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A second record of *Entoloma azureoviride* (Agaricales, Basidiomycota) from Brazilian Amazon

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ABSTRACT - (A second record of *Entoloma azureoviride* (Agaricales, Basidiomycota) from Brazilian Amazon). A new material collected and the holotype of *Entoloma azureoviride* were studied. The analysis of these specimens showed some discrepancies with the protologue which are discussed here. The diagnostic characters of this species are the following: fibrillose pileus, blue lamellae, cuboid spores, and abundant oleiferous hyphae in the lamellae trama. It was also observed a peculiar pseudoparenchymatous pileus trama consisting of inflated elements up to 25 µm in diam., that become more elongated towards the hymenium. A comparison of *E. azureoviride* with other species with blue tints in the subgenus *Inocephalus* and a discussion about its morphological peculiarity are provided.

Keywords: Agaricomycetes, Entolomataceae, neotropics, taxonomy

RESUMO - (Um segundo registro of *Entoloma azureoviride* (Agaricales, Basidiomycota) para a Amazônia brasileira). Um novo material coletado e o holótipo de *Entoloma azureoviride* foram estudados. A análise desses espécimes revelou algumas discrepâncias em relação ao protólogo que discutidassão discutidas no presente trabalho. As características diagnósticas da espécie são: píleo fibriloso, lamelas azuis, esporos cuboides e abundância de hifas oleíferas na trama da lamela. Observou-se também a ocorrência nessade uma peculiar trama pseudoparenquimatosa do píleo, consistindo de elementos inflados, atingindo 25 µm de diâmetro e tornando-se mais alongados em direção ao ápice do himênio. Uma comparação de *E. azureoviride* com outras espécies de tons azulados do subgênero *Inocephalus* e uma discussão sobre sua peculiaridade morfológica são apresentadas.

Palavras-chave: Agaricomycetes, Entolomataceae, neotropics, taxonomy

Introduction

Entoloma (Fr.) P. Kumm. is a species-rich genus in the order Agaricales and is easily recognized by its pink spore print and angular basidiospores (Noordeloos 1981, Singer 1986). More than 1500 species are currently known (e.g. Co-David et al. 2009, Morgado et al. 2013), of which at least 271 species are from Central and South America (Coimbra 2014).

In Brazil, the priest Johannes Rick carried out the first studies on the diversity of *Entoloma sensu lato*. He described several taxa from the State of Rio Grande do Sul (Rick 1919, 1920, 1930, 1938a, 1938b), which are compiled in his posthumous work published

by another priest Balduíno Rambo (Rick 1961). In a review of these works, Putzke and Cavalcanti (1997) listed 50 names distributed in the genera *Claudopus* Gillet, *Eccilia* (Fr.) P. Kumm., *Entoloma sensu stricto*, *Leptonia* (Fr.) P. Kumm., and *Nolanea* (Fr.) P. Kumm. As previously referred to by Singer (1953), very few of Rick's names were confirmed as valid. Considering these revisions, at least 57 species were known in Brazil (Putzke and Putzke 2000). Recently, de Meijer (2006), Wartchow (2006), Karstedt *et al.* (2007, as *Pouzarella* Mazzer) and Karstedt & Capelari [2010 as *Calliderma* (Romagn.) Largent, 2013 as *Inocephalus* (Noordel.) P.D. Orton], Alves and Nascimento (2012), Coimbra *et al.* (2013) and Karstedt & Capelari (2015)

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included additional taxa and hence more than 100 species of *Entoloma* sensu lato are presently known from Brazil (Coimbra 2014).

Entoloma sensu lato is relatively well known in the Brazilian and Bolivian Amazon from where the following taxa were described: E. azureoviride E. Horak & Singer, E. clitocyboides E. Horak & Singer, E. conspicuocystidiosum E. Horak & Singer, E. cutifractum E. Horak & Singer, E. dragonosporum (Singer) E. Horak, E. flavotinctum E. Horak & Corner, E. lowyi (Singer) E. Horak, E. lycopersicum E. Horak & Singer, E. obscurum Dennis, E. sparsicystis E. Horak & Singer, E. spadiceum E. Horak & Singer, E. spineum E. Horak & Singer, E. tucuchense Dennis, E. viscaurantium E. Horak & Singer, Leptonia cf. obscura (Dennis) Dennis, Inopilus speciosus (Romagn.) Pegler nom. inval., Nolanea sp. and Trichoplilus fasciculatus Largent & Aime (Singer 1965, 1973, Horak 1982, Singer & Aguiar 1986, Capelari & Maziero 1988, Coimbra & Gibertoni 2014).

The species treated here belongs to Entoloma subg. Inocephalus Noordel. There are different opinions about the taxonomic status of Inocephalus. Some authors (e.g. Baroni & Halling 2000, Largent et al. 2008, 2013, Karstedt & Capelari 2013) prefer to accommodate all taxa with mycenoid stature, conical pileus with radially fibrillose surface, nearly free lamellae, intracellular pigments and cuboid spores, in the segregated genus Inocephalus (Nopordel.) P.D. Orton. On the other hand, Pegler (1983) used the name Inopilus (Romagn.) Pegler, in accordance with the original concept of Romagnesi (see Noordeloos 1979) to accommodate the taxa with these characteristic and (sub)isodiametric basidiospores and inflated cystidia, with I. versatilis (Gillet) Pegler as the type species. However, since Romagnesi equivocally changed the type of the subgenus Inopilus from Rhodophyllus versatilis [type of the subgen. Pouzarella (Mazzer) Noordel.] into E. inocephalum without following the rules of the International Botanical Code (Noordeloos 1981), the name Inopilus is illegitimate and cannot be used for this group of species (Noordeloos 1979). So, E. inocephalum is the type species of the genus Inocephalus. Recent molecular studies (Co-David et al. 2009, Morgado et al. 2013), however, showed that Inocephalus forms an integral part of a monophyletic genus Entoloma, and is a morphologically highly specialized group, characterized by very abundant tramal granules and almost exclusively cuboid spores (Noordeloos 1981).

Pseudoparenchymatous pileitrama among *Entoloma sensu lato* is not well studied. Noordeloos (1981) already cited that the pileus context of this genus is regular, in general made up of the same type of elements as found in the lamella trama. However, in some thick-fleshed specimens the centre of pileus can be more irregular or even pseudoparenchymatous.

We examine a new collection of *Entoloma* azureoviride, a blue tinted species with mycenoid/inocybvoid habit bearing cuboid basidiospores, and studied the holotype for comparison and provide an account of an interesting anatomical characteristic of *Entoloma* subg. *Inocephalus*.

Material and methods

The basidiome was collected from Reserva Ducke, located in the outskirts of Manaus, Brazil $(02^{\circ}55^{\circ}S, 59^{\circ}59^{\circ}W)$. The reserve protects 10,000 ha $(10 \times 10 \text{ km})$ of "terra-firme" forests but is being isolated by the expansion of Manaus city. A detailed description of the collection site was given in Braga-Neto *et al.* (2008).

We followed the traditional methodology for the study of agarics (Singer 1986) and the colors of the basidiome were named using the British Fungus Flora Color Chart (Henderson *et al.* 1969). Basidiospores measurements follow the style proposed by Baroni & Lodge (1998), on which the cuboid basidiospores are measured using the flat sides of the 4-sided spores, from the apex to the base and from the adaxial to the abaxial facet while the spore was in lateral (profile) view.

The key is based on Horak (1976, 1982), Noordeloos & Hausknecht (2007), Li *et al.* (2009) and Karstedt & Capelari (2013). The basidiospores were measured without including the hilar appendix. The material is deposited at URM and the type of *E. azureoviride* from Z-ZT was also studied (Thiers, continuously updated).

Results and Discussion

Entoloma azureoviride E. Horak & Singer in Horak, Sydowia 35: 81. 1982. Figs. 1-3.

≡ *Inocephalus azureoviridis* (E. Horak & Singer) Karstedt & Capelari, Nova Hedwigia 96: 282. 2013.

Material examined: BRAZIL. AMAZONAS, Manaus, 30 km N of Manaus, 14.VI.1977, *R. Singer* (ZT 78/31, holotype!); Manaus, Reserva Ducke, 25.I.2008, *R. Braga-Neto and R. Zucaratto/RBN 585* (URM 80093).

Pileus ca. 22 mm in diam., conic-campanulate with a straight margin, warm brown (darker than "52 Buff") at the centre, ochraceous brown (darker than "9 H") towards the margin, strongly radially fibrillose, slightly splitting at the margin; margin not striate nor sulcate. Lamellae adnexed to almost free, thin, moderately distant, blue ("72 Blue") with blackish ("37 Olivaceous black") spots, with a distinctly fimbriate, paler edge; lamellulae common, truncate to subtruncate with diverse lengths. STIPE 42 × 3 mm, central, cylindric, fragile, mostly equal, pale beige (slightly darker than "52 Buff") with greenisholivaceous tints, subviscid, with longitudinally arranged grayish blue fibrils, with blue (paler than "71 Sky blue") basal mycelium. Context thin, fleshy, color not seen by the collector. Odor and taste not noted.

Basidiospores pinkish in mass, 8-10 μm, average 9 μm, cuboid, frequently with convex to plane and occasionally with depressed facets, pink, thinwalled, guttulate; hilar appendix conical. BASIDIA 32-44 × 10-12 μm, clavate, 4-spored. Pleurocystidia frequent, as hyphoid pseudocystidia. Lamella edge sterile, with crowded cheilocystidia. Cheilocystidia $30-60 \times 7-14 \mu m$, clavate to narrowly clavate, with a pale yellowish brown intracellular pigment, thin walled, clamped. Lamella trama regular, made up of wide hyphae ca. 17 µm in diam., which become narrow towards the margin; some with abundant vacuoles, frequently clamped; oleiferous hyphae common, mostly near the gill margin. Pileitrama pseudoparenchymatous in transversal section, consisting of very inflated elements up to 25 µm in diam., becoming more elongated to 30 × 8-10 µm downward in the direction of the hymenium. Pileipellis a trichocutis made up of anticlinal parallel hyphae with terminal slender clavate elements $80-107 \times 9-13 \mu m$, with brown vacuolar to sometimes parietal pigments. Clamp connections abundant.

Habitat: solitary on soil of mature tropical 'terrafirme' moist forest, along plateaus with clayish soils and sparse leaf litter layer.

Distribution: known from the type locality in Amazon and Atlantic Forest of southeast Brazil (Karstedt & Capelari 2013).

Remarks: *Entoloma azureoviride* belongs to the subgenus *Inocephalus* due to the distinctly fibrillose pileus and the cuboid basidiospores (Noordeloos 1981, 1987). It is easily recognized in the field by its ochraceous brownish pileus and blue lamellae

(although reported bluish then ochre-brown by Horak 1982 and Karsted & Capelari 2013). Microscopically, the cuboid basidiospores, presence of pseudocystidia and the relatively pseudoparenchymatous pileitrama are remarkable characters.

Although well delimited, the protologue of E. azureoviride was described with blue fading to green or ochre-green pileus and stipe, concolorous lamellae, a smooth and hygrophanous pileus and smaller basidiospores 5-8.5 μ m (Horak 1982). At first, we thought that our specimen was a new species, but Karstedt & Capelari (2013) already reviewed the holotype and no microscopic differences among these materials as well the material analyzed by us.

Our examination of the holotype of *E. azureoviride* revealed discrepancies in comparison to the protologue and suggested a closer relationship with our specimen: (1) somewhat larger basidiospores than reported in the protologue (6.5-)7-9.5(-10) µm (average 8 µm), with four but only very occasionally with five angles; (2) a pseudoparenchymatous pileitrama; and (3) a transitional pileipellis between a cutis and trichoderm (trichocutis). We also have observed hyphoid pseudocystidia, which frequently arise from the lamellar trama, but only infrequently higher than the rest of the structures of the hymenium. Although not mentioned in the protologue, Horak (1982, legend of fig. 3L) referred to these structures as 'pleurocystidia'. Recent studies by Karstedt & Capelari (2013) who provided an excellent description, also reported similar features in their type study, although they described the pileus trama of differtent way: "composed of radially arranged and parallel hyphae, with hyphae 5-26 mm diam., cylindrical or slightly inflated..." (Karstedt & Capelari (2013).

Before comparing this Amazonian fungus with other species, we need to comment on its remarkable pileitrama. The pileitrama of *E. azureoviride* consists of mostly inflated elements that give an appearance of a pseudoparenchymatous tissue in the middle of the pileitrama, that gradually become more elongate in the direction to top of lamellae (figure 3). Although this specialized trama is more frequent in thick-fleshed species (Noordeloos 1981: 130), it is an interesting new anatomical finding in this thin-flesh species. In the case of the holotype and our specimen, these rounded cells occur in almost all pileitrama.

Compiling our observations and those of Karstedt and Capelari (2013), *E. azureoviride* can be characterized by a brownish to ochraceous brown pileus fading to blue with greenish tints at maturity,



Figure 1. Basidiome of *E. azureoviride* (URM 80093). Scale bar 10 mm.

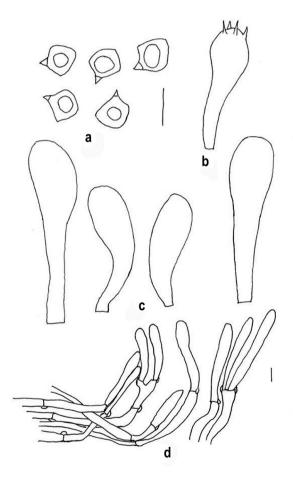


Figure 2. Entoloma azureoviride a. Basidiospores. b. Basidium. c. Cheilocystidia. d. Terminal elements of the pileipellis. Scale bars $10~\mu m$.

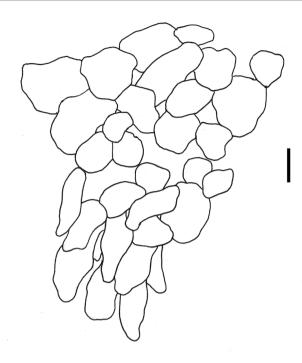


Figure 3. Pileitrama of *Entoloma azureoviride* and the top of the lamellae near midratio presenting more elongate elements towards the lamellae trama. Scale bar $10 \mu m$.

a fibrillose pileus surface hygrophanous with age, cuboid basidiospores, a pseudoparenchymatous pileitrama and a trichocutis-type pileipellis (in the sense of Noordeloos 1981: 128, fig. 4).

Several other taxa with blue lamellae, mycenoid stature, cuboid spores and brownish pigmented cystidia are known. They can be primarily segregated from the Amazonian taxon by the shape of the cystidia as well as the pileitrama features in some cases:

The type of *E. virescens* (Berk. & M.A. Curtis) E. Horak ex Courtec. nom illegit. (non Schaeff. 1774), originally known from Bonin Island off Taiwan, and was described as having a conic pileus with a conic papilla and cheilocystidia strangulated at the apex and $40-120 \times 6-20 \mu m$ in size [somewhat] rostrate in our interpretation of Horak's (1976, p. 201, fig. 19g) figure]. It is also reported from Japan, Papua New Guinea, New Zealand, Malaya, Sri Lanka and Madagascar (Horak 1976, 1980). In addition, it was described as having a blue color when young, fading to blue greenish or yellow-blue greenish, smooth to radially fibrillose and striate pileus, and basidiospores that are (7-) 8-10 μm in size (Horak 1976). Additionally, Noordeloos and Hausknecht (2007) described "E. virescens sensu lato" as being only slightly translucently striate with age when moist and vivid light blue to grayish blue

unchanging with age. Later, Courtecuisse (1986) and Pegler [1997 as *Inopilus virescens* (Berk. and M.A. Curtis) Pegler] also reported somewhat larger spores: $10.5-12.5 \times 10.5-12 \mu m$, $8.8-11 \mu m$ and $10-12 (-14) \times 9-11 \mu m$ respectively.

A recent description of 'Inocephalus virescens (Sacc.) Largent & Abell-Davis' from Northeastern Queensland, Australia, presented the average of the basidiospores measurement somewhat similar to *E. azureoviride* (9.31 × 8.84 µm), but that collection lacks the pseudoparenchymatous elements in the pileitrama and its color fades to greenish (Largent & Abell-Davis 2011). Alves & Nascimento (2012) referred larger basidiospores in a material identified as *Entoloma virescens* collected in the Brazilian semiarid, '9.75-13.75 × 9.75-12.5 µm (xm = 11.72 ± $1.16 \times 11.29 \pm 0.84$; E = 0.90-1.16 (-1.65); Q = 1.06 ± 0.136 ; n = 30/2)'.

Studies by Noordeloos & Hauksnecht (2007) concluded that *E. virescens sensu lato* probably has a very wide geographical distribution, from the paleotropics to the Neotropic, including the temperate regions of Japan, New Zealand and Australia. However, Largent & Abell-Davis (2011) and Karstedt & Capelari (2013) claimed that only careful morphological and molecular studies will clarify whether the degree of morphological variation can be used for species delimitation.

Entoloma hochstetterii (Reichert) G. Stev. was recently reviewed by Horak (2008) and he reported cheilocystidia with strangulate-rostrate apex and larger basidiospores (11-15 \times 11-14 μm). Unfortunately, no conclusions were taken for this species owing to the lack of type material and poorly preserved authentic specimens. Stevenson (1962), on the other hand, did not review the type.

Entoloma altissimum (Massee) E. Horak, originally described from Singapore differs in having cylindric to subclavate cheilocystidia ($50-130 \times 6-20 \,\mu\text{m}$) and a longer stipe (up to $100 \,\text{mm}$). It is also known from Sabah, Papua New Guinea and Madagascar (Horak 1976, 1980). It was described as having a deep blue pileus with brownish-green centre in the larger basidiomes and cuboid basidiospores measuring 7-10.5 $\,\mu\text{m}$ (Horak 1976).

Entoloma subaltissimum T.H. Li & Chuan H. Li from South China differs in having a pale to deep blue pileus with greenish to turquoise tints, distant blue lamellae that turn rust brown when bruised, larger subquadrate to quadrate basidiospores measuring

 $8\text{-}12.5 \times 8\text{-}12 \,\mu\text{m}$, and broadly clavate cheilocystidia $50\text{-}76 \times 15\text{-}29 \,\mu\text{m}$ (Li *et al.* 2009). The recently described *E. mengsongense* A.N. Ediriweera, Karun., J.C. Xu, K.D. Hyde & P.E. Mortimer also from China differs primarily by the consistently smaller basidiospores measuring $4\text{-}8 \times 4\text{-}6 \,\mu\text{m}$ (Ediriweera *et al.* 2017).

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