

# *Amanita viridissima* (Amanitaceae, Basidiomycota), a striking new species from highlands of the semiarid region of Bahia, Brazil

Felipe Wartchow

Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia/CCEN, 58051-970, João Pessoa, PB, Brazil  
E-mail: [fwartchow@yahoo.com.br](mailto:fwartchow@yahoo.com.br)

**Background** – *Amanita* is a well-established genus by morphological, biochemical, and molecular data, in which most of the taxa are ectomycorrhizal. The generic characteristics are bilateral lamellar trama (in the agaricoid forms), longitudinally acrophysalidic stipe tissue and schizohymenial development. In the Brazilian semi-arid region putative ectomycorrhizal agaric or chanterelle species are infrequently reported. *Amanita lippiae*, *Cantharellus guyanensis*, *C. rubescens* and *Lactarius rupestris* were recently described for that region. Here, an additional, morphologically striking new species of *Amanita* is described from the ‘Chapada da Diamantina’ highlands of Bahia, Brazil.

**Methods** – The new species was collected in a forest called ‘mata de neblina’ or ‘mata nebulosa’ (= misty forest), that occurs at elevations between 1650 and 1800 m a.s.l. in the region of Catolés. For morphological analysis standard methods for *Amanita* were followed.

**Key results** – *Amanita viridissima* is described as new species from the Bahian semiarid highlands. It belongs to *Lepidella* subsect. *Solitariae* stirps *Cinereoconia* and is characterized by the medium-sized green basidiomes, clampless basidia, pale subhymenium cells, pigmented universal veil and with elongate (but also sometimes cylindrical in our case) basidiospores. Other two taxa of stirps *Cinereoconia*, *Amanita odorata* and *A. pelioma* also have green pigments, but differ in several features.

**Key words** – Agaricales, Agaricomycetes, Fungi, neotropics, taxonomy.

## INTRODUCTION

*Amanita* Pers. is a well-established genus supported by morphological, biochemical, and molecular data (Weiß et al. 1998, Drehmel et al. 1999, Moncalvo et al. 2000, Zhang et al. 2004, Li & Oberlies 2005, Wolfe et al. 2012b). It comprises primarily agaricoid and a few secotioid forms (Justo et al. 2010). Most of the taxa of this genus are ectomycorrhizal (ECM) although it is known that basal lineage is represented by saprotrophic members (Wolfe et al. 2012a, 2012b). The main anatomical characterization of this pallid-spored genus is the presence of both bilateral lamellar trama (in the agaricoid forms) and the longitudinally acrophysalidic stipe tissue (Bas 1969). Alternatively, schizohymenial development (Bas 1969, Yang & Oberwinkler 1999) of the basidioma characterizes the genus, in which all structural elements develop within a solid primordium and are separated by development of gelatinizing or friable intermediary tissues allowing edges of lamellae to separate from a partial veil or stipe surface, and hymenial surfaces of lamellae to be segregated from each other (Bas 1969: 299–300).

In Brazil, the earliest records of this genus were given by Rick (1906), who cited *Amanita spissa* var. *alba* Rick and *A. spissa* var. *laeta* Rick. Since then, Rick (1937), Singer (1953), Homrich (1965), Bas (1978), Grandi et al. (1984), Capelari & Maziero (1988), Bas & de Meijer (1993), Stijve & de Meijer (1993), Pegler (1997), Giachini et al. (2000), de Meijer (2001, 2006) and Sobestiansky (2005) reported other taxa. Recent efforts by Wartchow & Maia (2007), Wartchow et al. (2007, 2009, 2013a, 2013b, 2015a, 2015b), Menolli et al. (2009a, 2009b) and Wartchow (2015) cited or described other Brazilian species.

The Brazilian semi-arid region has been proved to be an interesting hotspot for discovery of new putative ECM fungal species, including those in *Amanita*. Wartchow et al. (2009) described the first species from this region, *A. lippiae* Wartchow & Tulloss in ‘campo rupestre’ from the state of Pernambuco. Later, Wartchow & Cavalcanti (2010 as *Lactarius* Pers.) discovered *Lactifluus rupestris* (Wartchow) Sá & Wartchow nom. prov. from this same forest. The most recent discoveries of putative ECM taxa was given by Henkel et al. (2014), who cited *Cantharellus guyanensis* Mont.

in 'brejo de altitude' from Paraíba and Nascimento et al. (2014) with *C. rubescens* C.C.Nascimento, F.G.B.Pinheiro, Wartchow & M.A.Alves from Ceará. Nonetheless, *Amanita* records from the Brazilian semi-arid region are extremely rare. Although recently cited from the Atlantic Forest of Northeast Brazil (Wartchow & Maia 2007, Wartchow et al. 2007, 2013a, 2015b, Menolli et al. 2009b), the only cited taxon from semi-arid was *A. lippiae* (see above). Gusmão et al. (2005) reported 23 species of macrofungi from the region of 'Chapada da Diamantina', but no *Amanita* or any other agaricoid fungus.

The new species of *Amanita* described here was collected during a field trip (9–12 Jan. 2015) to 'Chapada da Diamantina', Bahia, Brazil, including a trip to the region of Catolés, in a forest called 'mata de neblina' or 'mata nebulosa' (= misty forest), that occurs at an altitude comprised between 1650 and 1800 m a.s.l. in the region of Catolés. The area is well known for its richness in epiphytes as well arboreal trees, as for example *Podocarpus* L'Hér. ex Pers. spp. (Podocarpaceae) and *Lamanonia ternata* Vell. (Cunoniaceae) (Zappi et al. 2003). In the region these authors also referred putative ECM trees genera as *Guapira* Aubl. (Nyctaginaceae) and *Coccoloba* P.Browne (Polygonaceae). This area was recommended for integral protection due extreme biological importance, high degree of endemism of rare/threatened species and having human activities mainly by agriculture and mining (Giulietti et al. 2004). Recently Santos et al. (2013) described *Marcetia paganucci* A.K.A.Santos & A.A.Martins from this region and they considered it vulnerable according to IUCN criteria.

#### MATERIAL AND METHODS

The usual methodology on studies in agaric fungi was followed (Singer 1986) and colours were named based on Kornerup & Wanscher (1978). Microscopic examinations were on a Coleman microscope from dried material rehydrated and mounted in 3% KOH and Congo Red solutions. For the biometric values, the emended methodology of Tulloss & Lindgren (2005), slightly modified by Wartchow & Gamboa-Trujillo (2012), was performed as follows: **wcs** = breadth of central stratum of lamella; **wst-near** = distance from one side of central stratum to nearest base of basidium; **wst-far** = distance from one side of central stratum to most distant base of basidium on the same side of the central stratum; **L (W)** = the range of average lengths (widths) of spores of each basidioma examined; **L' (W')** = the average of all lengths (widths) of spores measured of all basidiomata; **Q** = the ratio of length to width of a spore or the range of such ratios for all spores measured; **Q** = the average value of Q computed for one specimen examined and the range of such averages; **Qm** = average value of Q computed for all spores measured of all basidiomata (not applied here); specific terms used for *Amanita* are summarized by Wartchow et al. (2013a). Generic and infrageneric names and concepts follow Corner & Bas (1962) and Bas (1969), as modified by Yang (1997). The holotype is deposited at JPB (Thiers 2015).

#### SPECIES DESCRIPTION

*Amanita viridissima* Wartchow, sp. nov.

Mycobank No. 814157

**Diagnosis** – This species differs from *Amanita odorata* and *A. pelioma* in the green pileus and stipe, pale lamellae, elongate (sometimes cylindrical) basidiospores (9–)9.8–13(–13.5) × (5.5–)5.7–8.3(–8.5) µm, clampless basidia, pale subhymenium cells and velar elements greenish-olive pigmented. – Type: Brazil, Bahia, Chapada da Diamantina, Abaíra, Catolés de Cima, Serra do Barbado, Mata do Tijuquinho, 13°16'74"S 41°54'29"W, alt. 1757 m, 9 Jan. 2015, F. Wartchow et al. FW 01/2015 (holo-: JPB 60532!). Figs 1 & 2.

**Etymology** – From the Latin 'viridis' (= green) and '-issima' (= superlative ending).

**Basidioma** medium sized, solitary. **Pileus** 66 mm wide, plano-convex, green (27D7 'deep green') or sometimes slightly paler (28A7) or deep green (27D8) in some parts; surface dull but shine below a very thin scalp; margin non-sulcate, lacerate due to age, appendiculate; universal veil as green (27A8) floccose remnants ~ 0.5 mm high, more concentrated at centre, more pulverulent toward margin; context whitish with greenish (26A6) portion near pellis, unchanging, 3 mm thick near centre then gradually thinning and then abruptly thin near margin. **Lamellae** very narrowly adnexed, pale cream-buff (4C4), unchanging; edge smooth and concolorous; 5 mm broad (measured at half portion of the pileus radius); lamellulae attenuate in steps, frequent, with 3–4 lengths and 1–2 lamellulae between each main lamella. **Stipe** 45 (measured from above bulb) × 11 mm (width measured at base), subequal and very slightly flaring at apex; green (27C5) or slightly darker (27E7) toward base surface; floccose-subpruinose; bulb rooting, 40 mm long, 18 mm wide (at the widest point), concolorous with stipe; context solid, pale green (27A3), unchanging, insect or larvae tunnels dark green (27E8); universal veil as greenish (28A7), small squamules slightly concentrically arranged and more flocculose at stipe base near junction with top of bulb; partial veil absent. Odour and taste imperceptible.

**Basidiospores** [40/1/1] (9–)9.8–13(–13.5) × (5.5–)5.7–8.3(–8.5) µm, **L** = 11.5 µm; **W** = 6.3 µm; **Q** = (1.42–)1.53–2.12(–2.27); **Qm** = 1.82; ellipsoid to elongate then sometimes subcylindric; hyaline, amyloid, thin-walled; contents as 1–2 guttules; hilar appendix prominent, subapical. **Basidia** 33.5–43 × 9–12 µm, clavate, bearing mostly four sterigmata to 4 µm high, clampless. **Subhymenium** cellular, up to 20 µm thick, with 2–3(–4) layers of inflated cells 6.5–17.5 × 6.5–13.5 µm, these broadly clavate, piriform to (sub)isodiametric, hyaline; **wst-near** = up to 40 µm; **wst-far** = up to 45 µm. **Lamellar trama** bilateral; **wcs** = 25 µm thick; composed of filamentous hyphae to 4 µm wide, inflated terminal elements to 35–55 × 17.5–20 µm, clavate, colourless, thin walled; oleiferous hyphae frequent, to 5 µm wide, mostly non-diverging from central stratum; divergent inflated elements e.g. 25 × 12.5 µm, diverging gradually to sub-abruptly. **Marginal tissue of lamellae** with abundant broadly clavate to broadly clavate



**Figure 1** – *Amanita viridissima* (from Wartchow *et al.* FW 01/2015, holotype) : A, basidiome; B, pileus surface. Scale bars = 20 mm.



terminal elements elements  $43.5\text{--}58 \times 14\text{--}25 \mu\text{m}$  but sometimes catenulate with two cells, colourless, thin walled. Pileus context well rehydrated, distinctly acrophysalidic; acrophysalides  $70\text{--}204 \times 20\text{--}65 \mu\text{m}$ , abundant, very conspicuous, fusoid to clavate, sometimes slender-fusoid; filamentous hyphae  $2\text{--}10.5 \mu\text{m}$ , strongly interwoven, frequently branched, forming a matrix where other elements occur; hyaline then greenish parietal pigments near pileipellis; thin walled, clampless; oleiferous hyphae  $6 \mu\text{m}$  wide, unbranched, infrequent. Stipe context longitudinally acrophysalidic; acrophysalides  $143\text{--}300 \times 23\text{--}45.5 \mu\text{m}$ , slender clavate to fusiform, very conspicuous; filamentous hyphae  $2\text{--}13 \mu\text{m}$ , longitudinally oriented to frequently dichotomously branched, mostly hyaline, with green parietal pigments near stipitipellis, clampless; vascular hyphae  $5\text{--}8.5 \mu\text{m}$ , frequent. Pileipellis a cutis  $78 \mu\text{m}$  thick; filamentous hyphae  $1.5\text{--}6.5 \mu\text{m}$ , more or less interwoven, loosely compacted in a partially gelatinized matrix, pale coloured; over disc near universal veil elements periclinaly oriented, non-gelatinized; near context more distinctly radially oriented with hyphae sometimes thickening to  $10.5 \mu\text{m}$  wide, plasmatic greenish pigmented; oleiferous hyphae very infrequent, to  $9 \mu\text{m}$  wide, more or less radially oriented. Universal veil remnants on pileus up to  $500 \mu\text{m}$  thick, with abundant ovoid ( $19.5\text{--}66 \times 13\text{--}44 \mu\text{m}$ ) but also balloon shape (e.g.  $60 \times 38 \mu\text{m}$ ), subisodiametric (e.g.  $47 \times 44 \mu\text{m}$ ), or clavate to broadly clavate (e.g.  $56 \times 40 \mu\text{m}$ ) or clavate (e.g.  $75 \times 30.5 \mu\text{m}$ ), sometimes in rows of 3–5 inflated cells, greenish-olive pigment, wall thin sometimes thickening to  $0.5 \mu\text{m}$ ; filamentous hyphae ranging  $6 \mu\text{m}$ , interwoven, pale-colour or greenish, infrequent. Universal veil on top of bulb made of terminal slender clavate elements  $130\text{--}200 \times 22\text{--}27 \mu\text{m}$ , wall thin sometimes ranging to  $0.7 \mu\text{m}$ , some ellipsoid elements oriented in chains (e.g.  $95\text{--}105 \times 32\text{--}45 \mu\text{m}$ ) and some more elongate (e.g.  $26 \times 18 \mu\text{m}$ ), pale coloured, greenish to sometimes olive or olivaceous brown; hyphae  $3\text{--}10 \mu\text{m}$  wide, periclinal to surface, pale coloured to infrequently pale green to olivaceous brown parietal pigment, elements on the top of rows with obtuse apex, wall thin thickened ( $5 \mu\text{m}$ ); vascular hyphae absent. Partial veil on stipe absent.

**Habit and habitat** – Solitary on clay soil in a ‘misty forest’ (sensu Zappi et al. 2003) about 1700 m alt. According to these authors, *Guapira obtusata* (Jacq.) Little (1000–1850 m alt.) and *Coccoloba brasiliensis* Nees & Mart. (1000–1700 m alt.) are putative ECM trees reported as found in similar altitude to our new species.

**Known distribution** – Only known from the type locality, in the Chapada da Diamantina, Bahia, Brazil.

**Conservation status** – This species is only known from the type locality. No information is available on its population status, natural history or threats, and it is consequently listed as Data Deficient (IUCN 2012). The species might have a restricted range but further investigations of population trends and distribution are necessary for assessing its conservation status.

## DISCUSSION

*Amanita viridissima*, due the non-sulcate pileus, amyloid basidiospores and appendiculate pileus margin, is best placed

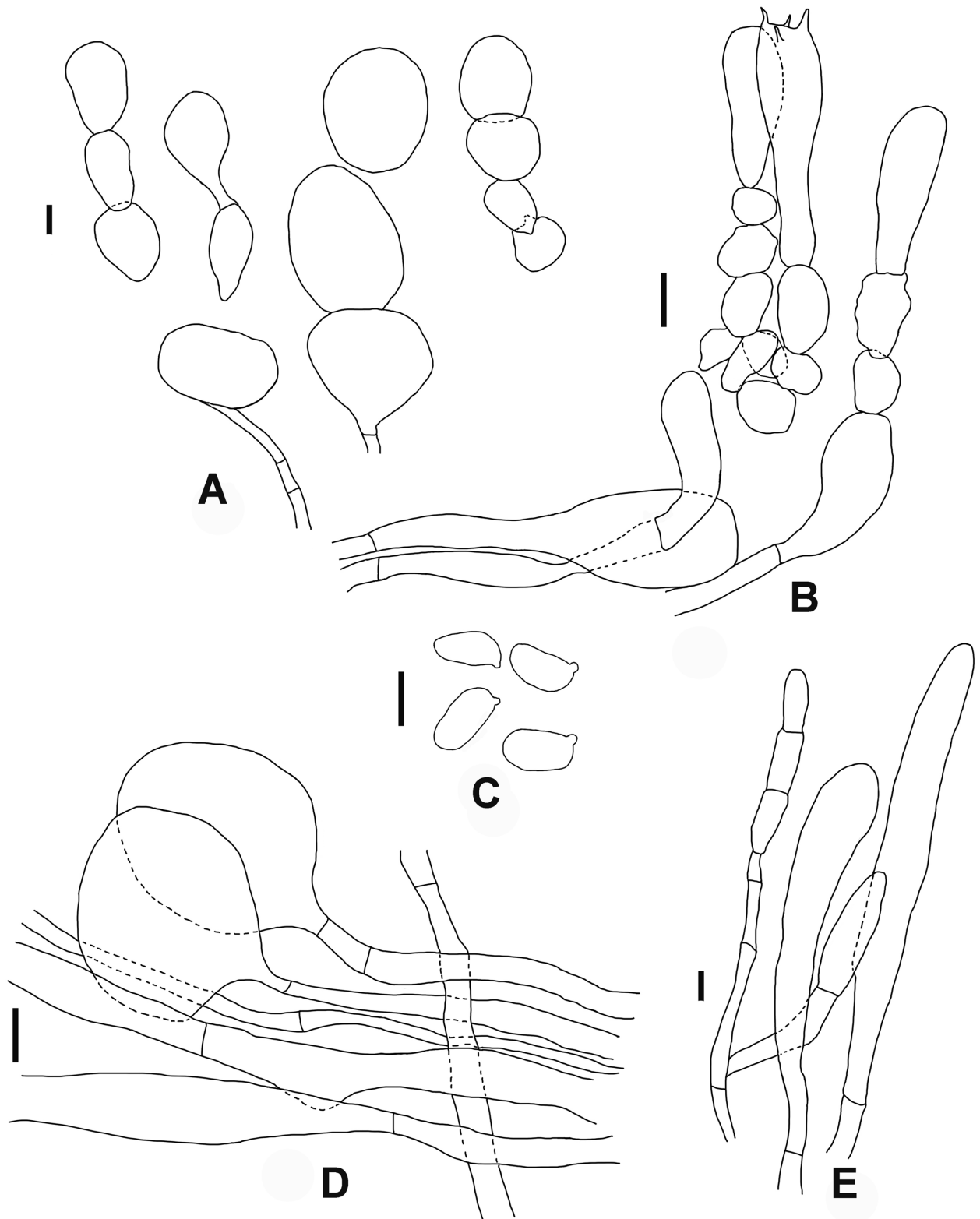
in subgenus *Lepidella* (Roze) Veselý emend. Corner & Bas (1962) sect. *Lepidella*. Into this group, the non-membranous universal veil that never forms limb at stipe base, but floccose warts on pileus that comprise erect-parallel inflated elements are features of subsect. *Solitariae* Bas (1969). The coloured basidiomes are very unusual in this subsection, but in Bas’ (1969: 386–388) key *A. viridissima* would appear to belong to stirps *Cinereoconia* on account of the clampless basidia, pale subhymenium cells, pigmented universal veil and medium-sized basidiomes with elongate (but also sometimes cylindrical in this case) basidiospores.

These features easily fit *A. viridissima* with two other species that also have greenish pigments in stirps *Cinereoconia*. These taxa are presented in table 1 and have the following key characteristics that are distinguished from our new species as follow:

*Amanita odorata* Beeli, from the Democratic Republic of Congo in *Gilbertiodendron dewevrei* forest (Beeli 1931, Gilbert 1940–1941, as ‘*Macrolobii dewevrei*’), was illustrated by Gilbert (1940–1941: pl. 68) and redescribed by Bas (1969: 468–469) with greyish-brownish olive to greyish olivaceous brown pileus, pulverulent then shapeless to conical universal veil warts at centre, pinkish-whitish lamellae, olivaceous-brown and hardly thickened and slender clavate bulb stipe, and presence of floccose-fibrillose fugacious partial veil. Microscopically, the basidiospores are narrower ranging to bacilliform [ $20/2$ ]  $8\text{--}9.5\text{--}(10.5) \times 4.5\text{--}5.5 \mu\text{m}$ ;  $Q = 1.90\text{--}3.00$ ;  $Qm = 2.40$ ; and the cells of the universal veil are brown (Bas 1969). Tulloss & Yang (2015) reported basidiospores [ $100/5/3$ ]  $(8.3\text{--})9.1\text{--}12\text{--}(12.4) \times (4.0\text{--})4.4\text{--}5.1\text{--}(5.5) \mu\text{m}$ ;  $L = 9.6\text{--}11.1 \mu\text{m}$ ;  $L' = 10.3 \mu\text{m}$ ;  $W = 4.7\text{--}4.9 \mu\text{m}$ ;  $W' = 4.8 \mu\text{m}$ ;  $Q = (1.70\text{--})1.86\text{--}2.49\text{--}(2.76)$ ;  $Q = 1.95\text{--}2.35$ ;  $Qm = 2.17$ . Note that although Tulloss & Yang (2015) did not observe bacilliform basidiospores, they are distinctly longer than in our species.

*Amanita pelioma* Bas from Eastern United States was firstly considered as ‘*A. chlorinosma* form A’ by Coker (1917), but later described as new species by Bas (1969). It also presents erect and parallel rows of cells in the pileus centre, but has greyish olivaceous buff tinged basidiomes with blue-green stain at top of bulb, somewhat shorter basidiospores [ $20/1$ ]  $(9\text{--})10\text{--}12.5 \times (6\text{--})6.5\text{--}8 \mu\text{m}$ ;  $Q = 1.45\text{--}1.85$ ;  $Qm = 1.65$ , ramose to subcellular subhymenium, and yellowish to yellow universal veil cells (Bas 1969). According to Tulloss & Yang (2015) the basidiospores of this North American taxon measure [ $165/7/5$ ]  $(6.5\text{--})9.5\text{--}13.6\text{--}(22.5) \times (5.4\text{--})5.8\text{--}9.1\text{--}(11.8) \mu\text{m}$ ;  $L = 10.5\text{--}12.3 \mu\text{m}$ ;  $L' = 11.4 \mu\text{m}$ ;  $W = 6.0\text{--}8.1 \mu\text{m}$ ;  $W' = 7.1 \mu\text{m}$ ;  $Q = (1.20\text{--})1.40\text{--}1.88\text{--}(2.22)$ ;  $Q = 1.49\text{--}1.76$ ;  $Qm = 1.62$ .

In South America another taxon with very distinguishable colour is known, *A. cyanopus* C.Simmons, T.W.Henkel & Bas from the Guiana Shield. The subhymenium is also described as having inflated cells and elements of the universal veil as slightly olivaceous brown. However, it differs in the dull greyish to bluish turquoise pileus, smaller and shorter basidiospores [ $25/1$ ]  $7.4\text{--}8.7\text{--}(9) \times (5\text{--})5.6\text{--}7.4 \mu\text{m}$ ;  $Q = (1.15\text{--})1.25\text{--}1.15\text{--}(1.60)$  and  $Qm = 1.36$  and the non-appendiculate margin. This last feature lead this entity to *Amanita* subgen.



**Figure 2** – *Amanita viridissima* (from Wartchow *et al.* FW 01/2015, holotype); A, elements of the universal veil from pileus (after crushed); B, basidium, basidioles, subhymenium and elements of the lamella trama; C, basidiospores; D, elements of the universal veil on pileus and the adjacent pileipellis; E, elements of the universal veil from top of bulb. Scale bars = 10  $\mu$ m.

**Table 1 – Comparison among taxa of *Amanita* stirps *Cinereoconia* with green tints and *A. cyanopus* (Bas 1969, Simmons et al. 2002, Tulloss & Yang 2015).**

	<i>A. viridissima</i>	<i>A. odorata</i>	<i>A. pelioma</i>	<i>A. cyanopus</i>
Pileus colour	greenish blue	with greyish-brownish olive to grayish olivaceous brown	greyish-olivaceous buff tinges	dull greyish to bluish turquoise
Pileus margin	appendiculate	appendiculate	appendiculate	non-appendiculate
Lamellae colour	pale cream-buff	pinkish-whitish	pale greyish olivaceous to pale brownish gray, sometimes with slight lavender tint	dull dark greyish tan (or pale cream buff)
Universal veil from pileus	green floccose remnants with about 0.5 mm high, more concentrated at centre, then pulverulent toward margin	pulverulent then shapeless to conical universal veil warts at centre	pulverulent-flocculose, sometimes form pulverulent warts over disc, rarely as blue-green squamules near margin	flat felted volval patches, particularly near margin, minutely, rather innately fibrillose
Universal veil from stipe	small squamules slightly concentrically arranged, more flocculose at stipe base near junction to top of bulb; difficult to discern in exsiccate	with or without olivaceous brown to brown pulverulent-subverrucose material near stipe base	irregularly detersile pulverulent covering, sometimes as inconspicuous pulverulent flocculose to (rarely) squamulose remains near top of bulb,	small bright blue, conical volval warts on a pale blue or whitish background
Bulb	bulb rooting, 40 mm long, 18 mm wide (at the widest point)	marked and turnip-shaped to spindle-shaped, and ± 25 mm wide	clavate to fusiform (c. 15–25 mm wide) or poorly defined, sometimes radicating	large, slender napiform, somewhat rooting
Partial veil in stipe	absent	floccose-fibrillose fugacious	absent	present
Basidiospores	[40/1/1] (9–)9.8–13(–13.5) × (5.5–)5.7–8.3(–8.5) µm L = 11.5 µm W = 6.3 µm - - - Q = (1.42–)1.53–2.12(–2.27) Q = 1.82 Qm = 1.82 (Tulloss & Yang 2015)	[100/5/3] (8.3–)9.1–12(–12.4) × (4.0–)4.4–5.1(–5.5) µm - - L = 9.6–11.1 µm L' = 10.3 µm W = 4.7–4.9 µm W' = 4.8 µm Q = (1.70–)1.86–2.49(–2.76) Q = 1.95–2.35 Qm = 2.17 (Tulloss & Yang 2015)	[165/7/5] (6.5–)9.5–13.6(–22.5) × (5.4–)5.8–9.1(–11.8) µm - - L = 10.5–12.3 µm L' = 11.4 µm W = 6.0–8.1 µm W' = 7.1 µm Q = (1.20–)1.40–1.88(–2.22) Q = 1.49–1.76 Qm = 1.62 (Tulloss & Yang 2015)	[25/1] 7.4–8.7(–9) × (5–)5.6–7.4 µm - - - Q = (1.15–)1.25–1.15(–1.60) - Qm = 1.36 (Simmons et al. 2002)
Subhymenium	cellular	cellular	ramose to subcellular	cellular
Colour of the elements of universal veil	greenish-olive	brown sometimes lacking pigment	yellowish to yellow	slightly olivaceous brown

*Validae* (Fr.) Quél. (Simmons et al. 2002); it is obviously phenetically distant from *A. viridissima*.

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