

Taxonomic studies of *Amanita muscaria* (L.) Lam (Amanitaceae, Agaricomycetes) and its infraspecific taxa in Brazil

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ABSTRACT

We analyzed specimens identified as *Amanita muscaria*, some recently collected and others already deposited in herbaria, in Brazil. We concluded that two subspecies of *A. muscaria* occur in Brazil: *A. muscaria* var. *muscaria*; and *A. muscaria* var. *flavivolvata*. The first taxon was found in association with *Castanea sativa*, and the second (one specimen only) was found in association with *Pinus* and *Eucalyptus* spp. Morphologically, *A. muscaria* var. *flavivolvata* is distinguished by a shallower subhymenium and by basidiospores that are more elongated than are those of *A. muscaria* var. *muscaria*, which is the more widely known subspecies. We present descriptions, discussions, illustrations and a dichotomous key for these two subspecies.

Key words: Agaricales, Basidiomycota, Mushroom, taxonomy

Introduction

Amanita muscaria, in a broad sense, is considered the world's most famous fungal species, often depicted in various media (Michelot & Melendez-Howell 2003). This species is referenced in relation to several ancient Old and New World cultures (Brough 1971, Dunn 1974; Lowy 1974; Whelan 1974; Saar 1991; Samorini 1992; Hajicek-Dobberstein 1995) and has been reported to cause psychosis in humans (Lampe 1979; Satora *et al.* 2005; Brvar *et al.* 2006), owing to the psychoactive compounds it contains, including muscimol (Wieland, 1968; Krogsgaard-Larsen *et al.* 1981; Stijve & Meijer 1993; Michelot & Melendez-Howell 2003; Tsujikawa *et al.* 2007). There have also been reports of *A. muscaria*-related accidents involving pets (Rossmesl *et al.* 2006).

Initially, *A. muscaria* was suspected to be a well-defined morphospecies with ample geographic distribution, also associated with ectomycorrhizal hosts dispersed across multiple genres of vascular plants (Trappe 1962). However, phylogenetic studies conducted by Oda *et al.* (2004) showed that *A. muscaria* occurring in Eurasia and North America correspond to phylogenetically distinct populations. Later, Geml *et al.* (2006, 2009) found that it is likely that cryptic, sympatric speciation occurred in the Beringia region of what is now Alaska, and Geml *et al.* (2008) concluded that *A. muscaria sensu lato* has a strong inter- and intra-continental

phylogeographic structure, mainly in North America, and that several phylogenetic species occur within *A. muscaria sensu lato*. The distribution of the species is listed by Tulloss & Yang (2012).

As a continuation of studies on the genus *Amanita* conducted by our group (Wartchow & Maia 2007; Wartchow *et al.*, 2007, 2009, 2013), the present study raises the possibility of the occurrence of various distinct subspecies of *A. muscaria* in Brazil. We also address morphological studies, as well as discussing the importance of using morphological and ecological criteria in order to distinguish between subspecies.

Materials and Methods

In May 2009, fresh *A. muscaria* specimens were collected in the São Francisco de Paula National Forest (29°23'S; 50°23'W), which covers an area of 1606 ha in the state of Rio Grande do Sul, Brazil. Although the composition of this nature reserve is classified as a mixed ombrophilous forest, there are also exotic plantation species of *Pinus*, *Eucalyptus* and *Castanea sativa* Mill. (Dobrovolski *et al.* 2006; Longhi *et al.* 2006, Ribeiro *et al.* 2007). Other materials examined were obtained from the collections of the herbaria of the following institutions (Thiers 2012): the Federal University

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of Santa Catarina (code, FLOR); the Blumenau Regional University Foundation (code, FURB); the University of Santa Cruz do Sul (code, HCB); the Federal University of Santa Maria (code, SMDB); the São Paulo State Department of the Environment (code, SP); and the Federal University of Pernambuco (code, URM).

The description of basidiospores observes the notation “[*a/b/c*]”, which is read as “*a* (number of) basidiospores were measured for *b* (number of) basidiomata from *c* (number of) collections”. Data related to size and shape (*Q*) were annotated as “(*m*-) *n*-*o* (-*p*)”, where *m* and *p* are the lowest and highest value (observed or calculated), respectively; *n* is the 5th percentile; and *o* is the 95th percentile. The descriptions of basidiospores also consider the following biometric variables (Tulloss *et al.* 1992; Tulloss 2000; Tulloss & Lindgren 2005):

- L** = the average length of basidiospores of a single basidiome
- W** = the average width of basidiospores of a single basidiome
- L'** = the average length of all basidiospores
- W'** = the average width of all basidiospores
- Q** = ratio of length to width (for a single basidiospore)
- Q** = the mean *Q* computed for all basidiospores of one basidiome
- Q'** = the mean *Q* computed for all basidiospores of all basidiomes
- w_{sc}** = width of the central stratum of the lamella
- w_{st}-near** = distance from one side of the central stratum to the base of the nearest basidium
- w_{st}-far** = distance from one side of central stratum to the base of the farthest basidium

Abbreviations in author citations follow Kirk & Ansell (1992). The infrageneric classification follows the proposal made by Corner & Bas (1962), subsequently revised by Bas (1969), and later modified by Yang (1997).

Results and Discussion

- Amanita muscaria* var. *muscaria* (L.) Lam., *Encycl. Méth. Bot.* 1: 111. 1783
- ≡ *Agaricus muscarius* L., *Sp. Pl.* 2: 1172. 1753.
- ≡ *Hypophyllum muscarius* (L.) Paulet nom. inval., *Hist. Soc. Roy. Med.* T.11, f. 2-3. 1779 ('1776').
- ≡ *Agaricus muscarius* L.: Fr, *Syst. Mycol.* 1:16. 1821.
- ≡ *Venenarius muscarius* (L.: Fr) Earle, *Bull. New York Bot. Garden* 5: 450. 1909.
- = *Agaricus imperialis* Batsch, *Elench. fung.* (Halle): col. 59. no. 55. 1783.
- = *Agaricus puellus* Batsch, *Elench. fung.* col. 59, no. 54. 1783.
- ≡ *Amanita muscaria* var. *puella* (Batsch) Pers., *Syn. Meth. Fung.* 2: 253.1801.
- = *Agaricus pseudoaurantiacus* Bull. *Herb. France* 11: pl. 122. 1794.

Amanita muscaria var. *muscaria* (L.) are medium-to-large basidiomycetes, dispersed across the ground.

- **PILEUS**: 70.0-140.0 (-180.0) mm; evolving from hemispheric to flat and eventually becoming plano-concave; dark red, then fading to pale orange; thinly striated margin, 10.0-20.0 mm long; invariably white context, approximately 10.0 mm thick at the center and tapering toward the edges; universal veil with invariably pyramidal whitish to cream-colored warts, moderately easy to remove
- **LAMELLAE**: closely spaced in younger basidia, forming a decurrent line at the apex of the stipe in older individuals and eventually separating, invariably white to creamy-white out to the edges, 16.0 mm wide, proximal; lamellulae truncate to obtusely truncate, varying in length
- **STIPE**: 8.0-22.0 × 75.0-150.0 mm, narrowing toward apex, invariably white, fine longitudinal ridges (observed only with a ≥ 10× lens); bulb 35.0-45.0 mm in length and 25.0-40.0 mm in width, fusoid; invariably white background, solid, central cylinder 10.0 mm diameter, insect or larva tunnels of reddish brown (salmon colored), partial veil white near the midpoint, smooth, with remnants of universal veil on margins; universal veil distributed as submembranous sheaths broken at stipe base and at bulb
- **ODOR**: undetectable
- **FLAVOR**: undetectable
- **BASIDIOSPORES**: [100/4/1] (8.5-) 9.0-12.2 (-13.0) × (5.7-) 6.5-9.3 (-9.4) μm, (**L** = (9.3-) 10.1-10.9 μm, **L'** = 10.2 μm, **W** = (6.9-) 7.6-8.3 μm, **W'** = 7.6 μm, **Q** = (1.17-) 1.21-1.50 (-1.63), **Q** = 1.33-1.37, **Q'** = 1.35), inamyloid, hyaline, typically ellipsoid, rarely elongated, smooth, thin-walled; apiculus obtuse, conical sublateral; containing a large guttula
- **BASIDIA**: 55.0-65.0 × 10.0-13.0 μm, bearing four sterigmata, each 4.0 μm in length, abundant clamp connections
- **SUBHYMENIUM**: rehydrating satisfactorily; approximately 40.0 μm thick, three cells deep; generally inflated and clavate; approximately 19.0 × 13.0 μm; **w_{st}-near** = 110.0-125.0 μm; **w_{st}-far** = 130.0-145.0 μm
- **LAMELLA TRAMA**: rehydrating satisfactorily; **w_{cs}** = 40.0-70.0 μm; filamentous hyphae 4.0-7.5 μm, sometimes branched, with frequent clavate elements approximately 115.0 × 36.0 μm; absent vascular hyphae
- **LAMELLAR EDGE**: elements not found
- **PILEUS CONTEXT**: rehydrating satisfactorily, distinctly acrophysalidic; acrophysalides approximately 150.0 × 30.0 μm clavate and elongated, abundant; filamentous hyphae 3.0-8.0 μm, very common, usually branched, very intertwined, forming a loose matrix where the other elements occur; vascular hyphae not observed.
- **STIPE CONTEXT**: rehydrating satisfactorily; longitudinally acrophysalidic; acrophysalides 300.0 × 40.0

μm , abundant; filamentous hyphae 2.0-9.0 (-20.0) μm , longitudinally oriented but sometimes branched, abundant; vascular hyphae 5.0-20.0 μm with a more or less longitudinal orientation, abundant

- **PILEIPELLIS:** cutis reaching 240.0 μm in the center; suprapellis an 80.0- μm thick ixocutis with 2.0-6.0 μm intertwined hyphae, hyaline, embedded in a gelatinous layer; subpellis (160.0 μm) a cutis, radially arranged, 2.0-5.0 μm hyphae, abundant, yellowish; vascular hyphae of approximately 10.0 μm , rare
- **UNIVERSAL VEIL:**
 - on pileus—terminal elements primarily balloon-shaped, 40.0 \times 25.0 μm , pale, abundant, sometimes thick-walled, occasionally in chains of two cells; filamentous hyphae (1.5-5.0 μm), often branched, pale, thin-walled, more abundant near the surface of the pileus; vascular hyphae not observed
 - on stipe base—inflated cells, typically subglobose (41.0 \times 40.0 μm), sometimes ovoid (44.0 \times 34.0 μm), elliptical (50.0 \times 40.0 μm), or clavate (60.0 \times 25.0 μm), pale, 1.0- μm thick walls, filamentous hyphae of approximately 2.0-8.0 μm , hyaline, abundant; vascular hyphae absent
- **PARTIAL VEIL:** intensely intertwined, branched filamentous hyphae of 2.0-4.0 μm , abundant; elongate-elliptical inflated terminal elements (60.0-92.0 \times 14.0-19.0 μm), difficult to locate; vascular hyphae absent; margin with abundant inflated cells from the universal veil mixed with hyphae, hyaline

Distribution in Brazil: Rio Grande do Sul (state)

Habitat: on soil under *Castanea sativa* Mill. (European chestnut)

Material examined: **BRAZIL. Rio Grande do Sul:** São Francisco de Paula National Forest, 19/V/2009, F. Wartchow FLONA2 (URM 82985).

Comments: The European subspecies is very similar to *A. muscaria* var. *flavivolvata*, which differs by having basidiospores that are more elongated, often $L' = 10.7$ μm and $Q' = 1.42$, and shallower subhymenium with $w_{\text{st-near}} = 75.0$ -90.0 μm and $w_{\text{st-far}} = 80.0$ -105.0 (-115.0) μm (Tulloss & Yang 2012). Data on basidiospores provided by Tulloss & Yang (2012) and Tulloss (unpublished data) for *A. muscaria* var. *muscaria*: [475/24/19] (7.4-) 8.5-11.5 (-13.1) \times (5.6-) 6.5-8.5 (-9,8) μm , $L = (8.7\text{-}) 9.1$ -11.2 (-11.4) μm , $L' = 10.0$ μm , $W = (6.5\text{-}) 6.9$ -8.1 μm , (-8,2), $W' = 7.5$ μm , $Q = (1.10\text{-}) 1.21$ -1.47 (-1.75), $Q = 1.26$ -1.41 (-1.42), $Q' = 1.34$. Also described for the European taxon $w_{\text{st-near}} = 110.0$ -125.0 μm and $w_{\text{st-far}} = 130.0$ -145.0 μm , similar to measurements reported previously. Another feature is the natural geographic distribution of taxa, *A. muscaria* var. *flavivolvata* being found in North America, whereas the natural distribution of *A. muscaria* var. *muscaria* is in Eurasia.

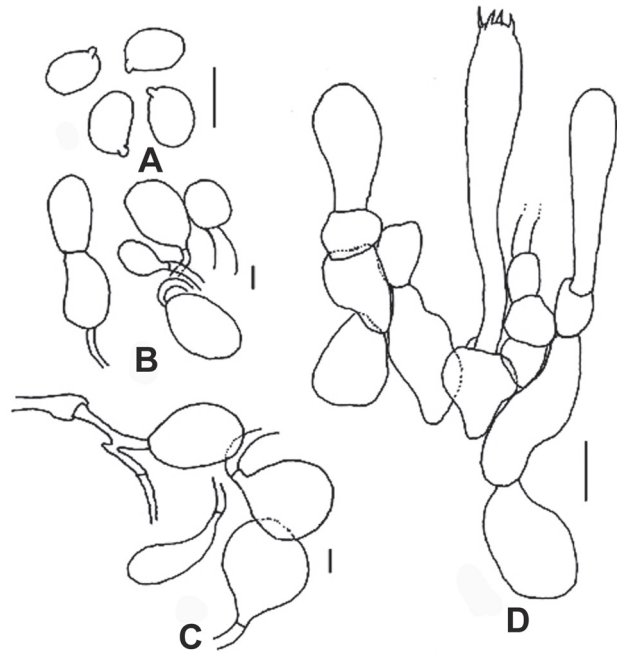


Figure 1. *Amanita muscaria* var. *muscaria*. A, basidiospores; B, elements of universal veil on pileus; C, elements of universal veil on stipe base; D, hymenium and subhymenium. Bar = 10 μm .

In comparison with that of the material studied by Tulloss & Yang (2012), the L' value of the material analyzed here was 2% higher (10.0 μm vs. 10.2 μm). It is noteworthy that we measured only 100 basidiospores from a single collection, and one of the basidiomes showed an L of 10.9 μm , which contributed to increasing the L' value. That basidiome was probably dehydrated at the beginning of sporulation (Tulloss, personal communication).

Geml *et al.* (2006, 2009) were the first to suspect a cryptic speciation in *A. muscaria sensu lato*, with the dispersion center located in Alaska, USA, in a region known as Beringia. In other studies, Geml *et al.* (2008) analyzed a larger sample and recognized at least six phylogenetically distinct clades (I to VI), which might represent distinct phylogenetic species. *A. muscaria* var. *muscaria* probably corresponds to clade II, with distribution from Eurasia to Alaska and the Pacific Northeastern region of the United States; in temperate, boreal and coastal forests with various species of conifers and deciduous trees, relatively common in Europe (Beardslee 1905; Jenkins & Petersen 1976; Breitenbach & Kränzlin 1995; Mattock 1995, Castro 1996; Neville & Poumarat 2001, 2004; Vaasma 2009). For other parts of the world, this taxon certainly corresponds to material collected from exotic plantations, growing under *Pinus* and other tree species (e.g., *Quercus*, *Picea* and *Pseudotsuga*) imported from Europe to Tanzania (Härkönen *et al.* 1994, Tulloss personal communication), Australia (Reid 1979; Grgurinovic 1997, Wood 1997; Hawkeswood 2006; Robinson 2010), South Africa (Pearson 1950; Reid & Eicker 1991) and New Zealand (Stevenson 1962; Ridley 1991). In

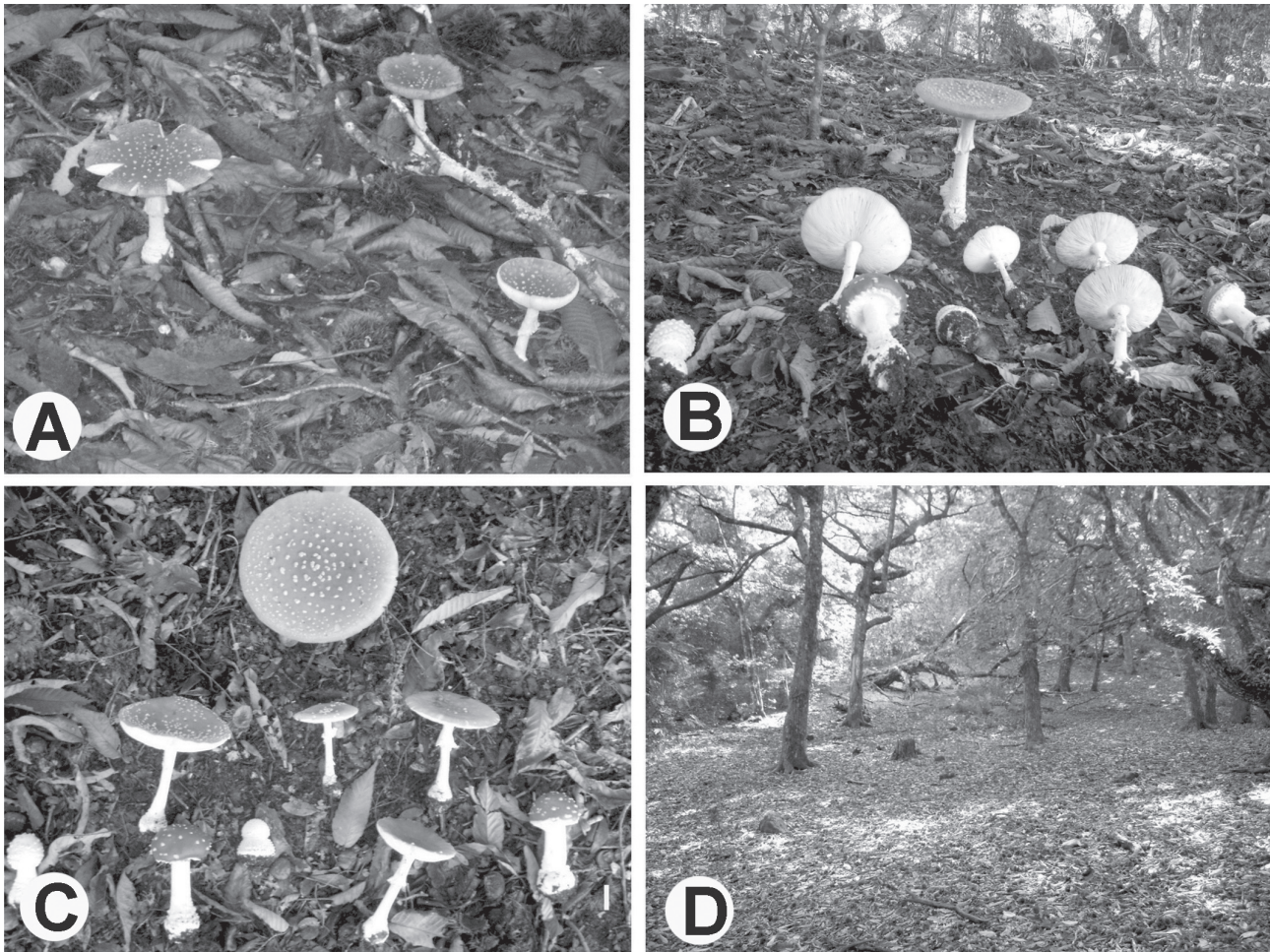


Figure 2. *Amanita muscaria* var. *muscaria*. A-C, basidiomes; D, aspect of *Castanea sativa* plantation. Photographs: F. Wartchow. Bar = 20 mm.

Brazil, this taxon is reportedly found in the plateau region of Rio Grande do Sul among the “European pines” planted there (Homrich 1965). Unfortunately, we could not locate the material needed in order to determine the true identity of this specimen.

The material examined, which was found growing under a *Pinus* sp. in the state of Santa Catarina [BRAZIL, Santa Catarina, Rancho Queimado, Campinho, 18.v.1986, F. Brugermann (no number) (FLOR 10328)], was very poorly preserved with few basidiospores: [19/1/1] (7.3-) 7.7-9 × 5.5-6.5 (-6.8) μm, (L = 8.3 μm; W = 6.1 μm; Q = (1.14-) 1.20-1.46 (-1.49), Q = 1.36. Although the characteristics of the subhymenium and the lamella trama are impossible to analyze, the values found for the basidiospores are characteristic of *A. muscaria* var. *muscaria*.

Amanita muscaria var. *flavivolvata* Singer, Sydowia 11: 374.1957 (‘1958’).

≡ *Amanita muscaria* var. *flavivolvata* (Singer) Dav.T. Jenkins, Biblioth. Mycol. 57: 56. 1977.

Amanita muscaria var. *flavivolvata* (Singer) are medium-to-large basidiomycetes.

- PILEUS: 70.0-110.0 mm, plano-convex expanding to plano-concave, dark red, becoming paler and eventually orange, shiny surface, slightly viscid, finely striated margin, reaching 11.0 mm in length; invariably white context, approximately 10.0 mm thick at the center and gradually tapering toward the margin and eventually tapering more abruptly in the furrows; universal veil invariably with white pyramidal warts that are moderately easy to remove
- LAMELLAE: ranging from closely spaced to completely separated, forming a decurrent line at the apex of the stipe in older individuals, invariably white from edge to edge, 10.0 mm wide, proximal; lamellulae truncate to obtusely truncate, varying in length
- STIPE: 80.0-100.0 × 25.0-30.0 mm, narrowing toward apex, invariably white, longitudinally thinly fibrillose (seen only with a ≥ 10× lens); bulb from 20.0-50.0 mm in length and 25.0-40.0 mm in width, fusoid; context white, unchanging, solid, central cylinder 10.0 mm diameter., insect or larva tunnels of reddish brown (salmon colored); partial veil white near the midpoint, smooth with remnants of universal veil on the edge;

- universal veil whitish, distributed as submembranous sheaths broken at stipe base and bulb
- ODOR: undetectable
 - FLAVOR: undetectable
 - BASIDIOSPORES: [295/10/8] (8.2-) 8.5-13.7 (-14.0) × (5.5-) 6.0-9.5 (-10.0) μm, (L = (10.0-) 10.3-11.1 μm, L' = 10.7 μm, W = 7.1-7.8 (-8.1) μm, W' = 7.6 μm, Q = (1.21-) 1.22-1.71 (-2.00), Q' = (1.35-) 1.41-1.48, Q'' = 1.43), inamyloid, hyaline, ellipsoid, sometimes ellipsoid and elongated, occasionally cylindrical, smooth, thin-walled; apiculus obtuse conical, sublateral to subapical; containing a large guttula.
 - BASIDIA: 41.0-47.0 × 11.0-13.0 μm, two to four sterigmata, each approximately 4.0 μm, abundant clamp connections
 - SUBHYMENIUM: rehydrating satisfactorily in most basidiomes; 29.0 mm thick, three cells deep, generally inflated, either clavate (13.0-23.0 × 7.5-16.0 μm) or elongate (14.0-20, 0 × 5.0-8.5 μm); w_{st}-near = 70.0-90.0 μm; w_{st}-far = 80.0-100.0 (-115.0) μm
 - LAMELLAE TRAMA: rehydrating satisfactorily in most basidiomes; w_{st} = 35.0-70.0; μm filamentous hyphae 4.0-7.5 μm, sometimes branched, with frequent clavate elements 85.0 × 25.0 μm; vascular hyphae absent
 - LAMELLAR EDGE: elements not observed
 - PILEUS CONTEXT: rehydrating satisfactorily, distinctly acrophysalidic; acrophysalides approximately 180.0 × 20.0 μm elongated and clavate, abundant; filamentous

- hyphae 2.0-12.0 μm, very common, usually branched, very intertwined, forming a loose matrix where other elements occur; vascular hyphae 14.0 μm, occasional
- STIPE CONTEXT: rehydrating satisfactorily; longitudinally acrophysalidic; acrophysalides 325.0 × 40.0 μm, abundant; filamentous hyphae 2.0-9.0 (-20.0) μm, longitudinally oriented, but sometimes branched, plentiful; vascular hyphae 5.0-13.0 μm with longitudinal orientation, quite abundant
 - PILEIPPELLIS: cutis reaching 170.0 μm at the center; suprapellis a 70.0-μm thick ixocutis with 1.5-4.0 μm intertwined hyphae, hyaline, embedded in a gelatinous layer; subpellis a 100.0-μm thick cutis, 2.0-4.5 μm hyphae radially arranged and sometimes intertwined, abundant, yellowish; vascular hyphae 10.0 μm, occasional
 - UNIVERSAL VEIL:
 - on pileus—terminal elements primarily balloon-shaped (60.0 × 43.0 μm) to subglobose (30.0 × 26.0 μm), pale to hyaline, abundant, sometimes thick-walled, occasionally in chains of two to three cells; filamentous hyphae (1.5-5.0 μm), often branched, pale, thin-walled, more abundant near pileus surface; vascular hyphae not observed in most specimens, abundant in one specimen 4.0-8.0 (-16.0) μm
 - on stipe base—inflated cells, typically subglobose (55.0 × 50.0 μm) or elongated-clavate (e.g., 50.0 × 20.0 μm), pale, thick-walled (1 μm); filamentous hyphae 2.0-8.0 μm, hyaline, abundant; vascular hyphae 4.0-8.0 μm, abundant
 - PARTIAL VEIL: filamentous hyphae 2.0-7.0 μm, abundant, intensely intertwined, branched; inflated elongate-elliptical terminal elements (100.0 × 22.0 μm), difficult to locate; vascular hyphae absent

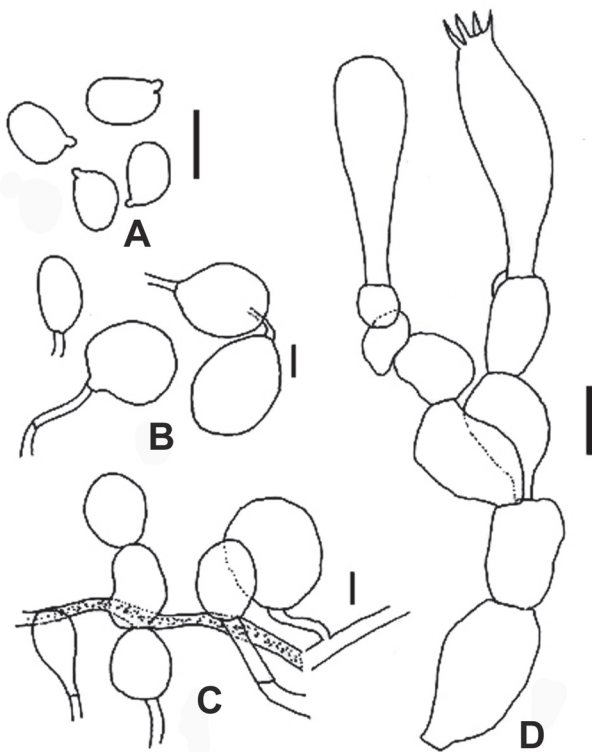


Figure 3. *Amanita muscaria* var. *flavivolvata*. A, basidiospores; B, elements of universal veil on stipe base; C, elements of universal veil on pileus; D, hymenium and subhymenium. Scale bar = 10 μm.

DISTRIBUTION IN BRAZIL: (states of) Paraná, Rio Grande do Sul, Santa Catarina, São Paulo.

HABITAT: often on soil under *Pinus* spp. (mainly *P. eliottii* Engelm., and *P. taeda* L.) natural distribution in North America (Silba 1986) and one of the most frequently planted in Brazil (Zanchetta & Diniz 2006), but also recorded in *Eucalyptus* sp. plantation (URM 82988) *Podocarpus* sp. (SP 307271) and *Araucaria* sp. (URM 75827). However, the latter two tree species are reported to associate predominantly with arbuscular mycorrhizal fungi (Oliveira & Ventura 1952; Breuninger *et al.* 2000; Moreira *et al.* 2007) and basidiomes of *A. muscaria* var. *flavivolvata* may be only occasionally present, although collectors do not mention the presence of *Pinus* nearby or excavating the rhizosphere where the basidiomes were in order to ascertain the ectomycorrhizal association.

Material examined: **BRAZIL. Paraná:** Cerro Azul, Fazenda Varanópolis, 19/VII/1985 U. Keutenedjian-Filho (no number) (SP 193903); **Rio Grande do Sul:** Minas do Leão, Agropecuária Condor, 26/V/2008, V.G. Cortez

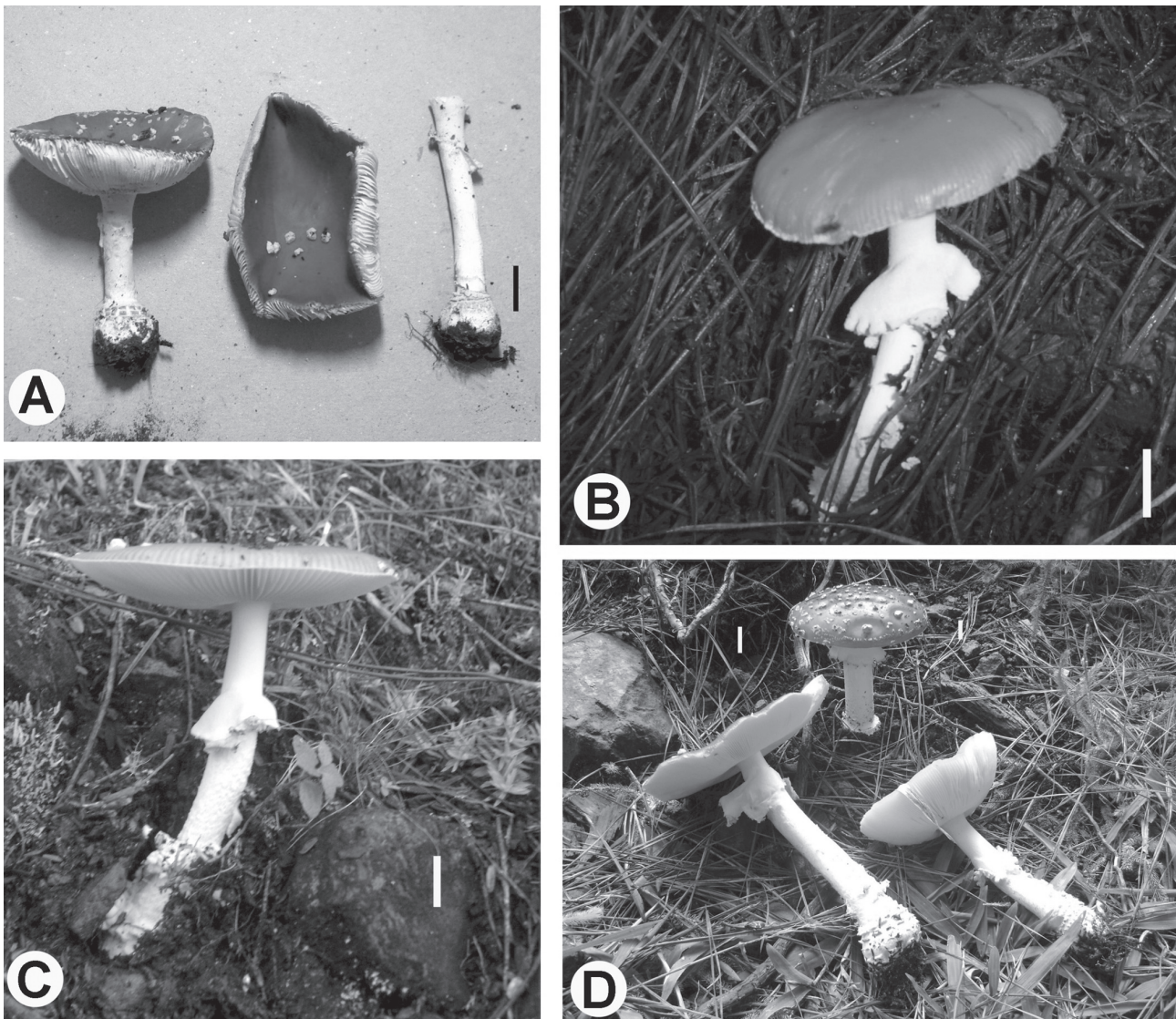


Figure 4. *Amanita muscaria* var. *flavivolvata*. A-D, basidiomes. Scale bar = 20 mm. Photographs: A, V.G. Cortez; B, F. Karstedt; C and D, F. Wartchow.

097/08 (URM 82988, RET), Santa Maria, Camobi, Federal University, Santa Maria Campus, 11/VII/2000 V.G. Cortez (no number) (SMDB 9169), São Francisco de Paula National Forest (road leading from entrance to office), 18/V/2009 F. Wartchow FLONA1 (URM 82987, RET), woods within the reserve (behind the office), 18/V/2009, F. Wartchow FLONA4 (URM 82986, RET); **Santa Catarina:** Joinville, RPPN Caetezal, 24/VIII/2004, F. Karstedt (FURB 840, 425); **São Paulo:** Campos do Jordão, Horto Florestal at 1350 m altitude, 28/VI/1997, M.H. Alves (no number) (URM 75827), Campos do Jordão, location unspecified, 12/X/1998, L.K. Okino (no number) (SP 307271).

Note: The macroscopic description of the subspecies is entirely based on collections made by the first author in the São Francisco de Paula National Forest (URM 82987 and URM 82986). Unfortunately, there is no information on collection notes for other records.

Comments: This subspecies, first found in San Francisco, California, was originally named for its yellowish volva (Singer 1958), and the name continued to be used by other authors in North America (e.g. Jenkins 1977, 1986; Thiers 1982) and Europe (Poumarat & Neville, 2001, 2004). Nevertheless, the color of the volva does not appear to be crucial to the definition of this subspecies. Jenkins & Petersen (1976) described a neotype of *A. muscaria* var. *muscaria* as having a cream-colored to yellowish volva, which raised the possibility that the entity *A. muscaria* var. *flavivolvata* occurs in Europe as well (Neville & Poumarat 2004).

Recent studies show that the most prominent features in distinguishing among these taxa are the L' and Q' of basidiospores, which are reported to be $L' = 10.8 \mu\text{m}$ and $Q' = 1.42$ in the American subspecies and $L' = 10 \mu\text{m}$ and $Q' = 1.34$ in the European subspecies (Tulloss, unpublished data; Tulloss & Yang 2012). Basidiospore data provided by Tulloss (unpublished data) for *A. muscaria* var. *flavivolvata*

are as follows: [957/48/37] (7.5-) 9.0-12.8 (-19.0) \times (5.5-) 6.5-8.5 (-11.5) μm $L = (8.9-)$ 9.41-12.1 (-14.6) μm $L' = 10.8$ μm , $W = (6.6-)$ 6.9-8.2 μm (-8.4), $W' = 7.6$ μm , $Q = (1.11-)$ 1.26-1.67 (-2.23), $Q = (1.29-)$ 1.31-1.65 (-1.95), $Q' = 1.42$). Other important features in the differentiation among subspecies are the w_{st} -near -and w_{st} -far of the subhymenium, on which *A. muscaria* var. *muscaria* are higher than the American strain of.

Apparently, information about the color of the volva and pileus are of little relevance, because it can be influenced by the climatic conditions to which the basidiomes are submitted. Those conditions can generate yellow or albino forms of pileus within each population (Geml *et al.* 2008). Fungal pigments include muscaflavin, which produces yellow; muscarine, which produces an orange-red color; muscapurpurin, which produces purple; and muscaaurin, which produces reddish-brown (Meléndez-Michelot & Howell 2003). The mixture or suppression of certain chemical components can determine what color prevails in a fungus, such as the yellow in the pilei of certain populations in the northeastern United States, which must be attributable to an abundance of muscaflavin.

Amanita muscaria var. *flavivolvata* is found in proximity to exotic plantations introduced into Australia (Sawyer *et al.* 2001), Chile (Garrido 1986) and Colombia (Tulloss *et al.* 1992); in Costa Rica, it is reported as occurring associated with *Quercus* (Tulloss *et al.* 2011). Daniele *et al.* (2005) cited *A. muscaria* in association with *Cedrus Deodara* (Roxb. ex D. Don) G. Don in Argentina, although the authors did not mention the infrageneric group to which it belongs. As demonstrated in the present study, *A. muscaria* var. *flavivolvata* occurs in the Brazilian states of Parana, Rio Grande do Sul, Santa Catarina and São Paulo. Specimens collected in Brazil by Guerrero & Homrich (1983), Fusco-Mucci & Yokomizo (1985), Figueiredo *et al.* (1996), Giachini *et al.* (2000, 2004), Meijer (2001, 2006) and Sobestiansky (2005) might represent *A. muscaria* var. *flavivolvata*, because the specimens were found primarily in proximity to *P. elliotii* and *P. taeda*, two species imported from North America. The material

F. Karstedt 425 (FURB 840) certainly matches the voucher for the material cited in Karstedt & Stürmer (2008). One of the specimens examined here (V.G. Cortez 097/08; URM 82988, RET) was collected under a *Eucalyptus* sp. It is of note that *A. muscaria sensu lato* has often been cited in Australia, albeit associated with exotic plantations (Reid 1979; Grgurinovic 1997, Wood 1997; Hawkeswood 2006, Robinson 2010). However, a study conducted by Malajczuk *et al.* (1982) demonstrated that this species, in its broader sense, is also associated with *Eucalyptus*.

The dichotomous key recently proposed by Menolli *et al.* (2009) emphasizes the yellow color of the universal veil elements in early development as a means of distinguishing *A. muscaria* var. *flavivolvata* from *A. muscaria* var. *muscaria*. Poumarat & Neville (2004) also considered the conditions of the universal veil a major distinguishing feature in *A. muscaria*. However, we consider the features of the universal veil irrelevant because they can result merely from environmental conditions to which the basidiome was submitted. The most important features distinguishing *A. muscaria* var. *muscaria* from *A. muscaria* var. *flavivolvata* are the depth of the subhymenium, the size of the basidiospores, and the geographic distribution (Tulloss & Yang 2012; Tulloss, personal communication). We have devised a new dichotomous key that focuses on characteristics that are more appropriate to differentiating between the two *A. muscaria* subspecies in Brazil (Table 1).

In agreement with our interpretation, Geml *et al.* (2008) suggested that clades corresponding to *A. muscaria* var. *muscaria* and *A. muscaria* var. *flavivolvata* belong to distinct phylogenetic species. In addition, Vellinga *et al.* (2009) called for more detailed taxonomic studies in order to elucidate which *A. muscaria sensu lato* occurs in the southern hemisphere.

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Table 1. Dichotomous key to the *Amanita muscaria* subspecies occurring in Brazil.

| Characteristic | <i>A. muscaria</i> var. <i>muscaria</i> | <i>A. muscaria</i> var. <i>flavivolvata</i> |
|-------------------------------|--------------------------------------------------------|---------------------------------------------------------|
| Subhymenium | | |
| w_{st} -near, μm | 110.0-125.0 | 75.0-90.0 |
| w_{st} -far, μm | 130.0-145.0 | 80.0-100.0 |
| Basidiospores, μm | (8.5-) 9.0-12.2 (-13.0) \times (5.7-) 6.5-9.3 (-9.4) | (8.2-) 8.5-13.7 (-14.0) \times (5.5-) 6.0-9.5 (-10.0) |
| L' , μm | 10.2 | 10.7 |
| W' , μm | 7.6 | 7.6 |
| Q' | 1.35 | 1.43 |
| Species in proximity | <i>Castanea sativa</i> | <i>Pinus</i> spp.* |

w_{st} -near – distance from one side of the central stratum to the base of the nearest basidium; w_{st} -far – distance from one side of central stratum to the base of the farthest basidium; L' – the average length of all basidiospores; W' – the average width of all basidiospores; Q' – the mean ratio of length to width computed for all basidiospores of all basidiomes.

* – natural distribution in North America.

portunity to visit and review his herbarium and examine exsiccates of *Amanita* from various parts of the world. We also thank the curators of the following herbaria who kindly allowed us to examine exsiccates of *Amanita muscaria sensu lato*: Adriana M. Gugliotta (SP); Clarice Loguercio-Milk (FLOR); Mara R. Ritter (ICN); Lucy Sevegnani (FURB); Thais S. Canto-Dorow (SMDB); and Jair Putzke (HCB). In addition, we thank the researchers Mara Rosa B. Silveira, Mateus A. Reck, Paula S. Silva and Gilberto Coelho for their assistance in collecting the specimens; Drs. Aristotle Goes-Neto, Iuri G. Baseia, José L. Bezerra, Laise H. Cavalcanti and Gilberto Coelho for reading and making suggestions to improve the draft of the manuscript; and Dr. Vagner Gularte Cortez and Fernanda Karstedt for authorizing the use of their photographs. This work received financial support from the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; PROTAX Grant no. 141073/2006-3 and INCT Herbário Virtual Grant no. 573.883/2008-4) and from the *Fundação de Amparo à Ciência e Tecnologia de Pernambuco* (FACEPE, Foundation for the Advancement of Science and Technology in the State of Pernambuco; Grant no. 0100-2.03/09 BFP).

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