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New species of *Amanita* subgen. *Lepidella* from Guyana

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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-α* are provided for each taxon, and β-*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.

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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 American *Amanita* species remain to be 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 odour of chlorine. *Amanita fulvoalba* sp. nov. produces robust

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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 characterise 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. Colours were described subjectively and coded according to Kornerup & Wanscher (1978), with colour 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-α)* and beta-tubulin (*β-tub*), 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), EF1-983F/EF1-2218R (Rehner & Buckley 2005), Am-*β-tubulin* F/Am-*β-tubulin* R (Cai *et al.* 2014) and Am-6F/Am-7R (Cai *et al.* 2014) were used to amplify ITS, nrLSU, *ef1-α*, *β-tub* and *rpb2*, respectively. Amplification 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 10 µL of clone/sterile water mixture. All amplicons were sequenced. Sequences were edited using Sequencher v. 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-α*, *β-tub* 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. Table 1 gives all taxa, collection information, GenBank numbers, and references for specimens used in the phylogenetic analysis.

Sequences were aligned in MEGA v. 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 v. 1.1.0 (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 were conducted using the program MrBayes 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 M 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

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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).

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

Species	Voucher	Locality	nrLSU	*rpb2*	*ef1-a*	*β-tub*
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]	--	--	--
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]	--	--	--
	TH 8056	Guyana	MK105499[c]	MK092925[c]	--	--
	TH 8455	Guyana	MK105500[c]	MK092927[c]	--	--
	TH 9043	Guyana	MK105501[c]	MK092928[c]	MK092946[c]	--
	TH 10395	Guyana	MK105497[c]	MK092926[c]	MK092942[c]	--
Amanita guyanensis	MCA 3155	Guyana	MK105504[c]	--	--	MK092935[c]
	TH 9767	Guyana	MK105502[c]	MK092929[c]	MK092948[c]	MK092937[c]
	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]	--	--	--
Amanita luteofusca	PSC 1093b	Australia	HQ539705[a]	--	--	--
Amanita luteolovelata	PSC 2187	Australia	HQ539706[a]	--	--	--

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Table 1. (Continued).

Species	Voucher	Locality	nrLSU	*rpb2*	*ef1-a*	*b-tub*
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]	--	--	--
	HKAS38716	China	AY436501[o]	--	--	--
Amanita sp.	HKAS77321	China	KJ466481[f]	KJ466646[f]	--	KJ466560[f]
	HKAS77322	Ohio, USA	KJ466470[f]	KJ466650[f]	KJ481984[f]	KJ466564[f]
	HKAS77339	South Korea	KJ466482[f]	KJ466647[f]	KJ481981[f]	KJ466561[f]
	HKAS77340	China	KJ466483[f]	KJ466648[f]	KJ481982[f]	KJ466562[f]
	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 *et al.*, unpubl. data; [c]this study; [d]Raspe, unpubl. data; [e]Thongbai *et al.* 2017, *PloS One* **12**: e0182131; [f]Cai *et al.* 2014, *Mycol. Prog.* **13**: 1008; [g]Vargas *et al.*, unpubl. data; [h]Matheny *et al.*, unpubl. data; [i]Smith *et al.* 2013, *PLoS One* **8**: e55160.; [j]Thongbai *et al.* 2016, *Phytotaxa* **286**: 211–231; [k]Vargas *et al.*, unpubl. data; [l]Weiss *et al.* 1998, *Canad. J. Bot.* **76**: 1170–1179; [m]Tulloss *et al.*, unpubl. data; [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, unpubl. data; [r]Drehmel *et al.* 1999, *Mycologia* **91**: 610–618; [s]Schoch *et al.* 2012, *Proc. Nat. Acad. Sci. USA* **109**: 6241–6246; [t]Hughes, unpubl. data; [u]Weiss, unpubl. data; [v]Smith *et al.* 2011, *New Phytol.* **192**: 699–712.

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RESULTS

Nine ITS (GenBank accessions: MK064186–MK064193, MK097470), 15 nrLSU, seven *ef1-α*, ten *rpb2* and seven *β-tub* 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-α* was 536, *rpb2* was 669 and *β-tub* 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).

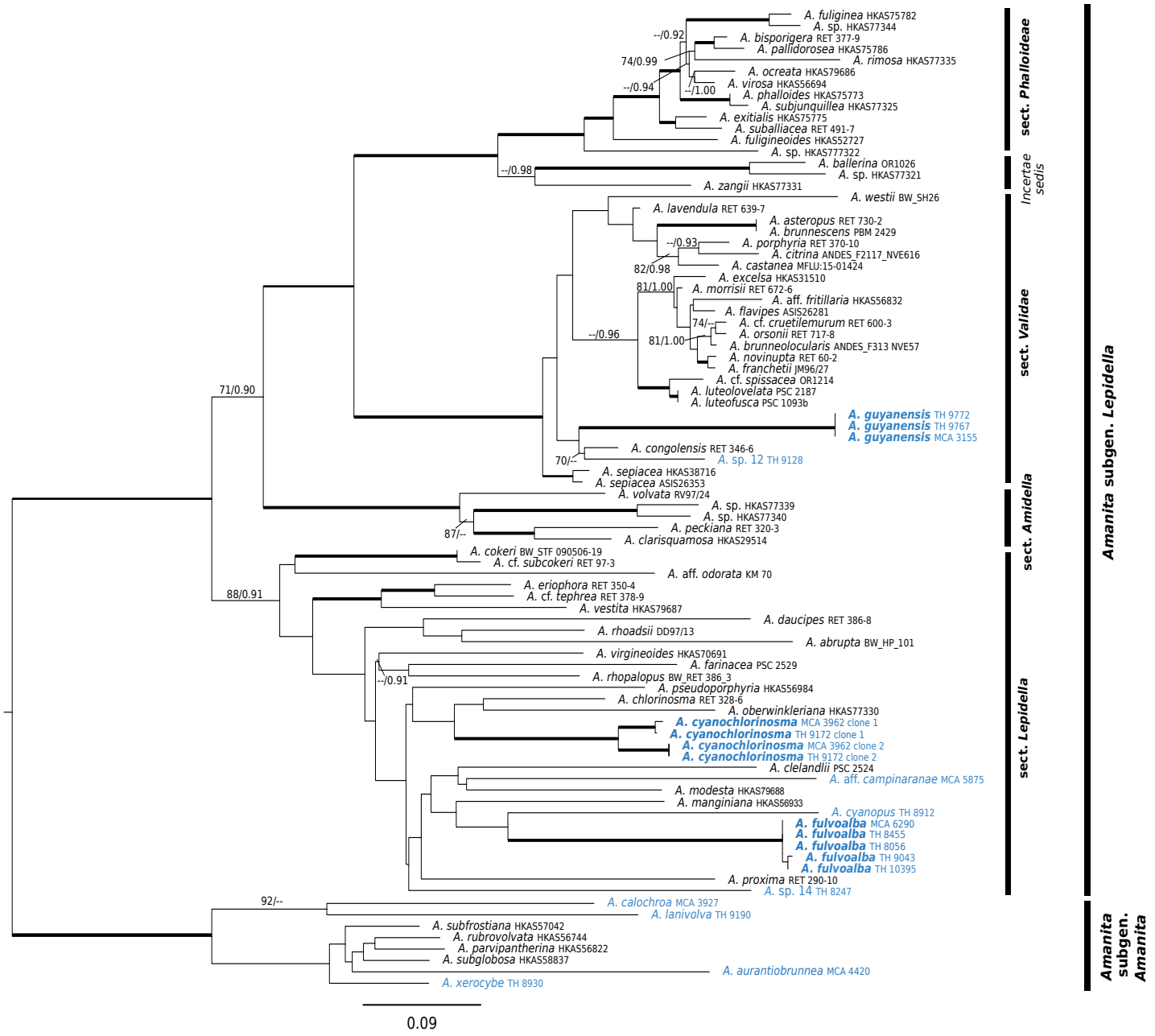

Fig. 1. Maximum-likelihood phylogeny generated from the analysis of four gene fragments (nrLSU, *ef1-α*, β-*tub* and *rpb2*) from 76 specimens in *Amanita* subgen. *Lepidella* and eight outgroup specimens in *Amanita* subgen. *Amanita*. The new species *A. cyanochlorinosma* and *A. fulvoalba* are resolved 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.

TAXONOMY

Amanita cyanochlorinosma Mighell & T.W. Henkel, ***sp. nov.*** MycoBank MB827394. Figs 2, 3.

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

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

Description: *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 concolourous, 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), discoloured 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. *Odour* 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 gelatinised, hyaline, thin-walled, loosely interwoven; subpellis 40–75 µm thick; hyphae 2–8 µm wide, non-gelatinised, 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.

Fig. 2. *Amanita cyanochlorinosma* (*Henkel 9172*, type). Scale bar = 1 cm.

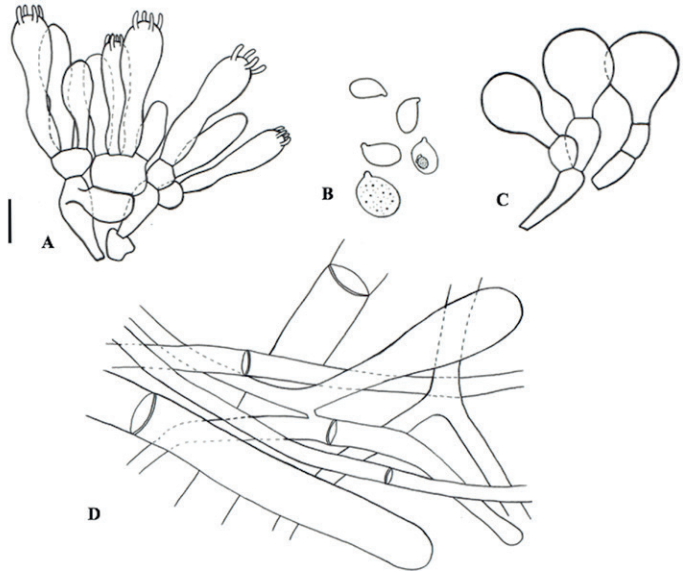

Fig. 3. *Amanita cyanochlorinosma*. **A.** Basidia and subhymenium. **B.** Basidiospores. **C.** Marginal tissue of lamellae. **D.** Slightly crushed tissue from volval limb. Scale bar =10 µm.

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

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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.

Typus: **Guyana**, Region 8 Potato-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* (**holotype** BRG 41298; **isotype** HSC G1229); GenBank accessions: ITS MK064187; nrLSU MK105493, MK105494; *rpb2* MK092933; *ef1-α* MK092956; *β-tub* MK092939.

Additional specimens examined: **Guyana**, Region 8 Potato-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 Jun. 2001, *Henkel 8375* (BRG 41301, HSC G1231); 3 km SE of base camp, 11 Jun. 2004, *Henkel 8669* (BRG 41302, HSC G1232); 0.4 km SW of base camp near Blackwater Creek, 1 Jul. 2006, *Aime 3147* (BRG 41303, PUL F24395); 14 Jun. 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 Jun. 2013, *Henkel 9737* (BRG 41306, HSC G1234).

Notes: *Amanita cyanochlorinosma* is a distinctive species recognised in the field by its medium-sized, solitary basidiomata, glabrous, viscid, greyish blue pileus contrasting with the white hymenophore, stipe, and veils, strong odour 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 odour, 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 odour, while known from a handful of sect. *Phalloideae* species, was emphasized by Bas (1969) as an important character of section *Lepidella*, occurring in about half of the known species. Additionally, the 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 odour, 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 odour 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 MB827395. Figs 4, 5.

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

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

Description: *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 concolourous, 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 concolourous, striate, floccose. *Odour* 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 gelatinised, periclinal, hyaline, thin-walled, loosely interwoven; subpellis 170–340 µm thick; hyphae 1.5–4 µm wide, slightly gelatinised, 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,

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Fig. 4. *Amanita fulvoalba* (*Henkel 10395*, type). Scale bar = 1 cm.

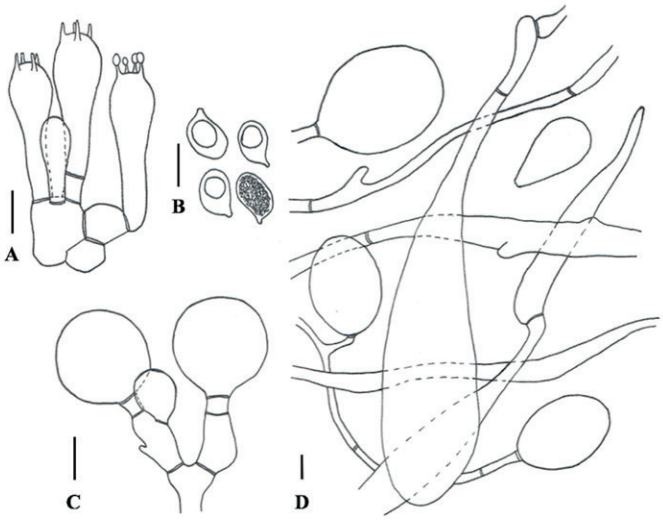

Fig. 5. *Amanita fulvoalba.* **A.** Basidia and subhymenium. **B.** Basidiospores. **C.** Marginal tissue of lamellae. **D.** Slightly crushed tissue from volval limb. Scale bars = 10 µm.

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 gelatinised. *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.

Typus: **Guyana**, Region 8 Potato-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* (**holotype** BRG 41307; **isotype** HSC G1235); GenBank accessions: ITS MK064190; nrLSU MK105497; *rpb2* MK092926; *ef1-α* MK092942.

Additional specimens examined: **Guyana**, Region 8 Potato-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 Jun. 2002, *Henkel 8455* (BRG 41309, HSC G1237); 28 Jun. 2004, *Henkel 8720* (BRG 41310, HSC G1238); 1.5 km SE of base camp, 30 Jun. 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 Jun. 2016, *Aime 6290* (BRG 41313, PUL F24397); 28 Dec. 2016, *Henkel 10394* (BRG 41314, HSC G1241).

Notes: *Amanita fulvoalba* is recognised 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 gelatinised 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, the 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. gelatinised) 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 MB827396. Figs 6, 7.

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

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

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Description: *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 concolourous 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 concolourous, 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 concolourous 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. *Odour* 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 gelatinised, 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 gelatinised, 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 *Pakaraimaea dipterocarpacea* 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.

Typus: **Guyana**, Region 8 Potato-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 Jun. 2013, *Henkel 9767* (**holotype** BRG 41315; **isotype** HSC); GenBank accessions: ITS MK064192; nrLSU MK105502; *rpb2* MK092929; *ef1-α* MK092948; *β-tub* MK092937.

Additional specimens examined: **Guyana**, Region 8 Potato-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 Jun. 2004, *Henkel 8712* (BRG 41318, HSC G1246); 0.4 km SW of base camp near Blackwater Creek, 1 Jul. 2006, *Aime 3155* (BRG 41319, PUL F24398); vicinity of base camp, 11 Jul. 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 Jun. 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 Jun. 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 Jun. 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. dipterocarpacea* 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 Jun. 2012, *Henkel 9674* (BRG 41326, HSC G1251).

Notes: *Amanita guyanensis* is recognised 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, the 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).

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Fig. 6. *Amanita guyanensis* (*Henkel 9767*, type). Scale bar = 1 cm.

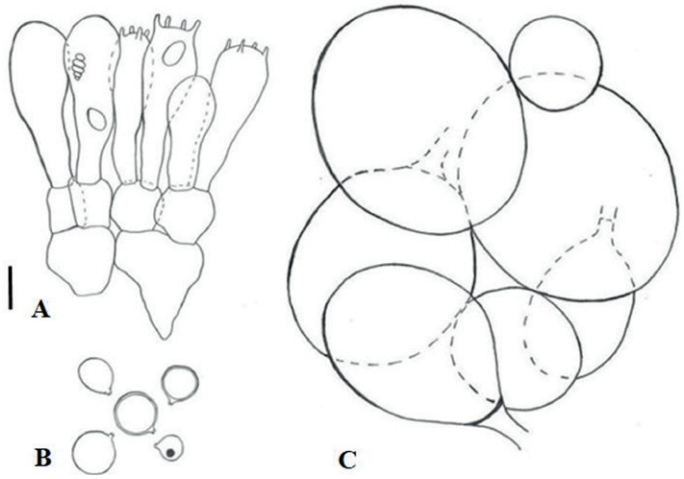

Fig. 7. *Amanita guyanensis.* **A**. Basidia and subhymenium. **B.** Basidiospores. **C.** Slightly crushed tissue of volval wart on pileus. Scale bars = 10 µm.

Worldwide there are numerous species of sect. *Validae* which resemble *A. guyanensis* in stature and colour. 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 colour 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 unpubl. 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 odour 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, colours, 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.

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