

First reports of fimicolous myxomycetes (Protozoa: Amoebozoa) from Brazilian Cerrado and Pantanal biomes

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Abstract

Fimicolous organisms are those that can grow on dung. These substrates offer conditions that favour colonization by microorganisms, such as high nutrient and moisture content and an alkaline-neutral pH that is especially advantageous in arid/desertic regions. There are about 250 species of myxomycetes known from Brazil, which are distributed in all geographic regions and biomes, obtained mainly from plant-derived substrates. However, there are some reports of fimicolous myxomycetes in Brazil. In this study, we expand this knowledge with new records of fimicolous myxomycetes in multiple Brazilian biomes. Between 2017 and 2018, horse and cattle dung samples were collected in municipal areas in the State of Goiás (Cerrado biome): Pirenópolis, Goiás, and Porangatu; and the State of Mato Grosso (Pantanal biome): Poconé. Samples were incubated in moist chambers and monitored for four months. Myxomycetous fructifications were observed, pho-

tographed under stereo and light microscopes, and morphologically identified. Vouchers were deposited at the HUEG Herbarium. A total of five species of myxomycetes were recorded: *Arcyria cinerea* and *Physarum viride* (Pirenópolis), *P. cinereum* (Goiás City), *P. melleum* (Porangatu), and *Perichaena corticalis* (Poconé). They represent the first records of fimicolous myxomycetes from the Brazilian Cerrado and Pantanal biomes. Additionally, *P. melleum* was reported as fimicolous for the first time in Brazil and the second time in the world; *P. corticalis* was reported for the first time in midwest region as well as for the first time as fimicolous in Brazil; and *P. viride* was reported for the first time as fimicolous in the world.

focused on the biology and ecology of species that occur on plant substrates (Lado and Basanta 2008; Schnittler et al. 2017). For instance, almost all of the 250 species of myxomycetes known from Brazil, recorded from all geographic regions and biomes, were obtained from plant substrates (e.g. Lado and Basanta 2008; Lima-Coelho 2019). Most of these studies were carried out in the Atlantic Forest or Caatinga biomes, with the other biomes remaining poorly studied (Lima-Coelho 2019). Knowledge on fimicolous myxomycetes is limited only to 10 species, most of them from northeast region of Brazil, in the areas of Caatinga (Calaça et al. 2020). This study is expanding this knowledge by reporting new records of fimicolous myxomycetes in other Brazilian biomes.

Introduction

Dung represents an important microhabitat for a wide variety of organisms, and can be a source of nutrients, substrate for growth, and dispersion (Bell 1983; Holter et al. 2016; Frank et al. 2017). This substrate offers conditions that favor colonization of microorganisms, such as high nutrient and moisture content, and an alkaline-neutral pH, which are especially advantageous in arid/desert regions. Although the term “fimicolous” is widely used as a synonym for coprophilous when referring to organisms that develop on dung, some authors classify coprophilous organisms as organisms that obligatorily inhabit feces in any moment of their life cycle and fimicolous as those that inhabit feces occasionally or opportunistically among other possible substrates (Bell 1983, Doveri 2004).

Fungi, bacteria, and slime molds (Protozoa: Amoebozoa) are the most common dung-inhabiting organisms, and they play a key role in the process of decomposition and nutrient cycling in these substrates (Bell 1983; Feest 1987; Novozhilov et al. 2017). Fimicolous myxomycetes can grow on dung as well as on living or decomposing plant substrates, soil, and rocks (Araújo et al. 2012; Calaça et al. 2020). Indeed, they are found practically in all terrestrial ecosystems from natural ecosystems to anthropogenic environments (Araújo et al. 2012; Novozhilov et al. 2017).

Studies of myxomycetes in tropical regions have

Materials and methods

Study areas and sampling

The Brazilian Cerrado (also referred to as Brazilian Savanna) biome comprises the most extensive woodland-savanna in South America, originally occupying more than two million km², predominantly in the central region of the country (IBGE 2004). A vegetation mosaic ranging from grassland to forest formations with high alpha and beta diversity characterizes the biome (Ribeiro & Walter 2008; Ferreira et al. 2017). Due to its high species richness, high level of endemism, and rapid loss of habitats, especially due to conversion to agricultural use, the Cerrado is considered a global hotspot for biodiversity conservation (Strassburg et al. 2017). The Pantanal biome (i.e. Brazilian wetlands) comprises the world's largest tropical wetland area, as well as the world's largest flooded grasslands, occurring in a plain with more than 140,000 km² in Central South America, with the majority in the Brazilian midwest region. This biome is characterized by having an annual and variable flood cycle (Signor et al. 2010). Figure 1 presents the original extension of both biomes in Brazil.

Dung samples of horse (*Equus caballus* L.) and cattle (*Bos taurus* L.) were collected between 2017-2018 during a field expedition to assess the diversity of coprophilous fungi in the region. These ex-

peditions were carried out in the municipalities of Goiás City, Pirenópolis and Porangatu, in the state of Goiás, in the Brazilian Cerrado, and in Poconé, state of Mato Grosso, in the Pantanal. A composite sample made up of dung from several individuals of each animal species was obtained in each studied location (Figure 1).

Samples were incubated in duplicate moist chambers and kept in the laboratory under ambient light cycles and temperature of $\pm 27^\circ$ C. These moist chambers were regularly moistened with distilled water. During the four months of incubation, the moist chambers were examined for the appearance of myxomycetous fructification with a stereomicroscope. Observed sporophores were photographed and removed with the aid of a sterile dissecting needle. Part of the material was utilized to prepare semi-permanent slides for microscopic examination. Both nomenclature and color codes used in the descriptions of the examined materials were based on Kornerup & Wanscher (1978).

The microscopic structures were photographed at $\times 1000$ magnification by using an Olympus CX31 optical microscope and a digital camera. Measurements were taken using Piximètre, v. 5.9 R 1532 (Henriot and Cheype, 2012) where Q: quotient between the length and width, Qm: medium value of Q, Me: medium values to all measured spores, and n: number of measured spores. Specimens of myxomycetes were identified up to the morphospecies using keys for fimicolous myxomy-

cete species (Eliasson & Lundqvist 1979; Eliasson & Keller 1999; Poulain et al. 2011). Scientific nomenclature followed Lado (2005-2020). Vouchers of studied material were deposited at the Collection of Myxomycetes from Herbarium HUEG. Geographic distribution of the species was based on data from the Global Biodiversity Information Facility database (GBIF, <https://www.gbif.org/>). The occurrence map (Figure 1A) was constructed with QGIS 3.10.2 and the geobr package in R (Pereira et al. 2019; R Core Team 2020) from the geographical coordinates obtained at the sampling sites, using a GPS device.

Results and Discussion

A total of five myxomycetes species were found, composed of three genera in three families: Arcyriaceae [*Arcyria cinerea* (Bull.) Pers.], Trichiaceae [*Perichaena corticalis* (Batsch) Rostaf.] and Physaraceae [*Physarum cinereum* (Batsch) Pers., *P. melleum* (Berk. & Broome) Masee, and *P. viride* (Bull.) Pers.]. These data represent the first occurrence records of fimicolous myxomycetes in the Brazilian Cerrado and Pantanal, as well as the first records of some of them as dung-inhabiting microorganisms in new geographic areas, expanding the known distribution range of these species (Table 1).

Table 1. Fimicolous myxomycetes recorded from Brazilian Cerrado and Pantanal biomes.

Species	Biome	Location	New record	
			as fimicolous	to location
<i>Arcyria cinerea</i>	Brazilian Cerrado	Pirenópolis (GO)	Brazilian Cerrado	-
<i>Physarum cinereum</i>	Brazilian Cerrado	Goiás (GO)	Brazilian Cerrado	Goiás state
<i>P. melleum</i>	Brazilian Cerrado	Porangatu (GO)	First to Brazil and second to world	-
<i>P. viride</i>	Brazilian Cerrado	Pirenópolis (GO)	World	midwest region
<i>Perichaena corticalis</i>	Pantanal	Poconé (MT)	Brazil	midwest region, first to genus in Pantanal

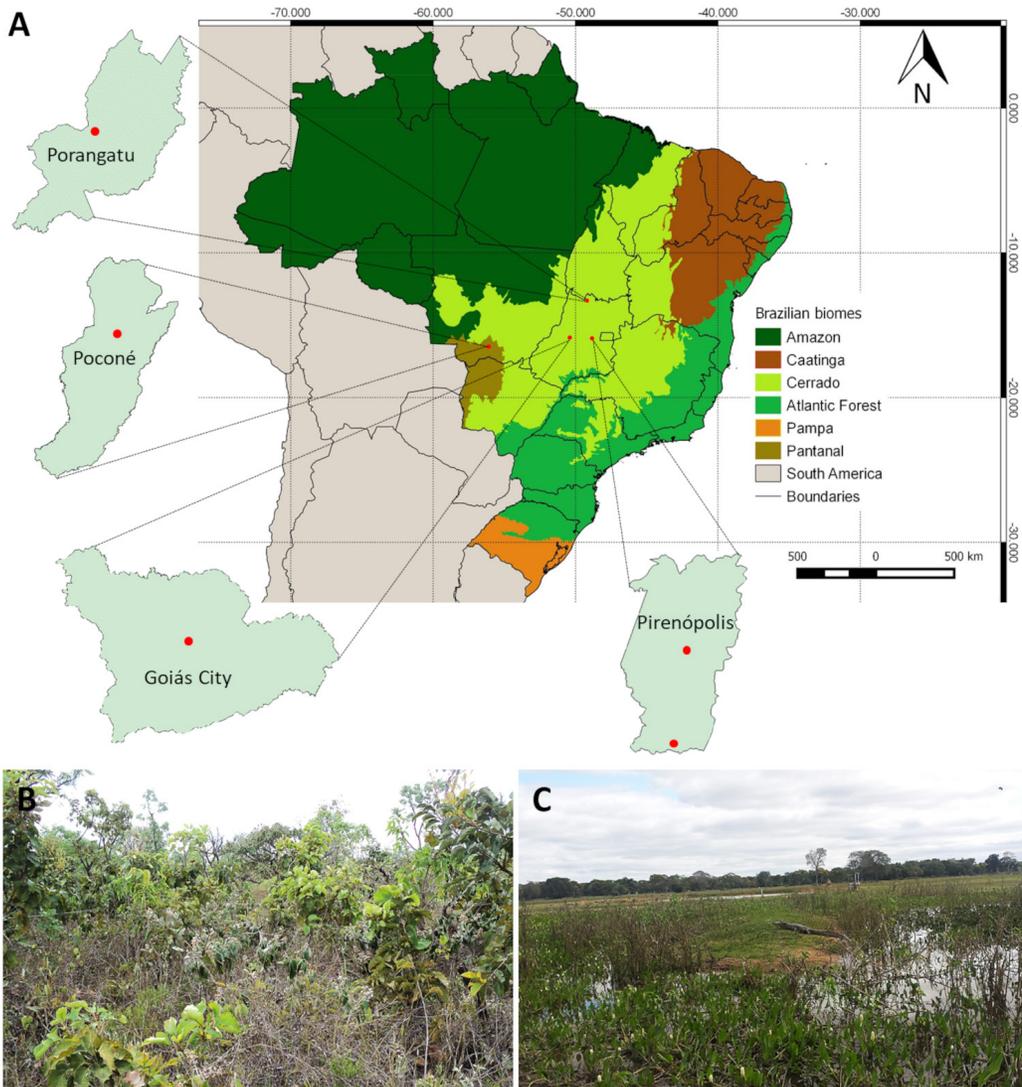


Fig. 1. Overview of the study area. **A:** Location of municipalities and locations sampled (red dots), **B:** View of Cerrado vegetation *stricto sensu*, in the Brazilian Cerrado, **C:** View of a common landscape in Pantanal, where the presence of broad-snouted caiman (*Caiman latirostris*: Alligatoridae) (in the center of the image) is frequent.

List of species

Arcyria cinerea (Bull.) Pers., Syn. meth. fung.
1:184 (1801) – Fig. 2.

DESCRIPTION: Sporangia gregarious or scattered, greyish brown (6D3) when immature to gray (2C1) to light gray (1C1) when mature, sporotheca sub-cylindrical or ovoid, 0.5–1.5 mm long total. Stalk longitudinally striated, gray (2C1), 0.2–0.5 mm long, filled with sporelike vesicles. Peridium single, shining. Calyculus gray (2C1) to light gray (1C1), plicate. Capillitium tubular, light gray (1C1) to yellowish gray (3B2), firmly attached to the calyculus, with many free ends, 4–5 μm in diameter, densely warted and/or spinulose. Spores globose to subglobose,

pale gray (1B1) or yellowish gray (2C2) colored in mass, nearly colorless and smooth, by transmitted light, (6.8) 7.7 – 8.8 (9.4) \times (6.8) 7.4 – 8.7 (9.3) μm , $Q = 1 - 1.08$ (1.1), $n = 20$, $Me = 8.2 \times 8 \mu\text{m}$, $Qm = 1$.

DISTRIBUTION: Worldwide.

SPECIMENS EXAMINED: BRAZIL. Goiás, Municipality of Pirenópolis, Santa Rita Farm (16°07'45.1"S and 49°02'38.5"W), on horse dung in moist chamber (HUEG 9636, SXS 6175). Usina Velha Waterfall (15°50'38.9"S and 48°55'34.6"W), on horse dung in moist chamber (HUEG 12988, FJSC 95).

COMMENTS: This is the first record of *Arcyria cinerea* as fimicolous from Brazilian Cerrado. In Brazil, previous records of this species as a dung-inhabiting myxomycete were reported on rabbit (*Sylvilagus brasiliensis* L.) dung (Bezerra et al. 2008b,

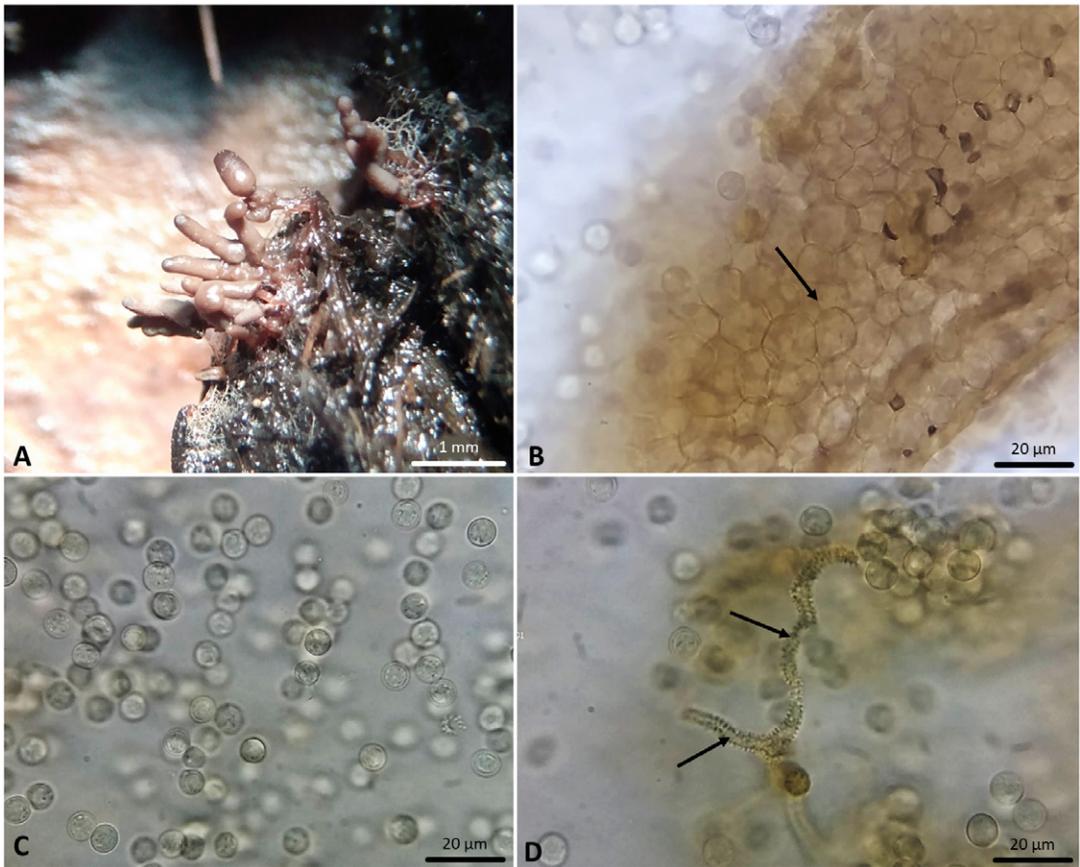


Fig. 2. *Arcyria cinerea* (HUEG 12988). **A:** immature sporangia on horse dung, **B:** detail of stalk, with spore-like structures (arrow), **C:** spores, **D:** capillitium, the arrows point the warted and/or spinulose composition of capillitium.

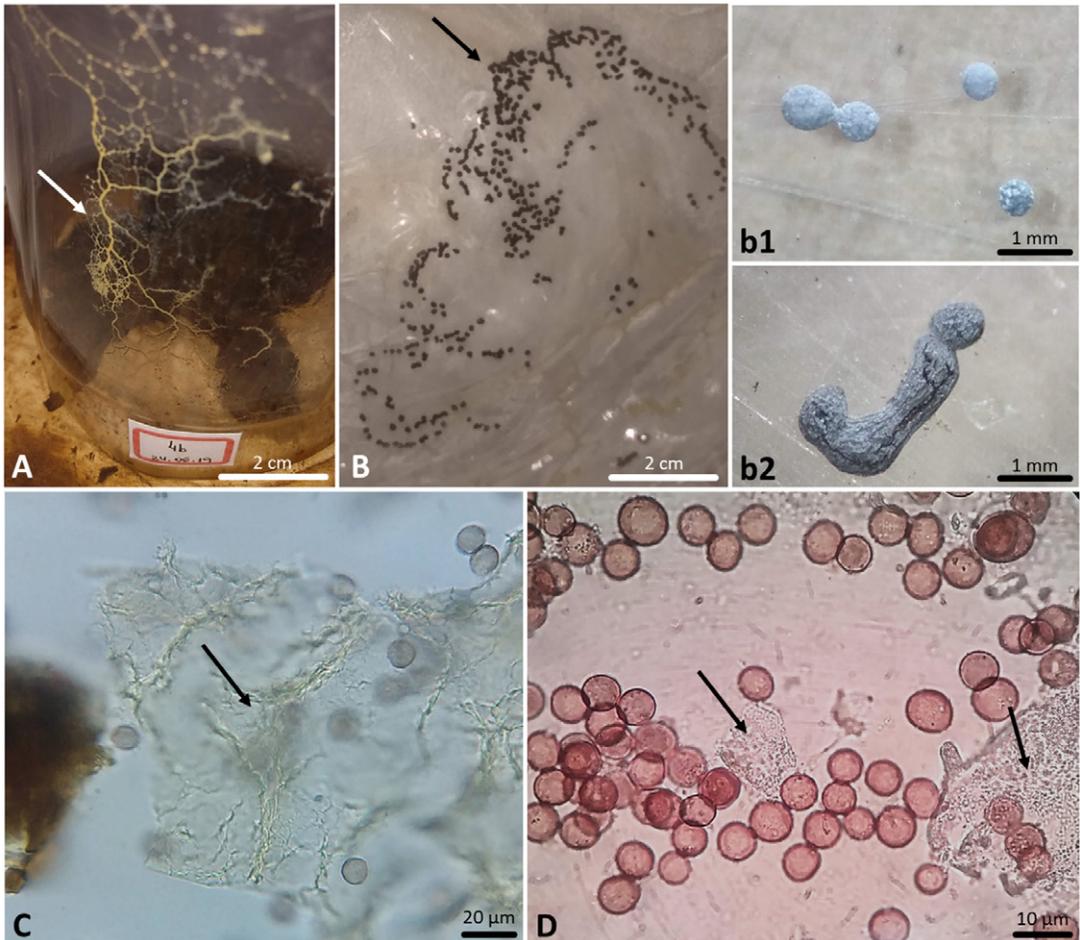


Fig. 3. *Physarum cinereum* (HUEG 12989). **A:** plasmodium in moist chamber, climbing the wall of the chamber, **B:** sporangia on the wall of the chamber, **b1** and **b2:** detail of sporangia, **C:** peridium, **D:** spores and capillitium, with calcareous nodes (arrows).

2010) and rock cavy (*Kerodon rupestris* Wied-Neuwied) dung (Parente and Cavalcanti 2017), both in the Caatinga biome in the northeast region of Brazil (Calaça et al. 2020).

Physarum cinereum (Batsch) Pers., Neues Mag. Bot. 1:89 (1794) – Fig. 3.

DESCRIPTION: Sporangia sessile, gregarious to crowded, subglobose or elongated in shape, which occasionally fuse forming short plasmodiocarps with 0.3 to 1 mm diam., white (1A1) or pale (cinereous) grey (1B1). Peridium single, membranous,

densely coated with lime. Capillitia abundant, composed of hyaline threads connected by angular nodes with calcareous deposits. Spores globose, spinulose, violet brown (10F8) in mass, greyish red (9C4) in light microscope, (7.8) 8 – 9.4 (10.4) × (7.5) 7.7 – 8.8 (9.3) μm, Q = 1 – 1.1, n = 30, Me = 8.7 × 8.3 μm, Qm = 1.

DISTRIBUTION: Worldwide.

SPECIMEN EXAMINED: BRAZIL. Goiás, Municipality of Goiás City, cattle pasture area in the campus of the Federal Institute of Goiás (15°55'58.1"S and 50°08'58.9"W), on cattle dung in moist chamber (HUEG 12989, FJSC 92).

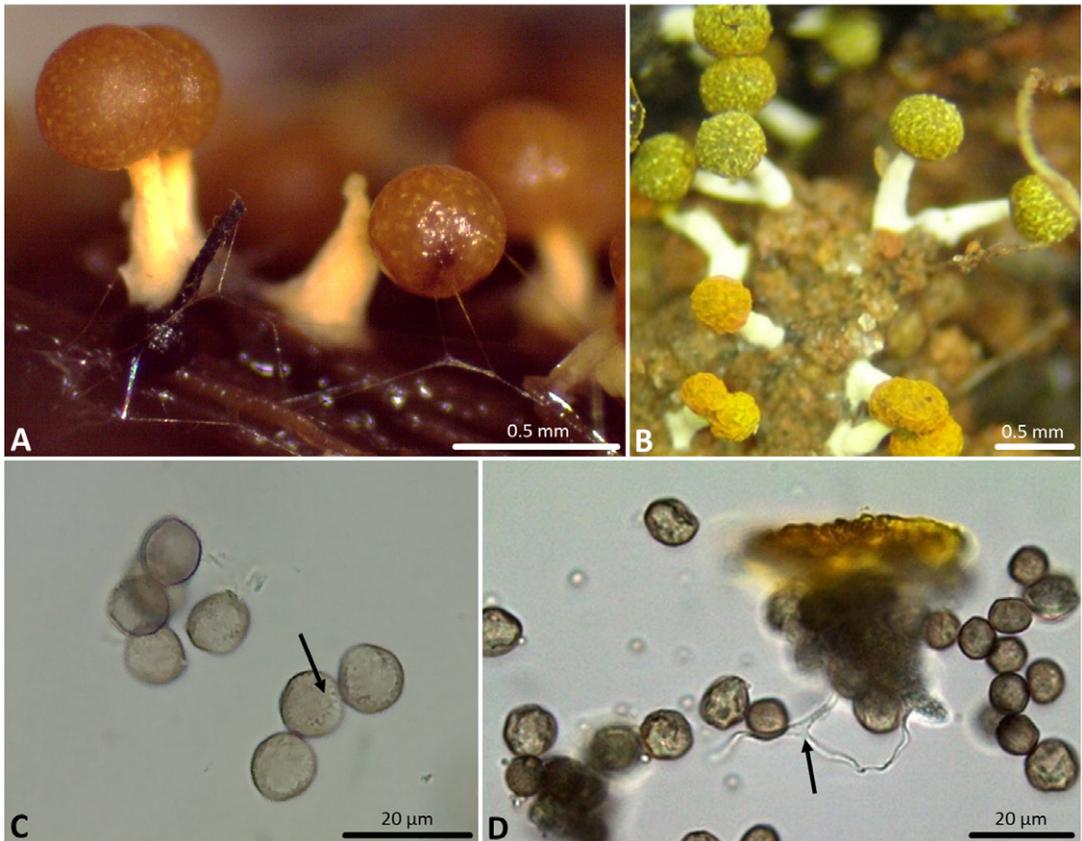


Fig. 4. *Physarum melleum* (HUEG 9289). **A:** immature sporangia on horse dung, **B:** mature sporangia, **C:** spores, the arrow indicates the almost inconspicuous ornamentation under the light microscope, **D:** spores and capillitium (arrow), with a calcareous greyish orange node.

COMMENTS: Previous records of this species as fimicolous, in Brazil, are from state of Sergipe, in the northeast region, Caatinga biome, on rabbit dung (Bezerra et al. 2008a, b; Calaça et al. 2020). This represents the first record as fimicolous from Brazilian Cerrado, as well as the first record for Goiás state.

Physarum melleum (Berk. & Broome) Masee, Monogr. Myxogastr. 278 (1892) – Fig. 4.

DESCRIPTION: Sporangia gregarious, sporotheca globose, up to 0.5 mm diam., olive yellow (3C8) to brownish yellow (5C8), up to 1.5 mm long total. Stalk cylindrical, calcareous, somewhat furrowed, up to 1

mm long, white (1A1) to greenish grey (1B2). Peridium single, membranous. Capillitium composed of irregularly branching hyaline threads connected by hyaline to greyish orange (5B5) nodes with calcareous deposits. Spores globose to subglobose, verruculose, violet brown (10F8) in mass, greyish brown (10E3) in light microscope, $(7.5) 8 - 9.5 (10.2) \times (7.4) 7.7 - 9.2 (10) \mu\text{m}$, $Q = 1 - 1.1 (1.2)$, $n = 50$, $Me = 8.9 \times 8.5 \mu\text{m}$, $Qm = 1$.

DISTRIBUTION: Worldwide.

SPECIMEN EXAMINED: BRAZIL, Goiás, Municipality of Porangatu, in a pasture area ($13^{\circ}25'38.2''\text{S}$ and $49^{\circ}08'53.7''\text{W}$), on horse dung in moist chamber (HUEG 9289).

COMMENTS: This is the second record of *P.*

melleum on dung worldwide and the first in Brazil. Yamamoto (2000) published the first record of this species as fimicolous, on cow dung in Japan (Calaça et al. 2020).

Physarum viride (Bull.) Pers., Ann. Bot. (Usteri) 15:6 (1795)

– Fig. 5.

DESCRIPTION: Sporangia gregarious, sporotheca globose, sometimes umbilicate below, fragmentary dehiscence, up to 0.5 mm diam., yellowish orange (4B7) to greyish yellow (4C7), up to 1.5 mm long total. Stalk subulate, striate, greyish yellow (4B2) to greyish beige (4C2). Peridium single, with some calcareous granules. Capillitium is an irregular network of slender and hyaline threads, with fusiform

golden yellow (5B7) nodes. Spores globose, smooth, violet brown (10F5) in mass, brownish grey (10D2) in light microscope, (9.1) 9.5 – 13.9 (15.4) × (8.4) 8.8 – 13.6 (14.9) μm, Q = 1 – 1.1, n = 30, Me = 10.9 × 10.4 μm, Qm = 1.

DISTRIBUTION: Worldwide.

SPECIMEN EXAMINED: BRAZIL, Goiás, Municipality of Pirenópolis, Santa Rita Farm (16°07'45.1"S and 49°02'38.5"W), on horse dung in moist chamber (HUEG 9288).

COMMENTS: These data expand both the geographical distribution and the substrates of *P. viride*, as this is the first record of this species on dung worldwide (Calaça et al. 2020) and also the first time that it is found in the midwest region of Brazil.

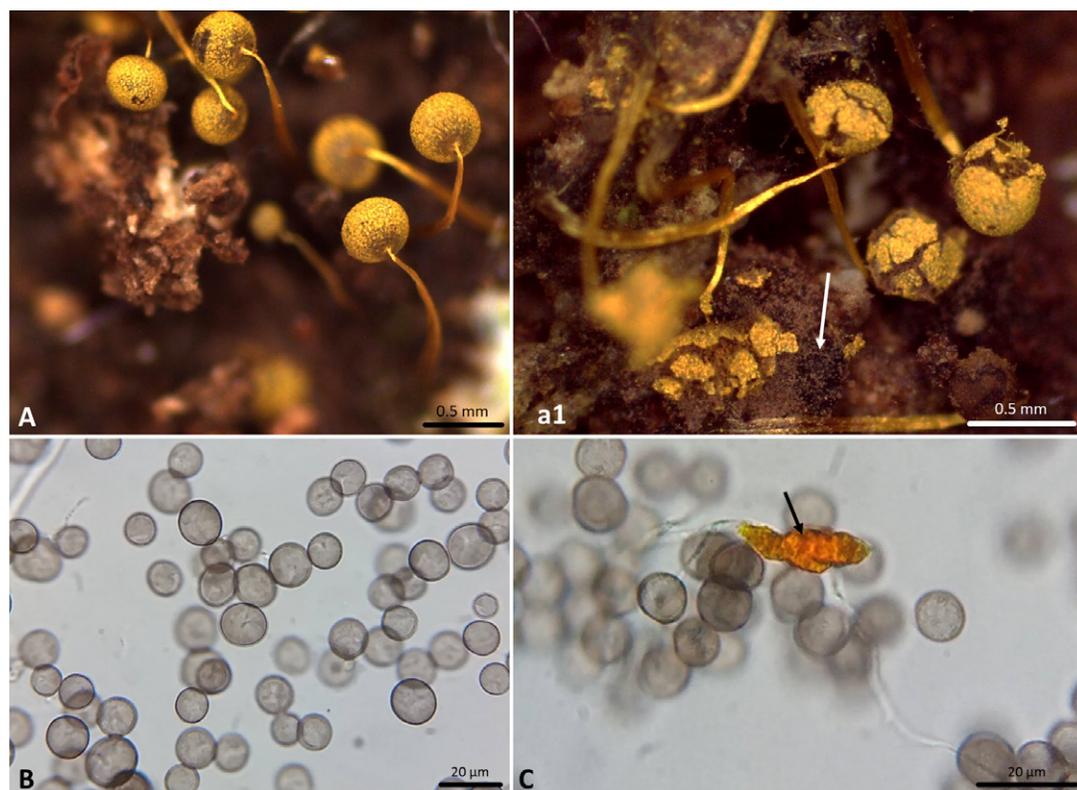


Fig. 5. *Physarum viride* (HUEG 9288). **A:** mature sporangia on dung, **a1:** the arrow shows the fragmentary dehiscence and golden yellow nodes in capillitium, **B:** spores, **C:** capillitium and a calcareous node (arrow).

Perichaena corticalis (Batsch) Rostaf.,
Sluzowce monogr. 293 (1875)

– Fig. 6.

DESCRIPTION: Sporophore sessile, gregarious to scattered, rarely isolated, globose to pulvinate, brownish orange (6C8) to light brown (6D8), 0.1 to 0.4 mm diam. Peridium double, inner layer membranous, smooth and hyaline, outer layer opaque, thickened with granules, dehiscence lines not observed. Capillitium scanty, with threads scattered, simple, poorly branched, pale yellow (1A3). Spores globose, minutely warted, golden yellow (5B8) in mass, grey-

ish yellow (4B5) to blond (4C4) in light microscope, (13.4) 14.1 – 15.6 (16.5) × (12.4) 12.8 – 14.9 (16) μm, Q = 1 – 1.1 (1.2), n = 20, Me = 15 × 14.2 μm; Qe = 1.1.

DISTRIBUTION: Worldwide.

SPECIMEN EXAMINED: BRAZIL. Mato Grosso, Municipality of Poconé, Pantanal area, next to Transpantaneira road (16°22'43.194"S and 56°37'18.8"W) on horse dung in moist chamber (HUEG 9287).

COMMENTS: This is the first record of *P. corticalis* as fimicolous in the midwest region of the Brazil. Moreover, this is the first record of the genus *Perichaena* in the Pantanal biome. Previous records of *P.*

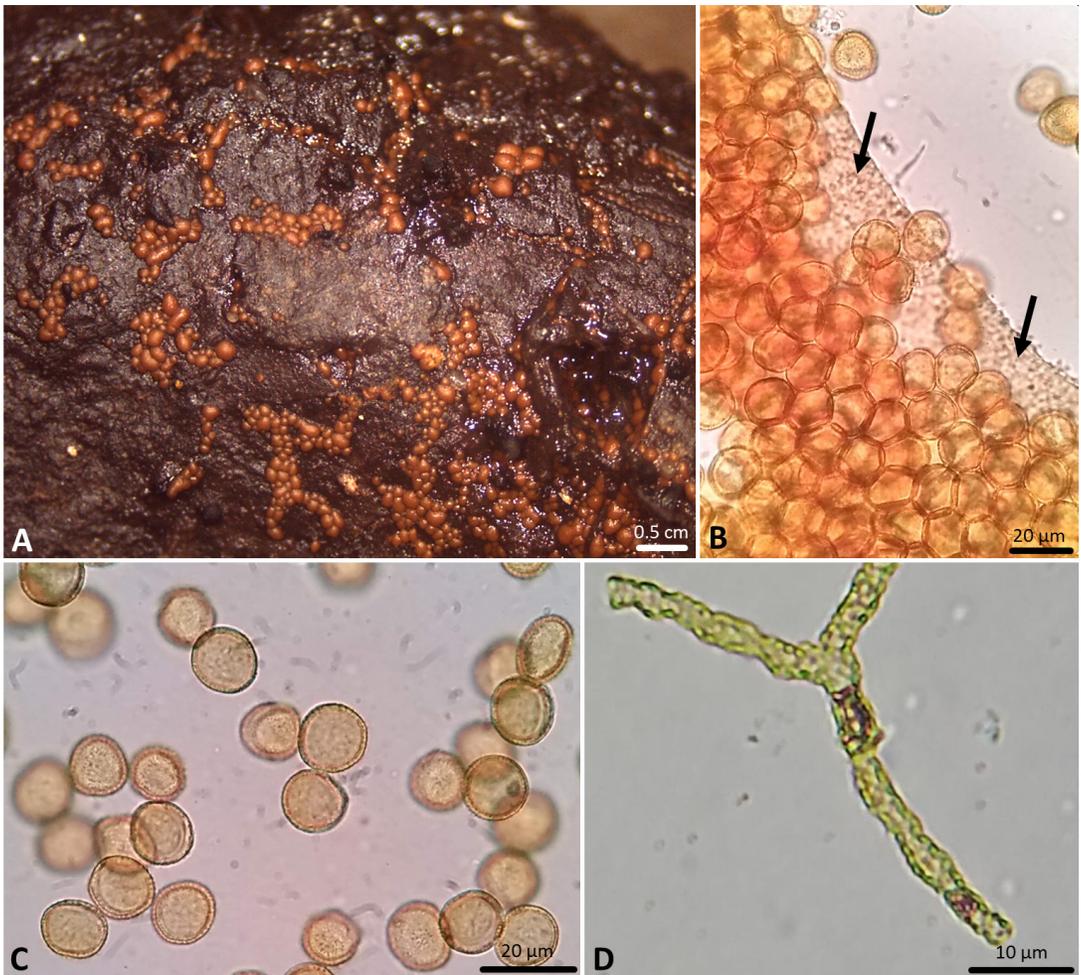


Fig. 6. *Perichaena corticalis* (HUEG 9287). **A:** sporophores on dung, **B:** membranous inner layer of peridium (arrow), **C:** spores, **D:** capillitium.

corticalis in Brazil were, until then, in the northeast region, on vegetal substrates (Cavalcanti et al. 2016). Records as fimicolous were reported in Algeria, Australia, Chile, China, Congo, Croatia, France, Hungary, Italy, Japan, Madagascar, Mongolia, Oman, Russia, Sri Lanka, Sweden, Tanzania, and USA (Calaça et al. 2020); therefore, this is the first record of the species as fimicolous for Brazil.

Besides presenting the first records of fimicolous myxomycetes from the Brazilian Cerrado and Pantanal biomes, our data increase the number of fimicolous myxomycete species known in Brazil to 13, with new records reported in the northeast and midwest regions and in the states of Sergipe (9 species), Goiás (4), Mato Grosso (1) and Piauí (1). Also, *P. melleum* is being reported as fimicolous for the first time in Brazil and second time in the world; *P. corticalis* was reported for the first time in midwest region, as well as for the first time as fimicolous in Brazil; and *P. viride* was reported for the first time as fimicolous in the world.

Recently, in an overview of the global distribution of fimicolous myxomycetes, Calaça et al. (2020) showed that most of the studies on these organisms rely on moist chamber culture use, and other techniques such as molecular approaches are still underused or inaccessible for many researchers in developing countries, such as Brazil. We highlight the need for more ecological studies focused on this group of myxomycetes, mainly on dung type preferences and biogeography, which will help clarify biological patterns for the 126 fimicolous species we know so far.

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