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New report of *Amanita* species (*Amanitaceae, Agaricales*) from Northern Nigeria

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Abstract

Three species of *Amanita* Pers. (gilled-mushrooms) in the order Agaricales were collected and described using morphological characteristics. Their taxonomic status was confirmed using keys proposed by Arora and Kerrigan. These were identified as *Amanita muscaria* var. *alba* Hook, *Amanita novinupta* Tulloss & J. Lindgr and *Amanita rubescens* (Pers. Ex Fr.) Gray. Although the species showed diverse fruiting body morphologies; however, they shared common characteristics such as divergent gill trama, inamyloid spore and lance-shaped cystidia. This study represents new records of *Amanita* from northern Nigeria. The original samples are kept in the Fungal Collection Centre in the Department of Botany, Ahmadu Bello University, Zaria-Nigeria.

Keywords: *Amanita*; Agaricales; basidiomycotina; description; morphology; taxonomy; Northern Nigeria.

Introduction

Mushrooms have been known by man since he became aware of his environment. They occur in different types of habitats and geographical areas, ranging from artic to tropics, with varying conditions that affect the development of their fruit bodies (Grimes 1978, Alexopoulous and Mims 1979, Bas 1991, Ali *et al*. 2010a).

Amanitaceae R. Heim ex Pouzar, contains two important genera, *Amanita* Pers. and *Limacella* Earle; the latter being distinguished by a gelatinous universal veil (Lewis and McGraw 1981). They are characterized by their mycorrhizal habit (Bas 1969, Ali *et al*. 2010a), and usually grow in the woods or near trees, with preference to slightly acidic soils (Ali *et al*. 2010a).

Amanitas include some of the world's best known and most attractive macro fungi which belong to an important genus in the order Agaricales, Phylum Dikaryomycotina, Subphylum Basidiomycotina (Singer 1986). They formed a large, ubiquitous genus of perhaps over 500 species (Kirk *et al*. 2001), occurring on every continent of the world except Antarctica. However, recent report has shown that species of *Amanita* might have occurred in the Antarctica during the Cretaceous era as mycorrhizal symbionts of such trees as *Nothofagus* (Arora 1986**)**.

The genus *Amanita* is distinct from other genera in the Agaricaceae by possession of white spore prints, lamellae that are usually white and free, pallid basidiospores, and presence of a universal veil that often creates a volva (Bas 1969). Many species have an annulus on the stem and warts or patches on their pileus, with exception of some species such as *A. fulva* that have no spots or patches of universal veil on the pileus. They share close resemblances especially among the white species (Dennis *et al*. 1999, Kuo 2013).

Several species of *Amanita* including novel species have been reported from different parts of the world. These include, Argentina (Niveiro and Alberto 2012), Brazil (Menolli *et al*. 2009, Wartchow *et al*. 2013), Colombia (Tuloss *et al*. 1992), China (Yan and Li 2001), Croatia (Mešić A. and Tkalčec 2002), India (Bhatt *et al*., 2003, Senthilarasu 2014), Japan (Yamashita *et al*. 2005), Nigeria (Zoberi 1972), Thailand (Sanmee *et al*. 2008) and Zambia (Pegler and Shah-Smith 1997). Recent reports of Amanitaceae have also emerged from California and the Pacific Northwest (Dimitar and Davis 2013), and Turkey (Sesli and Denchev 2014).

The taxonomy of Amanitas is problematic because of common similarities that exist within the genera and species. This is particularly true for white Amanitas which show much similarity hence are

not easily distinguished morphologically. For example, *Amanita ibotengutake* T. Oda, C. Tanaka & Tsuda described from Japan, is strikingly similar to *A. subglobosa* Zhu L. Yang which can be distinguished on the basis of molecular phylogeny (Yang 2007).

A few species of *Amanita* are edible like other mushrooms (Wienscyzk *et al*. 2001). For example, *A. chepangiana* Tulloss & Bhandary, is a staple part of diet of the Chepang people of Nepal, while the African species, *A. strobilaceovolvata* and *A. loosii* are much sought-after delicacy. *A. muscaria* (L.: Fr.) Lam., is a prized edible mushroom in the Nagano Prefecture of Japan, while some species are widely consumed by the local people of northern Thailand (Sanmee *et al.* 2003).

Certain beliefs are that, wood decaying saprotroph or tree associated mycorrhizal mushrooms are edible due to their capacity to digest easily. However, contrary to these widely accepted beliefs, many people shun the consumption of wild mushrooms due to fear of being poisoned (Kadiri 2008). This is based on the fact that, the poisonous species contain various kinds of toxic substances such as amatoxins and phallotoxins, among others (Wieland and Faulstich 1978). For example, *A. exitiallis* Z.L.Yang & T.H. Li, *A. phalloides* (Vaill. ex Fr.: Fr.) Link and *A. ibotengutake* are lethal; the latter being responsible for Pantherina syndrome in humans (Bresinsky & Besl 1990, Oda *et al*. 2002). The fruit body of this mushroom contains neurotoxic metabolites, ibotenic acid and muscimol, which though non-fatal to mammals, has insecticidal properties (Seeger and Stijve 1980, Oda *et al*. 2002).

Although there are upsurge in researches regarding the diversity of mushrooms worldwide, however, paucity of knowledge exists in the published literatures with regard to morphology and taxonomy of gilled mushrooms in Africa especially, Nigeria (Zoberi 1972; Osemwegie 2006, Osemwegie *et al.* 2009, Ali 2012, Ali *et al*. 2012). Therefore, the aim of this study was to describe *Amanita* species using morphological characteristics, and to evaluate their taxonomy.

Materials and Methods

Mushrooms Collection

Fungal specimens used in this study originated from field samples collected within the main campus of Ahmadu Bello University. Habitat and habit of growth of the mushrooms were recorded in a field notebook. Specimens were described based on characters observed in the fresh basidiocarps which included pileus, stipe and lamellae details. Spore prints were obtained by placing pilei over sterile papers as proposed by Christensen (1982). Spores were mounted in both water and Meltzer's reagent to note their shapes, surfaces, walls and ornamentations. Measurements of spores' sizes were done using a calibrated microscope. Ten matured spores were measured under oil immersion for each species, and the rate of widths and lengths of the spores were recorded following the methods by Kuo (2004). Microstructures such as lamellae trama, pileus cuticle, basidia and cystidia were also studied.

For taxonomic study, the keys of Arora (1986), Kenderick (2000) and Kuo (2004) were used to ascertain the taxonomic positions of the described species.

Results and Discussion

Microscopic Studies

The microscopic features of the *Amanita* species in this study were similar having thin, smooth and inamyloid spores, except *A. rubensis* (Pers. Ex Fr.) Gray, which had a slightly bigger spores that were dextrinoid. The cystidia and pileus cuticles in both *A. muscaria* var. *alba* (L.) Hook and *A. novinupta* Tulloss & J. Lindgr. were lanced shaped and filamentous respectively, whereas *A. muscaria* var. *alba* and *A. rubescens* had divergent lamellae tramas (Table1).

Table 1: Microscopic descriptions of *Amanita* species

Species	Characteristics							
	Spore size (µ)	Shape	THK	SUF	RMR	CST	LM-	PLC-
A.muscaria var. alba	7.5–10 x 5–7.5	round	thin	smooth with oil globuse	inamyloid	lanced-shaped	divergent	filamentous
A. novinupta	7.5–10 x 6.8–7.5	round to-oval	thin	smooth	inamyloid	lanced-shaped	lanced-shaped	filamentous
A.rubescens	8–12 x 5.5–8.5	spherical	thin	smooth	dextrinoid	-		

Key: SHP-shape, THK-thickness SUF-surface, RMR-reaction to Meltzer's reagent, CST-cystidia LM-lamellae, PLC-pileus cuticle.

Description of taxa

Amanita muscaria* var. *alba (L.) Hook.

Habit and habitat: solitary to gregarious, found growing on soil near *Casie samea*, *spore print* white in deposit. *Pileus* creamy, 9–12 cm in diameter, outline circular, convex when young expanding to plane at maturity; margin shape straight, surface wet with warts in concentric rings. *Lamellae* creamy, sub-distant with numerous lamellulae at varying lengths; intermediate, thin and soft, edges seceding. *Stipe* granulose and sticky, 1–13.5 cm long and 1–3 cm thick, centric, equal but often with a slightly bulbous end and tightly stuffed. *Annulus* superior, collapsing on the stem (Fig. 1a).

The description of *A. muscaria* var. *alba* in both anatomy and morphology was in complete conformity with the details given by Arora (1986). However, this species could attain a stipe length of 30cm and pileus up to 40cm broad (Arora 1986). It is similar to *A. muscaria* var. *flavivolvata* but differ significantly by having yellowish warts. This species resembled *Stropharia ambigua* but differ by having less prominent partial veil adhering on the margin of the pileus (Fig. 1c), and white spore print.

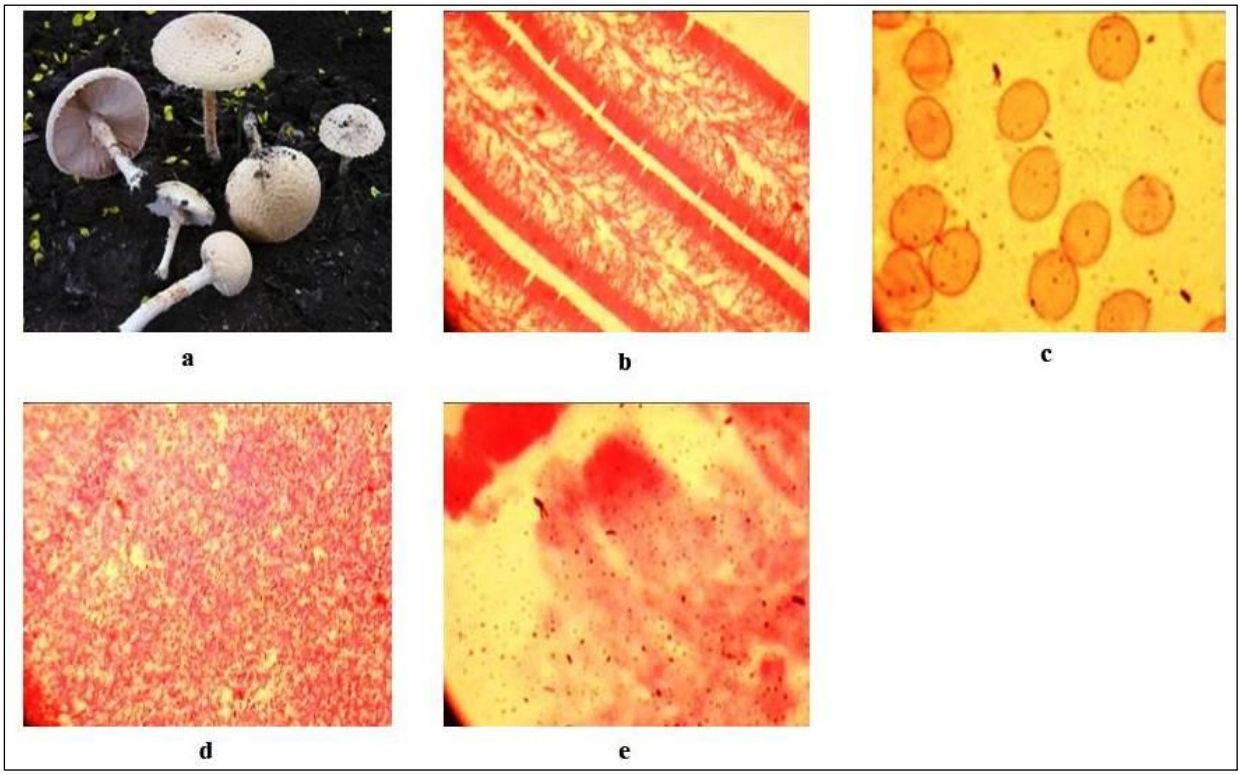

Figure1: Macroscopic and Microscopic features of *A. muscaria* var. *alba*. **a.** Basidiomata of *A. muscaria* var. *alba*. **b.** Divergent lamellae trama x 400. **c.** Basidiospores in Melzer's reagent x 1000. **d.** Filamentous pileus cuticle x 400. **e.** Cystidia x 1000.

Amanita novinumta Tulloss & J. Lindgr.

Habit and habitat: solitary, found growing soil. Spore print cream in deposit. *Pileus* cream, 14.2 cm in diameter, convex when young becoming plane, and slightly upturned margin at maturity; umbonate with yellow discolouration after exposure to air, dry with granulose and sticky patches, edges entire. *Lamellae* cream, close, intermediate, free, thick and waxy, edges crenulate. *Stipe* granulose and sticky, 19.5 cm long; 2.3 cm thick, centric, brittle and breaks easily at the point of attachment to the pileus, equal but sometimes with slightly bulbous base, dark brown below the annulus, exudates colourless latex. *Annulus* collapsed on the stem and superior.

The macroscopic and microscopic features recorded for *A. novinupta* conformed to the description provided by Kerrigan (1986), but the fruiting bodies are smaller in stature and sometimes the species attain a stipe length of 16 cm and pileus of 15 cm broad depending on the geographical location (Kerrigan 1986). Similar to *A. rubescens* var. *alba*, but *A. novinupta* has universal veil that sometimes leaves patches on the pileus that easily wash away by rain; the partial veil is not yellowish on the underside and spore are larger (Fig. 2b). Also similar to *A. rubescens* var. *rubescens* in terms of spore size, but var. *rubescens* has an originally pigmented pileus with universal veil which turns grey with age (Kenderick 2000).

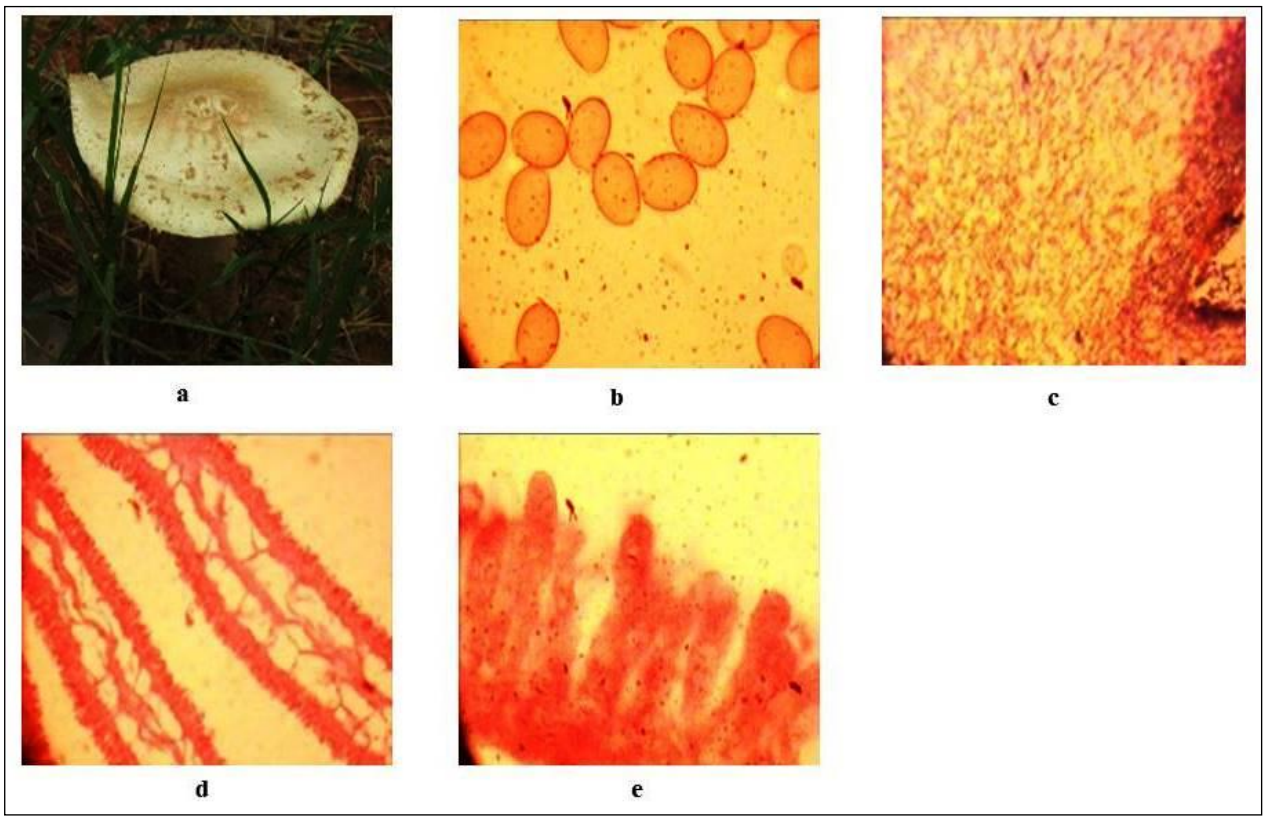

Figure 2: Macroscopic and microscopic features of *Amanita novinumta*. **a.** Basidiocarp of *A. novinumta*. **b.** Basidiospores in Melzer's reagent x 1000. **c.** Filamentous pileus cuticle x 400. **d.** Divergent lamellae trama x 400. **e.** Cystidia x 1000.

Amanita rubescens (Pers. Ex Fr.) Gray

Habit and habitat: solitary, found growing in soil. *Spore print*: ash/grey in deposit. *Pileus* 16 cm broad, bell-shaped to slightly convex with an incurved margin, white to bluish, soft, entire covered with wooly patches, edges entire. *Lamellae* creamy, sub-distant, intermediate, free, slightly thick; edge crenulate. *Stipe* cream, centric, slightly stuffed, 10 cm long, 1.5 cm thick, bulbous, covered with powdery substances; latex turns yellow when exposed to air. *Annulus* collapsing on the stem.

Morphological and anatomical descriptions of *A. rubescens* conformed to the earlier recorded species by Arora (1986). However, this collection differs by having free lamellae, and larger spore sizes, inamyloid spores with conspicuous oil globule (Fig. 3c), and

pileus wholly covered with somewhat wooly to fibrillose warts (Fig. 3a). This species could attain 20 cm of pileus-breath and 20 cm stipe-lengths due to influence of growth conditions (Arora 1986). The blushing of the pileus, stem, and flesh is one of the infallible field-mark of this species (Arora 1986). Matured specimens are sometimes mistaken for *A. patherina* or *A. muscaria*, but can be distinguished from them by its indistinctive volva, reddish stains, and dextrinoid spores. Pure white buttons can also resemble *A. strobiformis* group but lacks the tapered, rooting base of those species (Arora 1986).

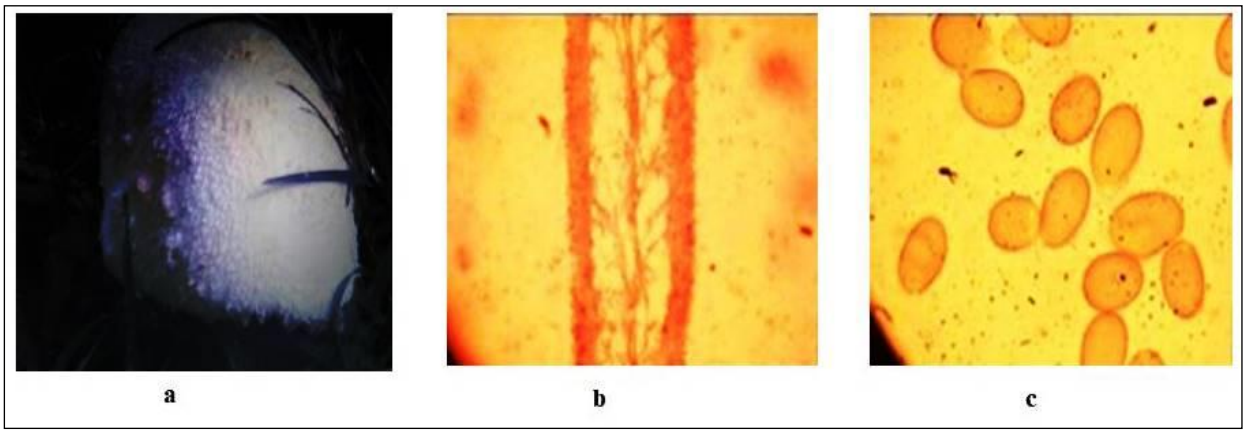

Figure 3: Macroscopic and microscopic features of *Amanita rubescens*. **a.** Basidiocarp of *Amanita rubescens*. **b.** Divergent lamellae trama x 400. c**.** Basidiospores in Melzer's reagent x 1000**.**

Although morphological variation was recorded among the described species, overall, close resemblances were observed. This agrees with the report of Dennis *et al*. (1999) that *Amanita* show higher level relationships among its species.

Conclusion

Amanita species in this study showed diverse fruiting body morphologies that placed them at different species level. However, common anatomical characters were shared among the species. Morphological variations observed in this study as compared with documented species by Arora (1986) could be due to differences in environmental factors prevailing in the different locations where they grew. This invariably affected their growth and fruiting body development. All the three species namely, *A. muscaria* var. *alba*, *A. novinupta* and *A. rubescens* are new records from northern Nigeria.

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