

Dung fungi from Brazil: Agrocybe pediades (Fr.) Fayod (Basidiomycota) in Cerrado

Fungos coprófilos do Brasil: Agrocybe pediades (Fr.) Fayod (Basidiomycota) no Cerrado

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(Recebido em 27 de fevereiro de 2020; aceito em 05 de junho de 2020)

In the survey of copromycodiversity from Brazil, we present the first records of the genus *Agrocybe* for the Cerrado biome, represented by the species *A. pediades*, found on cattle dung in the state of Goiás, central Brazil. We also present detailed morphological descriptions, illustrations, and comments about its distribution and the finicolous habitat, as well as the cultural characteristics of the specimens. Keywords: Agaricales, dung-inhabiting fungi, Strophariaceae.

No levantamento da copromicodiversidade brasileira, apresentamos os primeiros registros do gênero *Agrocybe* para o bioma Cerrado, representado pela espécie *A. pediades*, encontrada sobre esterco bovino no estado de Goiás, Brasil. Apresentamos descrições morfológicas, ilustrações e comentários detalhados sobre sua distribuição e hábito fimícola, bem como as características dos espécimes em meio de cultura. Palavras-chave:Agaricales, fungos de esterco, Strophariaceae.

1. INTRODUCTION

Dung fungi, also described as coprophilous or fimicolous fungi in accordance with the choice of substrates [1], are an artificial group encompassing the fungi with physiological and ecological adaptations, at different levels, to grow on the dung of a diverse animals assembles, mainly herbivores. In Brazil, there are few studies reporting species of dung fungi and, until 2017, the fungal diversity of this group accounted with 225 species [2]. Among the representatives of agaricoid dung fungi, a few species were recorded in Brazil, mostly in the genera *Coprinellus* P. Karst., *Coprinopsis* P. Karst., *Deconica* (W.G. Sm.) P. Karst., *Panaeolus* (Fr.) Quél. and *Psilocybe* (Fr.) P. Kumm. [3].

Agrocybe Fayod is a mushrooms genus belonging to the family Strophariaceae (Basidiomycota: Agaricales), comprising about 100 described species [4, 5]. The genus has wide distribution, occurring in natural (forests, on plant debris and soil, sand dunes) or anthropic (gardens, manured soil) areas, being considered as a poorly selective genus in substrate choice [6, 7, 8]. The fimicolous species of *Agrocybe*, which grows on dung or heavily manured soils, are poorly recorded, since they are a few species and because they do not develop on moist chambers, as observed in other dung fungi species [9].

Nine species of *Agrocybe* were recorded in dung or manured soil worldwide: *A. allocystis* Singer, *A. amara* (Murrill) Singer, *A. coprophila* (Rick) Singer, *A. fimicola* (Speg.) Singer, *A. hortensis* (Burt) Singer, *A. perfecta* (Rick) Singer, *A. platensis* (Speg.) Singer, *A. procera* Singer, and *A. stercoraria* Pegler [1, 9, 10]. According to Coimbra (2015) [11], 21 species of *Agrocybe* occur in Brazil and only four are considered fimicolous: *A. allocystis*, *A. fimicola* (Speg.) Singer, *A. neocoprophila* Singer, and *A. subpediades* (Murrill) Watling. However, until now there were no records of this genus for Brazilian Cerrado. Herein, we present the first occurrence of both, the genus *Agrocybe* and the species *A. pediades* for the biome, contributing to the increase in the knowledge of the mycobiota from Cerrado.

2. MATERIAL AND METHODS

We found the basidiomes of an *Agrocybe* species, growing on cattle dung (*Bos taurus* L.) in an organic vegetable garden in the municipality of Anápolis, Goiás state, Brazil (16°16'27.26"S, 48°54'27.13"W). The basidiomes were photographed, collected with part of the substrate, packaged in paper boxes, and taken to the laboratory. Macroscopic features were described from fresh basidiomes. Color names and codes used in the macroscopic descriptions were based on Kornerup and Wanscher (1978) [12]. The remaining specimens were dried in a drying oven at about 40°C for one week. Microscopic structures were observed from freehand cuts of dried material using a razor blade. The sections were rehydrated in 3% KOH and dyed with floxine or Melzer's reagent, when necessary. The microscopic structures were photographed at ×1000 magnification by using an Olympus CX31 optical microscope and a digital camera. Measurements were taken by using the software Piximètre, v. 5.9 R 1532 [13], where for basidiospores measurements, Q refers to the quotient between the length and width, *Qm* is the medium value of *Q*, and *n* is the number of measured spores. Voucher material was preserved and deposited at HUEG Herbarium [14].

Pure cultures of *A. pediades* was obtained inoculating a spore suspension on potato dextrose agar culture medium (PDA, 3.9 g in 100 mL of water) with the addition of 0.25 mg/mL of chloramphenicol in plate Petri dish and incubated in BOD at 26°C. Macro morphological aspects of culture were described according to Olmos-Fuertes and Herrera (1973) [15]. For microscopical description, fragments of mycelium were mounted on slides, with the aid of lactophenol cotton blue staining, photographed and measured with the same methodology adopted to microscopical descriptions of the basidiomes.

3. RESULTS AND DISCUSSION

Agrocybe pediades (Fr.) Fayod, Annales des Sciences Naturelles Botanique 9: 358 (1889) ≡ *Agaricus pediades* Fr., Systema Mycologicum 1: 290 (1821) Figures 1-2.

Macroscopic features: Pileus 10–20 mm diam., hemispheric to convex, yellowish grey to greyish beige in wet condition (4B2, 4C2), surface smooth to slightly rugulose, with a uniform margin, no veil remnants observed. Lamellae yellowish white to yellowish grey (4A2, 4B2), crowded, slightly wavy, entire edge, adnate; lamellulae present. Stipe pale yellow to greyish yellow (4A3, 4B3), central, fibrous in consistency, cylindrical, sometimes slightly bulbous at the base and partially squamulose; annulus absent; context 2–3.5 mm, fibrous to slightly fleshy when fresh, yellowish white (4A2); spore-print dark brown (7F4, 7F5).

Microscopic features: Basidiospores (10.3) 11.2 - 12.8 (14) × (6.3) 6.9 - 8.2 (8.7) µm, (Q = 1.5 - 1.7 (1.8), Qm = 1.6, n = 50), smooth, broadly ellipsoid in face view and ellipsoid in side view, thick-walled up to 1.1 - 1.3 µm, with a 1.3 - 1.5 µm wide germ pore, light-brown (7D6) under light microscope. Basidia $25 - 30 \times 9.4 - 13$ µm, clavate, mixed two and 4-spored. Pleurocystidia absent. Cheilocystidia $20 - 30 \times 8.7 - 13.5$ µm, abundant, crowded, irregularly lageniform to narrowly utriform with subcapitate to capitate apex 2.5 µm average width, hyaline and thin-walled. Pileipellis hymeniform, composed by clavate, hyaline, thin to slightly thickened-walled elements, (12.5) 14.51 - 19.77 (19.8) × 6.7 - 11.59 (11.6) µm. Stipitipellis is a dry and regular cutis of rarely branched, 5 - 7 µm wide hyphae with cylindrical elements, clamp-connections present.

Cultures in Potato Dextrose Agar medium (PDA): after 14 days at 26°C somewhat restricted, with slow growing, attaining 7 – 8 cm in four weeks. Surface downy, white (1A1), not becoming zonate with time; reverse uncolored. Hyphae system monomitic, hyphae hyaline 2 – 2.5 μ m diameter, few clamp connections observed (Figure 1, G-I).

Known distribution – widespread in subtropical and temperate regions.

Material examined – Brazil, Goiás, Anápolis, Vale das Antas farms sector, rural zone of municipality Anápolis, 16°16'27.26"S 48°54'27.13"W, found growing directly on cattle dung (*Bos taurus* L.) in an organic vegetable garden, 21 Nov 2018, F. J. S. Calaça FJSC86 (HUEG: 12037).

Notes – This is the first record of the genus *Agrocybe* in Brazilian Cerrado, with the occurrence of *A. pediades* in this domain. The species was observed occurring in a finicolous substrate (cattle

dung), increasing the records of coprophilous/fimicolous basidiomycetes that occur in Brazil Central to four species [16]. Before that, the validated records of this species were only for the Southern Region of Brazil, with the predominance of the Pampa and Atlantic Forest biomes [11, 16].



Figure 1: Agrocybe pediades. (A) Basidiomes. (B) Two and four-sterigmate basidia with young spores in formation (arrows, dyed with floxine). (C) Basidiospores (not stained). (D) Clamp-connection (arrow). (E) Irregularly lageniform to narrowly utriform cheilocystidia. (F) Clavate elements of pileipellis (arrows). (G) Two weeks of A. pediades culture in PDA medium. (H) Three weeks culture in PDA medium. (I) Four weeks culture in PDA medium, with 7–8 cm (Ø). (J) Monomitic hyphae system from culture in PDA medium, dyed with lactophenol cotton blue. Scale bars (A) = 2 cm, (B–F) = 10 μm, (J) = 20 μm.

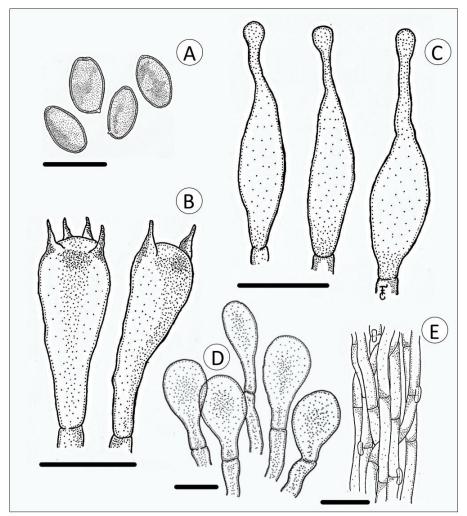


Figure 2: Microscopic features line drawings of Agrocybe pediades. (A) Basidiospores. (B) Four and twosterigmate basidia. (C) Cheilocystidia. (D) Pileipellis elements. (E) Stipitipellis. Scale bars 10 µm.

Some close related species, as *Agrocybe cubensis* (Murrill) Singer and *A. fimicola* (Speg.) Singer differ from our material by macro or microscopic characteristics and substrate choice. *Agrocybe cubensis* grows on dead vegetable residues, presents an appendiculate veil and 4-spored basidia [6]. This species was found in Brazil, in the state of Paraná [17]. *Agrocybe fimicola* was established as a variety of *A. pediades* by Nauta (2004, 2005) [6, 18], who considered this species within a complex of three varieties (*A. pediades* var. *fimicola*, *A. pediades* var. *pediades* and *A. pediades* var. *cinctula*), macroscopically distinguishable by the presence or absence of conspicuous appendiculate veil, annulus, and occurrence or not in dung. Meijer (2008) [19] collected and described specimens of *A. fimicola* from state of Paraná, Brazil, found growing on pasture in dung of horse and buffalo. Currently, *A. fimicola* remains an independent species and *A. pediades* var. *fimicola* is yet considered its synonymy [8, 18, 20, 21].

Table 1 shows the distribution of *Agrocybe* species with fimicolous habit recorded in Brazil, all from the South Region of the country. Thus, our data adds the first record of this genus outside a region with predominance of subtropical climate Cfa and/or Cfb, according to Köppen-Geiger classification [22], occurring in a region of seasonal tropical climate (Aw type), with well-defined dry and rainy season (Brazilian savanna). This record expands the geographical distribution of this genus, which probably is very widespread in Brazil, including its occurrence as dung-inhabiting genus.

Species	Fimicolous substrate types	Brazilian state	Key references
A. allocystis	on dung or soil	PR	Singer (1969) [23], Coimbra (2015) [11]
A. fimicola	on dung (cow, buffalo, horse), soil with grass, or as saprotrophic (plant debris)	PR, RS	Meijer (2006, 2008) [24, 19], Coimbra (2015) [11]
A. neocoprophila	on dung or soil	RS	Singer (1965) [25], Coimbra (2015) [11]
A. subpediades	on dung	PR	Watling (1992) [17], Meijer (2006) [24], Coimbra (2015) [11]
A. pediades	on manured soil with cattle dung	GO	Calaça et al. (present study)

Table 1: Agrocybe species with fimicolous habit (occurring on dung or highly manured soil), reported from Brazil in accordance with the literature.

The fungal diversity of Cerrado, it is still underexplored, with the most recent survey including about over 640 species confirmed, mostly phytopathogenic fungi [26, 27]. The biome is composed by a phytophysiognomic mosaic along a topographic gradient that allows the existence of a gradient of vegetation, allowing formations that vary between grasslands, savannas and forests formations [28]. Due to its biological diversity, high degrees of endemism, rapid habitat loss and land-use changes, the Cerrado is classified as a global biodiversity hotspot [29, 30]. Whereas much of the unknown fungal biodiversity may be in underexploited geographical areas or habitats, like biodiversity hotspots [31], efforts to survey the fungi of the Cerrado should be encouraged, aiming the search for new species.

These efforts should be directed mainly to those species considered as cryptic or that develop mostly on substrates little explored by mycologists, as dung for example. Unfortunately, the study of dung fungi in Brazil lacks human resources in quantity and quality to explore more of diversity of these fungi in the country. The estimated diversity for the group, as we have shown in previous data [2, 16], tends to increase considerably by including unexplored areas of the country (i.e. almost total Brazilian territory in regards to this fungal group) as well as the study of new substrates, especially from native hosts.

4. CONCLUSION

We presented here the first record of the occurrence of the agaricoid mushroom genus *Agrocybe*, with the record of *A. pediades* occurring in a fimicolous environment in the Brazilian Cerrado biome, contributing with new data on the biology and ecology of dung-inhabiting fungi from Brazil. Furthermore, we reinforce the need for additional studies in different locations and substrates that favor the occurrence of dung-inhabiting fungi, especially in threatened biomes, such as the Brazilian Cerrado, with the purpose of surveying the richness of this group of organisms that are still little studied in the Cerrado, which may contribute to reinforcing the need to preserve it.

5. ACKNOWLEDGEMENTS

This work was financed by the Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG, process nº. 201810267000595). We thank the reviewers for their insightful comments and efforts towards improving the final version of the manuscript.

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