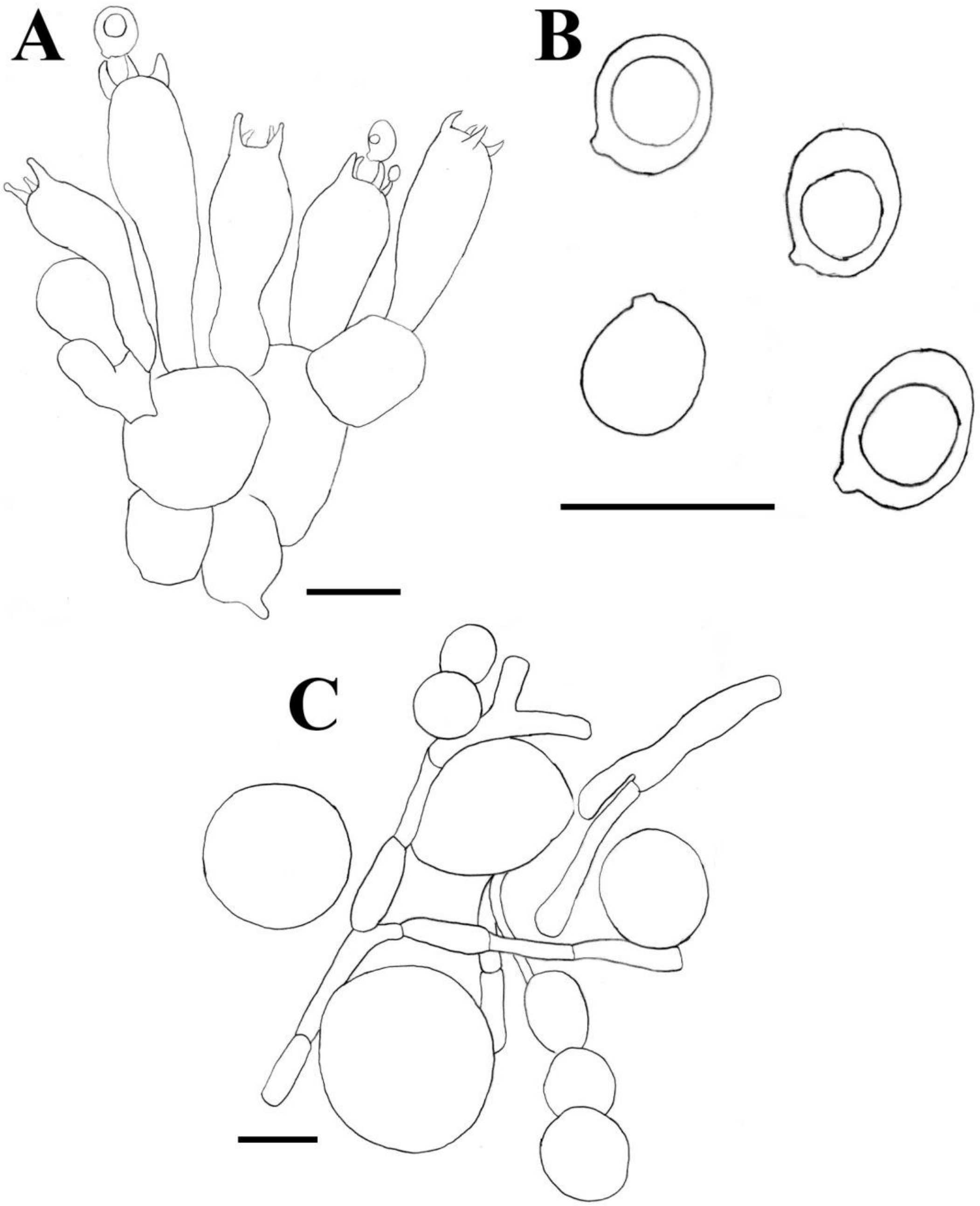

Figure 18. *Amanita minima.* **A.** Basidia and subhymenium. Scale bar = 10 µm. **B.** Basidiospores. Scale bar = 10 µm. **C.** Slightly crushed tissue from the universal veil. Scale bar = 20 µm.

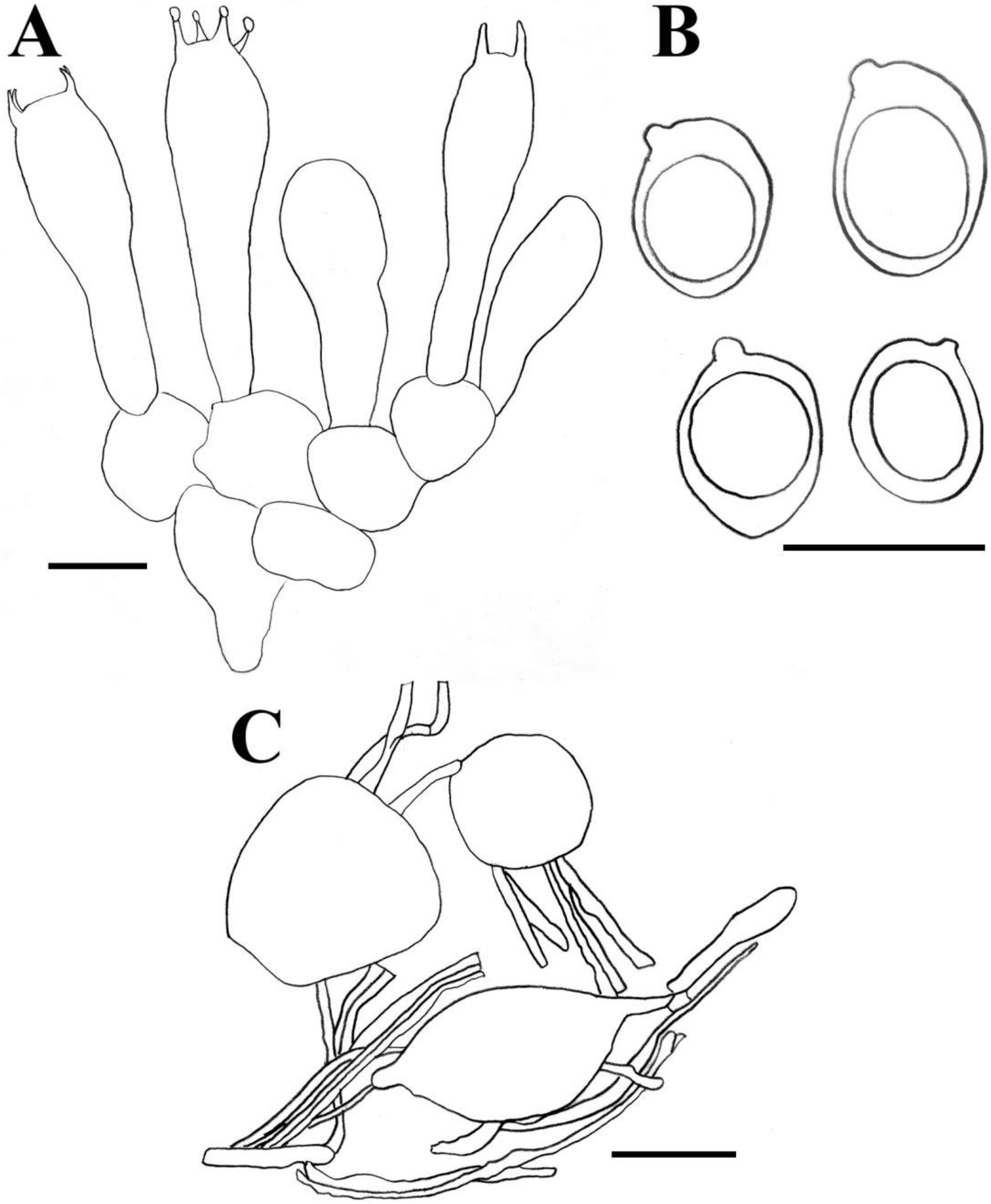

Figure 19. *Amanita luteolamellata.* **A.** Basidia and subhymenium. Scale bar = 10 μm. **B.** Basidiospores. Scale bar = 10 μm. **C.** Slightly crushed tissue from universal veil at stipe base. Scale bar = 50 μm.

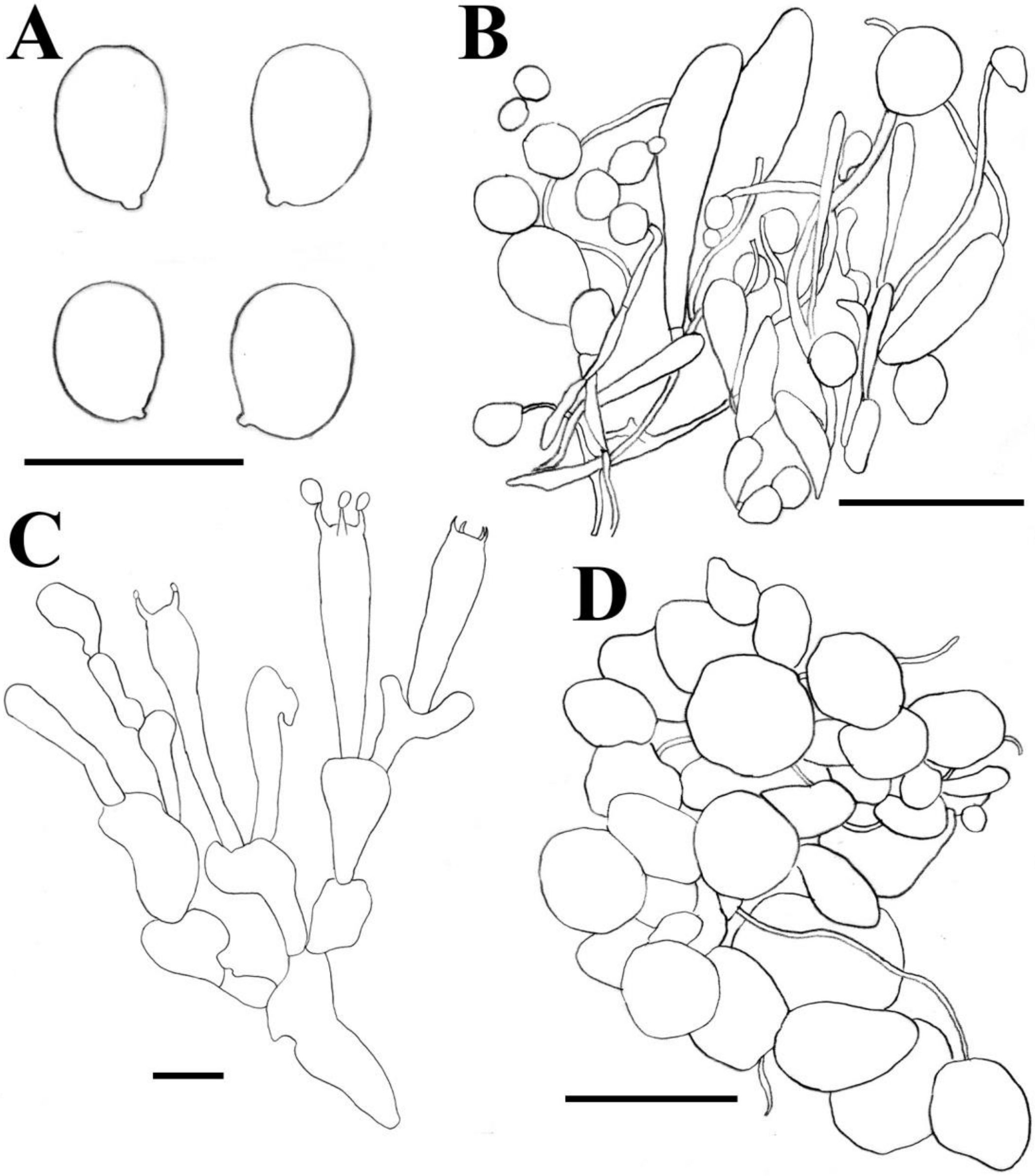

Figure 20. *Amanita goossens-fontanae.* **A.** Basidiospores. Scale bar = 10 µm. **B.** Slightly crushed tissue from universal veil on stipe. Scale bar = 100 µm. **C.** Basidia and subhymenium. Scale bar = 10 µm. **D.** Slightly crushed tissue from universal veil on pileus. Scale bar = 50 µm.

TABLES

Table 1. Taxa, voucher information and GenBank accession numbers for specimens used in the phylogenetic analysis, chapter 1. Taxa described here and type specimens are indicated in bold.

Taxon	Voucher	Locality	nrLSU	*rpb2*	*ef1-α*	β–*tubulin*
Amanita abrupta	BW_HP_101	Massachusetts, USA	HQ539660[a]	--	--	--
Amanita asteropus	RET 730-2	France	KY274804[b]	--	--	--
Amanita aurantiobrunnea	MCA 4420	Guyana	MK105506[c]	MK092931[c]	--	MK092938[c]
Amanita ballerina	OR1026	Thailand	MH157079[d]	KY656884[e]	--	KY656865[e]
Amanita bisporigera	RET 377-9	Tennessee, USA	KJ466434[f]	--	KJ481936[f]	KJ466501[f]
Amanita brunneolocularis	ANDES_F313 NVE57	Colombia	FJ890044[g]	--	--	--
Amanita brunnescens	PBM 2429	AY631902[h]	AY780936[h]	AY881021[h]	--	
Amanita calochroa	MCA 3927	Guyana	KC155375[i]	--	--	--
Amanita aff. *campinaranae*	MCA 5878	Guyana	MK105507[c]	MK092934[c]	MK092947[c]	MK092940[c]
Amanita castanea	MFLU:15-01424	Thailand	KU877539[j]	--	--	--
Amanita chlorinosma	RET 328-6	New York, USA	HQ539676[a]	--	--	--
Amanita citrina	ANDES_F2117_ NVE616	Colombia	KT008032[k]	--	--	--
Amanita cokeri	BW_STF 090506-19	Massachusetts, USA	HQ593113[a]	--	--	--
Amanita congolensis	RET 346-6	Gambia	HQ539736[a]	--	--	--
Amanita clarisquamosa	HKAS29514	AF024448[l]	--	--	--	--
Amanita clelandii	PSC 2524	Australia	HQ539680[a]	--	--	--
Amanita cf. *cruetilemurum*	RET 600-3	California, USA	KP711840[m]	--	--	--

Taxon	Voucher	Locality	nrLSU	*rpb2*	*ef1-α*	β–*tubulin*
Amanita cyanochlorinosma	MCA 3962	Guyana	MK105495[c]	MK092931[c]	MK092943[c]	MK092936[c]
			MK105496[c]	--	--	--
Amanita cyanochlorinosma	TH 9172	Guyana	MK105493[c]	MK092933[c]	MK092945[c]	MK092939[c]
			MK105494[c]	--	--	--
Amanita cyanopus	TH 8912	Guyana	KT339210[n]	--	--	--
Amanita daucipes	RET 386-8	Pennsylvania, USA	HQ539688[a]	--	--	--
Amanita eriophora	RET 350-4	Cambodia	HQ539672[a]	--	--	--
Amanita excelsa	HKAS31510	China	AY436491[o]	--	--	--
Amanita exitialis	HKAS75775	China	JX998053[p]	KJ466592[f]	JX998002[p]	KJ466504[f]
Amanita farinacea	PSC 2529	Australia	HQ539692[a]	--	--	--
Amanita flavipes	ASIS26281	KU139456[q]	--	--	--	--
Amanita franchetii	JM 96/27	North Carolina, USA	AF097381[r]	--	--	--
Amanita aff. *fritillaria*	HKAS56832	China	KJ466479[f]	KJ466644[f]	KJ481979[f]	KJ466558[f]
Amanita fuliginea	HKAS75782	China	JX998049[p]	KJ466597[f]	JX997996[p]	KJ466509[f]
Amanita fuligineoides	HKAS52727	China	JX998047[p]	KJ466599[f]	--	KJ466511[f]
Amanita fulvoalba	MCA 6920	Guyana	MK105498[c]	--	--	--
Amanita fulvoalba	TH 8056	Guyana	MK105499[c]	MK092925[c]	--	--
Amanita fulvoalba	TH 8455	Guyana	MK105500[c]	MK092927[c]	--	--
Amanita fulvoalba	TH 9043	Guyana	MK105501[c]	MK092928[c]	MK092946[c]	--
Amanita fulvoalba	TH 10395	Guyana	MK105497[c]	MK092926[c]	MK092942[c]	--
Amanita guyanensis	MCA 3155	Guyana	MK105504[c]	--	--	MK092935[c]
Amanita guyanensis	TH 9767	Guyana	MK105502[c]	MK092929[c]	MK092948[c]	MK092937[c]
Amanita guyanensis	TH 9772	Guyana	MK105503[c]	MK092930[c]	MK092944[c]	MK092941[c]
Amanita lanivolva	TH 9190	Guyana	KT339292[n]	--	--	--
Amanita lavendula	RET 639-7	Ontario, Canada	KR865979[m]	--	--	--

Taxon	Voucher	Locality	nrLSU	*rpb2*	*ef1-α*	β–*tubulin*
Amanita luteofusca	PSC 1093b	Australia	HQ539705[a]	--	--	--
Amanita luteolovelata	PSC 2187	Australia	HQ539706[a]	--	--	--
Amanita manginiana	HKAS56933	China	KJ466438[f]	KJ466603[f]	KJ481943[f]	KJ466515[f]
Amanita modesta	HKAS79688	China	KJ466440[f]	KJ466605[f]	KJ481944[f]	KJ466516[f]
Amanita morrisii	RET 672-6	New Jersey, USA	KR919770[m]	--	--	--
Amanita novinupta	RET 60-2	Oregon, USA	KU248118[m]	--	--	--
Amanita oberwinkleriana	HKAS77330	China	KJ466441[f]	KJ466606[f]	KJ481946[f]	--
Amanita ocreata	HKAS79686	California, USA	KJ466442[f]	KJ466607[f]	KJ481947[f]	KJ466518[f]
Amanita aff. *odorata*	KM 70	Cameroon	MK105505[c]	--	--	--
Amanita orsonii	RET 717-8	India	KX270345[b]	--	--	--
Amanita pallidorosea	HKAS75786	China	JX998054[p]	KJ466627[f]	JX998011[p]	KJ466539[f]
Amanita parvipantherina	HKAS56822	China	JN941163[s]	JQ031115[s]	KJ482005[f]	KJ466566[f]
Amanita peckiana	RET 320-3	New York, USA	HQ539720[a]	--	--	--
Amanita phalloides	HKAS75773	California, USA	JX998060[p]	KJ466612[f]	JX998000[p]	KJ466523[f]
Amanita porphyria	RET 370-10	Newfoundland, Canada	KP866187[t]	--	--	--
Amanita proxima	RET 290-10	France	HQ539728[a]	--		--
Amanita pseudoporphyria	HKAS56984	China	KJ466451[f]	KJ466613[f]	KJ481952[f]	KJ466524[f]
Amanita rhoadsii	DD97/13	North Carolina, USA	AF097391[r]	--	--	--
Amanita rhopalopus	BW_RET 386-3	West Virginia, USA	HQ539733[a]	--	--	--
Amanita rimosa	HKAS77335	China	KJ466455[f]	KJ466393[f]	KJ481957[f]	KJ466532[f]
Amanita rubrovolvata	HKAS56744	China	JN941156[u]	JQ031117[s]	--	--
Amanita sepiacea	ASIS26353	KU139443[q]	--	--	--	--
Amanita sepiacea	HKAS38716	China	AY436501[o]	--	--	--
Amanita sp.	HKAS77321	China	KJ466481[f]	KJ466646[f]	--	KJ466560[f]
Amanita sp.	HKAS77322	Ohio, USA	KJ466470[f]	KJ466650[f]	KJ481984[f]	KJ466564[f]

Taxon	Voucher	Locality	nrLSU	*rpb2*	*ef1-α*	β–*tubulin*
Amanita sp.	HKAS77339	South Korea	KJ466482[f]	KJ466647[f]	KJ481981[f]	KJ466561[f]
Amanita sp.	HKAS77340	China	KJ466483[f]	KJ466648[f]	KJ481982[f]	KJ466562[f]
Amanita sp.	HKAS77344	China	KJ466465[f]	KJ466634[f]	KJ481969[f]	KJ466548[f]
Amanita sp. 12	TH 9128	Guyana	JN168681[v]	--	--	--
Amanita sp. 14	TH 8247	Guyana	KT339281[n]	--	--	--
Amanita cf. *spissacea*	OR1214	Thailand	KY747478[e]	KY656886[e]	--	KY656867[e]
Amanita suballiacea	RET 491-7	Michigan, USA	KJ466486[f]	KJ466602[f]	KJ481942[f]	KJ466514[f]
Amanita cf. *subcokeri*	RET 97-3	New Jersey, USA	HQ539747[a]	--	--	--
Amanita subfrostiana	HKAS57042	China	JN941162[s]	JQ031118[s]	KJ482003[f]	KJ466565[f]
Amanita subglobosa	HKAS58837	China	JN941152[s]	JQ031121[s]	KJ482004[f]	KJ466567[f]
Amanita subjunquillea	HKAS77325	China	KJ466490[f]	KJ466656[f]	KJ481988[f]	KJ466574[f]
Amanita cf. *tephrea*	RET 378-9	New York, USA	HQ539751[a]	--	--	--
Amanita vestita	HKAS79687	China	KJ466494[f]	KJ466662[f]	KJ481995[f]	KJ466581[f]
Amanita virgineoides	HKAS79691	China	KJ466495[f]	KJ466663[f]	KJ481996[f]	KJ466582[f]
Amanita virosa	HKAS56694	Finland	JX998058[p]	KJ466664[f]	JX998007[p]	KJ466583[f]
Amanita volvata	RV97/24	Virginia, USA	AF097388[r]	--	--	--
Amanita westii	BW_SH26	Texas, USA	HQ539759[a]	--	--	--
Amanita xerocybe	TH 8930	Guyana	KC155384[i]	--	--	--
Amanita zangii	HKAS77331	China	KJ466500[f]	KJ466669[f]	KJ482001[f]	KJ466589[f]

[a] Wolfe *et al.* 2012, *Mycologia* **104**: 22–33; [b] Tulloss RE, Kudzma LV and Goldman NR, unpublished; [c] this study; [d] Raspe O, unpublished; [e] Thongbai *et al.* 2017, *PloS One* **12**: e0182131; [f] Cai *et al.* 2014, *Mycol Prog* **13**: 1008; [g] Vargas N, Grajales A, Bernal A, Sarria V, Jaimes AP, Cepero de GMC, Franco-Molano AE, Restrepo S, unpublished; [h] Matheny PB, Hibbett DS, unpublished; [i] Smith *et al.* 2013, *PLoS One* **8**: e55160.; [j] Thongbai *et al.* 2016, *Phytotaxa* **286**: 211–231; [k] Vargas N, Pardo-da La Hoz CJ, Franco-Molano AE, Jimenez P, Restrepo S, Grajales A, unpublished; [l] Weiss *et al.* 1998, *Can J Bot* **76**: 1170–1179; [m] Tulloss RE, Kudzma LV, Rodriguez Caycedo CE, Goldman NR, unpublished; [n] Smith *et al.* 2017, *New Phytol* **215** 443–

453; [o]Zhang *et al.* 2004, *Fungal Diversity* **17**: 219─238; [p]Cai *et al.* 2012, *Plant Diversity and Resources* **34**: 614─622; [q]Seok SJ, Jin YJ, Yoo KB, Kwon SW, unpublished; [r]Drehmel *et al.* 1999, *Mycologia* 91: 610─618; [s]Schoch *et al*. 2012, *Proc Nat Acad Sci USA* **109**: 6241–6246; [t]Hughes KW, Tulloss RE, Rodriguez Caycedo C, Goldman N, unpublished; [u]Weiss M, unpublished; [v]Smith et al. 2011, *New Phytol* **192**: 699─712

Table 2. Taxa, voucher information and GenBank accession numbers for specimens used in the phylogenetic analysis, chapter 2. Taxa described here and type specimens are indicated in bold.

Species	Collection	Location	28S	ITS
A. abrupta	BW_HP_101	Massachusetts, USA	HQ539660[a]	
A. cf. *albopulverulenta*	KM 48	DBR, Cameroon	MT446282[b]	
A. altipes	Yang 2431	Sichuan, China	HQ539667[a]	
A. cf. *annulatovaginata*	KM 71	DBR, Cameroon	MT446265[b]	
A. cf. *annulatovaginata var. citrina*	KM 83	DBR, Cameroon	MT446265[b]	
A. asteropus	RET 730-2	France	KY274804[c]	
A. ballerina	OR1026	Thailand	MH157079[d]	
A. banningiana	BW_Shopkins#16	New Jersey, USA	HQ539671[a]	
A. bingensis	**KM 104**	DBR, Cameroon	MT446281[b]	MT446264[b]
A. bisporigera	RET 643-5	Tennessee, USA	KJ466434[e]	
A. calochroa	MCA 3927	Guyana	KC155375[f]	
A. aff. *campinaranae*	MCA 5875	Guyana	MK105507[f]	
A. castanea	MFLU:15-01424	Thailand	KU877539[g]	
A. clarisquamosa	HKAS29514		AF024448[h]	
A. cokeri	BW_STF 090506-19	Massachusetts, USA	HQ593113[a]	
A. cf. *congolensis*	KM 17	DBR, Cameroon	MT446292[b]	
A. constricta	BW_Mycoblitz IV.2	California, USA	HQ539684[a]	
A. crenulata	BW_PH82906-1	Massachusetts, USA	HQ539687[a]	
A. cyanochlorinosma	TH 9172	Guyana	MK105493[f]	
A. cyanopus	TH 8912	Guyana	KT339210[f]	
A. daucipes	RET 386-8	Pennsylvania, USA	HQ539688[a]	
A. echinulata	KM 36	DBR, Cameroon	MT446290[b]	

Species	Collection	Location	28S	ITS
A. echinulata	**KM 87**	DBR, Cameroon	MT446291[b]	MT446259[b]
A. echinulata	KM 141	DBR, Cameroon		MT446255[b]
A. echinulata	TH 9920	DBR, Cameroon		MT446256[b]
A. echinulata	TH 10253	DBR, Cameroon		MT446257[b]
A. egregia	NY 66253	Queensland, Australia	HQ539690[a]	
A. cf. *elegans*	KM 114	DBR, Cameroon	MT446294[b]	
A. excelsa	HKAS31510	China	AY436491[i]	
A. farinacea	PSC 2529	Australia	HQ539692[a]	
A. flavipes	ASIS26281		KU139456[j]	
A. friabilis	PAM 06082601	Savoie, France	HQ539695[a]	
A. fuliginea	HKAS75782	China	JX998049[k]	
A. fuligineoides	HKAS52727	China	JX998047[k]	
A. fulva	BW_PH82906-7	Massachusetts, USA	HQ539697[a]	
A. fulvoalba	TH 10395	Guyana	MK105497[f]	
A. fulvopulverulenta	KM 79	DBR, Cameroon	MT446286[b]	
A. fulvopulverulenta	KM 90	DBR, Cameroon	MT446287[b]	
A. fulvopulverulenta	**TH 9887**	DBR, Cameroon	MT446285[b]	MT446250[b]
A. fulvopulverulenta	TH10323	DBR, Cameroon		MT446251[b]
A. goossensfontanae	**TH 10938**	DBR, Cameroon	MT446283[b]	MT446262[b]
A. goossensfontanae	KM 134	DBR, Cameroon	MT446284[b]	MT446263[b]
A. aff. *goossensiae*	KM 12	DBR, Cameroon	MT446289[b]	
A. guyanensis	TH 9767	Guyana	MK105502[f]	
A. jacksonii	BW_PH17	Massachusetts, USA	HQ539703[a]	
A. lanivolva	TH 9190	Guyana	KT339292[f]	
A. luteolamellata	**KM 75**	DBR, Cameroon	MT446266[b]	MT446258[b]

Species	Collection	Location	28S	ITS
A. luteovelata	PSC 2187	Australia	HQ539706[a]	
A. magniverrucata	RET 387-10	California, USA	HQ539712[a]	
A. manginiana	HKAS56933	China	KJ466438[e]	
A. minima	Dja 70	DBR, Cameroon		MT446260[b]
A. minima	KM 33	DBR, Cameroon	MT446279[b]	
A. minima	**KM 38**	DBR, Cameroon	MT446280[b]	MT446261[b]
A. modesta	HKAS75405	China	KJ466440[e]	
A. morrisii	RET 672-6	New Jersey, USA	KR919770[l]	
A. muscaria subsp. *flavivolvata*	BW_Viess_01	California, USA	HQ539712[a]	
A. novinupta	RET 060-2	Oregon, USA	KU248118[l]	
A. oberwinkleriana	HKAS77330	China	KJ466441[e]	
A. aff. *odorata*	KM 70	DBR, Cameroon	MK105505[b]	
A. orsonii	RET 717-8	India	KX270345[c]	
A. parvipantherina	HKAS56822	China	JN941163[m]	
A. peckiana	RET 320-3	New York, USA	HQ539720[a]	
A. phalloides	HKAS75773	California, USA	JX998060[k]	
A. populiphila	RET 266-9	Colorado, USA	HQ539724[a]	
A. porphyria	RET 370-10	Newfoundland, Canada	KP866187[n]	
A. praecox	BW_PH082906-9	Massachusetts, USA	HQ539725[a]	
A. proxima	RET 290-10	Gironde, France	HQ539728[a]	
A. pseudoporphyria	HKAS56984	China	KJ466451[e]	
A. pulverulenta	RET 348-9	Northern Province, Zambia	HQ539731[a]	
A. cf. *rhodophylla*	KM 35	DBR, Cameroon	MT446293[b]	
A. rhopalopus	RET 386-3	West Virginia, USA	HQ539733[a]	
A. rimosa	HKAS77335	China	KJ466455[e]	

Species	Collection	Location	28S	ITS
A. ristichii	BW_FR2_2006	Massachusetts, USA	HQ539734[a]	
A. robusta	**KM 41**	DBR, Cameroon	MT446278[b]	MT446252[b]
A. robusta	KM 143	DBR, Cameroon		MT446253[b]
A. robusta	TH 9945	DBR, Cameroon		MT446254[b]
A. rubrovolvata	HKAS56744	China	JN941156[o]	
A. sepiacea	HKAS38716	China	AY436501[i]	
A. sp.	HKAS777322	Ohio, USA	KJ466470[e]	
A. sp.	HKAS77340	China	KJ466483[e]	
A. sp. 12	TH 9128	Guyana	JN168681[p]	
A. sp. 14	TH 8247	Guyana	KT339281[q]	
A. sp. 22	KM 95	DBR, Cameroon	MT446275[b]	
A. sp. 30	KM 30	DBR, Cameroon	MT446274[b]	
A. sp. 37	KM 72	DBR, Cameroon	MT446272[b]	
A. sp. 40	KM 49	DBR, Cameroon	MT446271[b]	
A. sp. 41	KM 96	DBR, Cameroon	MT446273[b]	
A. sp. 42	KM 78	DBR, Cameroon	MT446270[b]	
A. sp. 46a	KM 18	DBR, Cameroon	MT446288[b]	
A. sp. 46b	KM 13	DBR, Cameroon	MT446276[b]	
A. sp. 48	KM 76	DBR, Cameroon	MT446277[b]	
A. sp. 68	KM 77	DBR, Cameroon	MT446296[b]	
A. spissacea	ASIS24949	Thailand	KY747478[g]	
A. cf. *strobilaceovolvata*	KM 34	DBR, Cameroon	MT446269[b]	
A. aff. *strobilaceovolvata*	KM 93	DBR, Cameroon	MT446268[b]	
A. aff. *strophiolata*	KM 50	DBR, Cameroon	MT446297[b]	
A. cf. *strophiolata*	KM 92	DBR, Cameroon	MT446295[b]	

Species	Collection	Location	28S	ITS
A. suballiacea	RET 490-1	Michigan, USA	KJ466486[e]	
A. subfrostiana	HKAS57042	China	JN941162[m]	
A. subglobosa	HKAS58837	China	JN941152[m]	
A. tephrea	RET 378-9	New York, USA	HQ539751[a]	
A. aff. *vaginata*	BW_PH82906-11	Massachusetts, USA	HQ539754[a]	
A. vestita	HKAS79687	China	KJ466494[e]	
A. virgineoides	HKAS70691	China	KJ466495[e]	
A. volvata	RV97/24	Virginia, USA	AF097388[r]	
A. wellsii	RET 001-5	Maine, USA	HQ539758[a]	
A. xerocybe	TH 8930	Guyana	KC155384[s]	
A. xylinivolva	NY 460959	San José, Costa Rica	HQ539760[a]	
A. yuaniana	Yang 2489	Yunnan, China	HQ539761[a]	
A. zambiana	RET 343-10	Copper Belt Province, Zambia	HQ539762[a]	
A. zangii	HKAS77331	China	KJ466500[e]	

[a] Wolfe *et al.* 2012, *Mycologia* **104**: 22–33; [b] this study; [c] Tulloss RE, Kudzma LV and Goldman NR, unpublished; [d] Raspe, unpubl. data; [e] Cai *et al.* 2014, *Mycol. Prog.* **13**: 1008; [f] Mighell *et al.* 2018, *Fungal Systematics and Evolution* **3**: 1–12; [g] Thongbai *et al.* 2016, *Phytotaxa* **286**: 211─231; [h] Weiss *et al.* 1998, *Can J Bot* **76**: 1170─1179; [i] Zhang *et al.* 2004, *Fungal Diversity* **17**: 219─238; [j] Seok SJ, Jin YJ, Yoo KB, Kwon SW, unpublished; [k] Cai *et al.* 2012, *Plant Diversity and Resources* **34**: 614─622; [l] Tulloss RE, Kudzma LV, Rodriguez Caycedo CE, Goldman NR, unpublished; [m] Schoch *et al*. 2012, *Proc Nat Acad Sci USA* **109**: 6241–6246; ; [n] Hughes KW, Tulloss RE, Rodriguez Caycedo CE, Goldman NR, unpublished; ; [o] Weiss M, unpublished; [p] Smith *et al.* 2011, *New Phytol* **192**: 699─712; [q] Smith *et al.* 2017, *New Phytol* **215** 443─453; [r] Drehmel *et al.* 1999, *Mycologia* 91: 610─618; [s] Smith *et al.* 2013, *PLoS One* **8**: e55160

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