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Some strange substrates for macrofungi in the Pampa Biome – Brazil

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ABSTRACT - Macrofungi usually are found growing on vegetal substrate, soil or dung, but sometimes they have the opportunity to explore some very unusual food. Studying mushrooms in São Gabriel municipality, southern Brazil (Pampa Biome) allow us to collect specimens in different substrata rarely or still not reported in the literature. The collections include *Amanita muscaria* subsp. *flavivolvata* growing in *Eucalyptus* plantation, *Clitopilus argentinus* growing on a wasp nest, *Cheimonophyllum candidissimum* growing on rock, *Marasmius neosessilis* on the shrub *Asparagus officinalis* (Angiosperm) and *Panaeolina foenisecii* in Capibara dung. The implications of such ecological relationship are discussed. To *Clitopilus* a review of the species reported to Brazil is presented.

Keywords: mushroom, ecology, relationship, distribution.

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INTRODUCTION

Mushrooms are usually found growing on wood or other vegetal residuals or directly on soil or dung. But sometimes the substrate can be very unusual and the relationship is still not stablished. Antarctic dry valleys, high Arctic glaciers, salt flats and salterns, hypersaline microbial mats and plant trichomes are some of those inhospitable niches colonized by fungi (Cantrell et al. 2011).

Unusual substrates for fungi are poorly studied and usually ignored in the discussions of relationships and ecological aspects, so only a few study cases can be found. Dung fungi are normally introduced with exotic domesticate animals and can interact with other dung and respective native fungi.

Some mycorrhizal fungi are already found associated to tree species not usually connected with before, so the impact of their occurrence on the native mushroom's species can be expressive. As for example, *Amanita muscaria* (Amanitaceae) is one of the iconic fungi found in *Pinus* spp. plantations in Brazil (introduced) but was already found associated to native oak forest (*Quercus humboldtii*) in Colombia (Vargas et al., 2019). The Pampa Biome is found only in Rio Grande do Sul state (Southern Brazil), Uruguay and Argentina being an area of massive *Eucalyptus* plantation nowadays allowing the introduction of exotic mushroom species which can associate to native trees the same way as in the oak forest.

MATERIAL AND METHODS

The specimens were collected on the ecological trail of the Federal University of Pampa, located in the municipality of São Gabriel - RS (30°20'07.3"S and 54°21'47.3"W) and Parque da Bica, a municipality conservation unit.

The identification of the material via optical microscopy was carried out at the Fungal Taxonomy Laboratory (LATAF), at the Federal University of Pampa, São Gabriel campus.

To carry out the review of published occurrences of the target genera, the search tools Google Scholar, SciELO, Academia.Edu, Portal Capes, book collection of Fundación Miguel Lillo - Lilloa were used.

Photographic records were taken with a Canon EOS REBEL T5 camera and Canon Ef-S 18-55mm 1:3.5-5.6 Is II Macro 0.25m/0.8Ft lens. Notes were also made regarding the habit of the species and its interaction with the substrate.

The material was macro and microscopically studied at the Fungal Taxonomy Laboratory - LATAF and to identify the species, specialized bibliography of Putzke & Putzke (2018), Singer (1951; 1953) and their references were used. The samples were deposited at the HBEI Herbarium.

RESULTS AND DISCUSSION

There were found 5 species associated with unusual substrates in the studied area, all referred in Table 1. The relationship and implications are discussed below.

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Table 1. Unusual substates for mushrooms found in the area studied.

Species	Substrate
Amanita muscaria subsp. *flavivolvata*	Mycorrhizal with *Eucalyptus* plantation
Clitopilus argentinus	Nest of the wasp *Polybia* sp.
Marasmius neosessilis	On and alive shot of *Asparagus officinalis*
Cheimonophyllum candidissimum	On rock
Panaeolina foenisecii	On capibara dung

1. *Amanita muscaria* subsp. *flavivolvata* Singer (Figure 1)

Description

Pileus 50 – 200 mm diam., strongly red, campanulate to hemispherical, covered with large patches of scales originated form the volva remnants, yellowish to whitish. Lamellae free to narrowly adnexed, crowded, pale cream. Stipe 45 – 190 x 10 – 25 mm, annulate, bulbose, vulvate. Spores (7.5-) 9.0 – 12.8 x (5.5-) 6.5 – 8.5 (-11.5) µm, broadly ellipsoid to ellipsoid, hyaline and inamyloid. Basidia 41.0 - 47.0 × 11.0-13.0 µm, two to four sterigmata. Cortical layers of the pileus a cutis or prostrate hyphae.

There are only two species of *Amanita* reported as associated as mycorrhizal in southern Brazil: *Amanita muscaria* var. *flavivolvata* (Wartchow et al., 2013) e *Amanita aliena* (Wartchow & Cortez, 2016). In our screening through *Eucalyptus* plantation, we found only *Amanita muscaria* subsp. *flavivolvata* Singer as common in the UNIPAMPA campus area. The species is usually found as symbiotically associated to *Pinus* spp. plantations, being this report, a good example of substrate change in a new environmental.

The introduction of species worldwide is changing the mycorrhizal composition of the native areas by introducing fungi species not known as naturally occurring in those areas. The species may adapt to the new hosts and even exclude usually found species by competition. One good example of invasion is that of the native oak forests in Colombia were *Amanita muscaria* is now found associated, competing with the native fungi species (Vargas et al., 2019).

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Figure 1. *Amanita muscaria* subsp. *flavivolvata* in situ.

2. *Cheimonophyllum candidissimum* (Berk. & Curt.) Singer

Description

Pileus 2-7 (-10) mm in diameter, convex, reniform or irregularly lobed, pure white, minutely velutinous, glabrous, smooth. Lamellae radiating from a lateral attachment point, white, somewhat wide, subdistant, with lamellae of two sizes. Stipe lateral, rudimentary or absent, solid, with a white, pruinose, attached to basal mycelium next to the substrate. Spores measuring 5.0-6.5 x 4.5-5.5 µm, subglobose, hyaline, inamyloid, smooth, thin-walled. Basidia 23 – 30 x 5 – 6 µm, clavate, with 4 sterigmata up to 3 µm long. Pleurocystidia absent. Edge of the lamellae sterile, with abundant cheilocystidia, which are filiform, measuring 40-60 x 3 – 6 µm, hyaline, thin-walled, with a pointed apex and a somewhat inflated base. Hymenophoral trama more or less regular, hyaline, formed by hyphae 1.5-2.5 µm in diameter Well-developed subhymenial layer, with intertwined hyphae up to 10 µm in diameter. Cortical layer of the pileus formed by a trichoderm, with hyphae 30 – 70 µm long. Context white, thin, non-gelatinized, formed by slightly inflated, narrow hyphae, 1.5-3.0 µm in diameter, with slightly thickened walls and fibulate. They usually occur in dead wood, bark and on small branches in forest interiors.

There are no difficulties in differentiating this species from others. Our material has few cheilocystidia, which made identification difficult at first, including the fact that it grew on rock surface. Some authors prefer to include it in *Pleurotellus*. Due to its habit, it can be confused with *Marasmiellus*, from which it is differentiated by the cortical layer.

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It occurs in Europe, North, Central and South America. RICK (1938; 1961 - both as *Pleurotus hoedinus* Berk.) and PEREIRA (1988 - based on specimens of *P. hoedinus* Berk. sensu Rick- "possibly" this genus) reported this species to Rio Grande do Sul state. CAPELARI & MAZIERO (1988) cited it for Rondônia. PEGLER (1983) refers this species to the USA, Cuba and the Antilles. DELIVORIAS & GNOU-ZAGOU (2008) cite it for Greece. DENNIS (1986) cites it for Europe, South and North America. PUTZKE (2002) cites it for RS.

STERNER et al. (1994) isolated at least 6 new antibacterial and nematicidal substances from *C. candidissimum*, demonstrating the potential of this fungus in this area. DELIVORIAS & GNOU-ZAGOU (2008) cite wood from *Populus, Alnus, Fraxinus, Corylus, Fagus, Sorbus, Tilia, Salix, Ilex, Quercus* and *Platanus* as substrate for the species.

3. *Marasmius neosessilis* Sing. (Figure 2)

Description

Pileus at first almost white but soon salmon flesh-color, pinkish cinnamon or sordid salmon, glabrous cross-veined and radially deeply sulcate-grooved when mature, reniform in outline, 1-19 mm broad. Lamellae white, distant, with numerous veins on intralamellar surfaces but veins rarely reaching neighboring lamellae, intermixed with about 1-5 lamellulae, not intervenose and not intermixed in small carpophores, narrow, adnate or in smaller caps concurrent. Stipe absent to present but then rudimentary and appressed to both substrata, whitish at apex, pallid to pale fuscous at base, later fuliginous, slightly subtomentose, insititious, 0.5-2 x 0.1-1 mm. Context thin, white, inodorous. Spores 7-10 x 3.3-4.8 µm, ellipsoid to oblong-subfusoid, smooth, hyaline, in amyloid. Basidioles fusoid. Pleurocystidia absent. Cheilocystidia like epicuticular broom cells but all hyaline or merely with pale golden yellow setulae, absent over part of the edges. Hyphae fibulate, in the pileus thin-walled, later becoming mixed thin-walled and amyloi. Cortical layer of the pileus hymeniform, indistinct yellowish golden-melleous, mostly vesiculose.

Usually found on various dead twigs and forest according to Singer (1976). One particularly interesting species found in Parque da Bica, São Gabriel municipality, was this sessile *Marasmius* growing on shoots of *Asparagus officinalis* plants, an introduced weed. These were dead but still attached to the alive plant.

This ornamental plant was introduced in Brazil and is nowadays an environmental problem since it is occupying the soil under native forest, avoiding the development of under forest species. This species could be used as a natural controller of the plant if the pathogenicity could be proved, what demands mores studies. Up to now only 13 microfungi are reported associated to this Angiosperm as pathogens, but no mushroom (Mendes et al., 1998).

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Figure 2. *Marasmius neosessilis* in *Asparagus* dead branches still attached to an alive plant.

4. *Panaeolina foenisecii* (Pers. ex Fr.) R. Maire

Description

Pileus 0.3-1.0 cm in diameter, hemispherical to conical, smooth, cracking in dry weather, hygrophanus, white to smoke grey, turning mouse grey, black and buff when drying, with margin slightly striate. Lamellae adnexed, distant, black with white margin. Stipe 1.2-5.8 x 1-1.5 mm, dark brown, smooth. Spore print dark brown. Spores 12.8-18.3 x 10.4-11.3 µm, ellipsoid to lemon shaped, thick walled, reddish brown in KOH. Basidia 20.2-25.1 x 14.7-15.1 µm, tetraspored. Cheliocystidia 22-49.5 × 8.3-22 µm, clavate to lageniform. Cortical layers of pielus cellular, with rounded cells 22.5-31.2 µm in diameter.

This species was found on capibara (*Hydrochoerus hydrochaeris*) dung. There are reports of this species growing on soil and on cattle and elephant dung (Ediriweera et al., 2015). The species is toxic and usually not hallucinogenic, although some studies confirm the presence of substances with these properties (Ediriweera et al., 2015; Putzke & Putzke, 2017). It is the first time it is found in this kind of substrate and probably is competing with the native mycota found usually associated to capibara dung. The implications of this competition need to be studied.

***5. Clitopilus argentinus* Singer (Figure 3 - 4)**

Description

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Habit clitocyboid, 12–45 mm in diameter, plano-convex to slightly concave or concave; surface brownish gray in the center, gray at the edge or uniformly gray, usually smooth to slightly squamulose, margin convex, usually uniform; context 2–5 mm thick, whitish, not changing color when bruised; lamellae decurrent, white to slightly yellowish, clusters, 2/mm, edges entire and colorless, lamellae numerous. Stipe 20–30 x 5–6 mm, eccentric, surface yellowish white to grayish white, fibrillose. Spores 8–12(–13) × 5–7(–8) µm, hyaline, subfusiform to broadly fusiform, sometimes subovoid to ellipsoid in lateral view, with 5–6 longitudinal ridges; basidia 22–36 × 8–15 µm, clavate, hyaline, tetrasporate, sterigmata 2.5–5 µm; regular lamella branch, formed by hyaline hyphae 4–10 µm in diameter. Cortical layer of the pileus formed by a cutis composed of generally dry or slightly gelatinized, more or less radially arranged or slightly intertwined, thin-walled, cylindrical hyphae, 3–6 µm wide, with yellowish to yellow intracellular or parietal pigment; fibulae absent.

It grows solitary to gregarious on wood or bryophytes. Our sample was found in a decomposing nest of wasps of the genus *Polybia* in a remnant of native forest mixed to a *Eucalyptus* plantation.

Distribution - Cerro Alto de Taficilo, Argentina (26°56'52.1"S - 65°46'43.1"W), Singer (1950) n° T. 822 LIL; Cerro Muñoz, Tucuman - Argentina (26°55'45.7"S - 65°51'16.1"W), R, Singer (1950) n° T 861, LIL; La Plata, Buenos Aires - Argentina (34°54'34.1"S - 57°53'41.2"W), C. Spegazzini, March 1888, LPS; Los Nevados National Park - Colombia (4°47'22.8"N - 75°22'35.1"W), Pulido & Boekhout (1989), Niveiro & Albertó (2014).
Material examined: São Gabriel, Rio Grande do Sul, Brazil (30°20'07.3"S and 54°21'47.3"W), in the months of April-May.

Unlike *C. argentinus*, originally described as *Pleurotus pusillimus*, by Spegazzini in 1898 in Argentina growing on bryophytes, the specimen described in this study was found in a decomposing *Polybia* wasp nest. Singer (1952) reported the occurrence of the species in wooden substrate and dead tree bark.

C. argentinus has a brownish gray surface in the center, gray on the edge or uniformly gray, unlike *C. fuscogelatinosus* which is found in decomposing wood, has a grayish pileus, lamellae with whitish edges, produced by the cheilocystidia The species also differ in the size of spores (Baroni, 1992). *C. fuscogelatinosus* as 7.5-8.3 x 4.9-5.5 µm.

Clitopilus amygdaliformis described by Zhu L. Yang in 2007, has a basidiome between 20 – 80 mm in diameter while *C. argentinus* has 12 – 45 mm in diameter. In relation to the color of the basidiome, the first species is described as having a whitish to chalky white surface, in relation to the second, which has a brownish gray surface in the center to completely gray. Both species have 5-6 longitudinal ridges on the spores. *C. amygdaliformis* sterigmata are 1.5–4 µm long, smaller when compared to *C. argentinus*, which presents 2.5–5 µm.

Checklist of *Clitopilus* species to Brazil

According to He et al. (2019), 140 species of *Clitopilus* are known, of which 14 species occur in Brazil (Coimbra, 2014). Investigating the subject in national articles already published on reviews and records of Entolomataceae and Agaricales, it was possible to verify the presence of 21 occurrences, 19 of which were identified at species level (Singer 1969, Singer 1973, Singer 1989, Pegler 1983, Pereira 1990, Putzke & Putzke 2000, Meijer 2006, Meijer 2008,

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Meijer 2010, Coimbra 2014, Silva-Filho et al. 2018, Valões-Araújo & Wartchow 2021, Bertazzo-Silva et al, 2022) (Table 2).

Table 2. Species of *Clitopilus* reported to Brazil.

Species	**Area**
Clitopilus argentinus Singer	Paraná- PR
Clitopilus aff. *albovelutinus* (G. Stev.) Noordel. & Co-David	Paraná-PR
Clitopilus hobsonii (Berk. & Br.) Orton	Amazônia- AM; Rio Grande do Sul -RS
Clitopilus incrustatus Singer	Rio Grande do Sul- RS
Clitopilus rhodotrama Singer	Rio Grande do Sul-RS; São Paulo-SP
Clitopilus scyphoides (Fr.) Singer	Paraná-PR
Clitopilus caelatoideus (Dennis) Noordel. & Co-David	Paraná-PR
Clitopilus caelatus (Fr.) Kuhner & Romagn.	Paraná-PR
Clitopilus conchatus (E. Horak) Noordel. & Co-David	Paraná-PR
Clitopilus conicus (Singer) Noordel. & Co-David	Amazonas-AM
Clitopilus crepidotoides (Singer) Noordel. & Co-David	Pernambuco-PB
Clitopilus hobsonii (Berk. & Br.) Orton	Amazonas-AM, Rio Grande do Sul-RS
Clitopilus hobsonii (Berk. & Br.) Orton var. *hobsonii*	Paraná-PR
Clitopilus levisporus (de Meijer) de Meijer	Paraná-PR
Clitopilus mycenoides (Singer) Noordel. & Co-David	Paraná-PR
Clitopilus pseudonitellinus (Dennis) Noordel. & Co-David	Paraná-PR
Clitopilus scyphoides var. *submicropus* (Rick) Singer	Paraná-PR
Clitopilus melleus (T.J. Baroni & Ovrebo) Noordel. & Co-David	Paraná-PR
Clitopilus sp.	Rio Grande do Sul-RS
Clitopilus sp.	Rio Grande do Sul- RS
Clitopilus galerinoides (Singer) Noordel. & Co-David	Paraná-PR

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In a review on Entolomataceae, *Clitopilus argentinus* had been cited in Latin America only for Colombia and Argentina (Coimbra et al., 2014). However, of the macrofungal species found in pine forests in Paraná, Meijer (2008) highlights that the majority of the species found are saprobic fungi and mentions three species of *Clitopilus*: *Clitopilus argentinus, Clitopilus hobsonii* and *Clitopilus scyphoides*.

The genus as being cosmopolitan, and *Clitopilus argentinus* is cited as growing on wood and bark from dead trees and unidentified liverworts and mosses, in addition to defining mountainous regions and subalpine regions as places where the mushroom is likely to be found (Singer, 1951; 1953). The association of the species with wasp nests of the genus *Polybia* sp. is unprecedented, considering that the material that constitutes the nests is different from the substrates known for *Clitopilus* spp. The nests are composed of a mixture of plant materials such as fibers and trichomes, macerated with water and eventually with glandular secretion and clay (Somavilla et al., 2012). The species was collected in the pampa biome area in April, during autumn.

Revision of the genus *Clitopilus* to Brazil

In relation to works published nationally focused on the systematics and taxonomy of the genus *Clitopilus*, the collections carried out in the state of Rio Grande do Sul by Rick (1906; 1907; 1920; 1938) and reviewed by Singer (1952; 1953) stand out. Putzke & Putzke (2000) reviewed all the materials present in the PACA Herbarium, and confirmed the occurrence of five *Clitopilus* species in Rio Grande do Sul. Meijer (2006) published the occurrence of one species and three varieties for the state of Paraná. Although there is no more recent review focused on the genus, Coimbra et al. (2014), when reviewing the Entolomataceae family for South America, cited 21 species to Brazil.

According to the Specieslink and GBFI search platforms, there are deposits of preserved specimens identified of the genus *Clitopilus* sp. However, it was possible to highlight materials identified at the species level, which were collected, cataloged and preserved in an herbarium, but the collection locations and description of the specimens were not published in scientific literature, therefore, they were not included in the distribution map. These are: *C. hobsonii* collected in the state of Rio de Janeiro (F-Fungi), Amazonas (INPA), Bahia (INPA), *C. incrustatus* in Amazonas (INPA), Rio de Janeiro (F-Fungi).

In relation to the species *Clitopilus mundulus* Rio Grande do Sul (PACA-Fungi), *Clitopilus prunulus* (Scop.) P. Kumm. (IPA) and *Clitopilus abortivus* (Berk & M.A. Curtis) Sacc. (R-Fungi), they are not mentioned in scientific literature to Brazil, however, they were collected and cataloged in Brazilian herbaria. *Clitopilus subfascicularis* from Rio Grande do Sul (PACA-Fungi), was collected and identified by Rick, however, when analyzing the specimen, it was found that it is not part of the Entolomataceae family (PUTZKE & PUTZKE, 2000).

Forms of Wasp-Fungi-Nest interactions

The findings regarding Wasp-Fungi-Nest interactions involving species of *Polybia* sp. and their forms of association are summarized in Table 3.

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Table 3. Forms of Wasp-Fungi-Nest interactions of *Polybia* sp. described in the literature.

Wasp species	**Fungi**	**References**
Polybia snitellaris	Spores on unidentified fungi on honey	2
Polybia occidentalis	*Cryptococcus neoformans* var. *gattii* isolated on a was nest	1
Polybia plebeja	A wasp infected by the fungi *Hirsutella* sp.	6
Polybia ignobilis	*Rhodotorula* sp., *Candida* sp., *Cryptococcus* sp. and *Metschnikowia* sp. Were isolated from a wasp nest.	3
Polybia platycephala	Wasps infected by *Beauveria bassiana* and *Metarhizium anisopliae*.	5
Polybia ignobilis	*Auriculibuller* sp. and *Papiliotrema* sp. Associated to intestinal tract and to the insects.	4

1. Gezuele et al. (1993), 2. Telleria (1996), 3. Sousa (2011), 4. Dayo-Owoyemi (2013), 5. Venâncio (2015), 6. Matarrita-Carranza et al. (2017).

To Erthal et al. (2007) insects and fungi have many forms of associations, however, some stable interactions come from evolutionary forces that provide adaptations between them, which leads to the perpetuation of the association. This placement is related to the findings of *Polybia* sp. together with yeasts (Table 3). For yeast, wasp nests can provide a safe environment, favored by the resources of temperature, humidity and nutritional availability (SOUSA, 2011).

As dispersing agents, the spread of fungal spores can be favored by wasps, which have a characteristic foraging habit involving flight over short and long distances (HUGHEY et al., 2012). These associations can be considered transient, as the wasp acts as a vector, and as a benefit, depending on the fungal species, nutritional acquisition can occur (SOUSA, 2011). In general, nests of *Polybia* sp. have one or more combs surrounded by a structure called envelope (BARRAVIERA, 1994). Also, they are built in places sheltered from wind and rain, being called *calyptodromous phragmocyte*, due to the fact that the comb is fixed directly to the substrate and contains a protective casing (CARPENTER & MARQUES, 2001). Therefore, much of the fungus-wasp-nest connection occurs through the mechanical dispersion of spores from the source zone to the new zone by the wasp.

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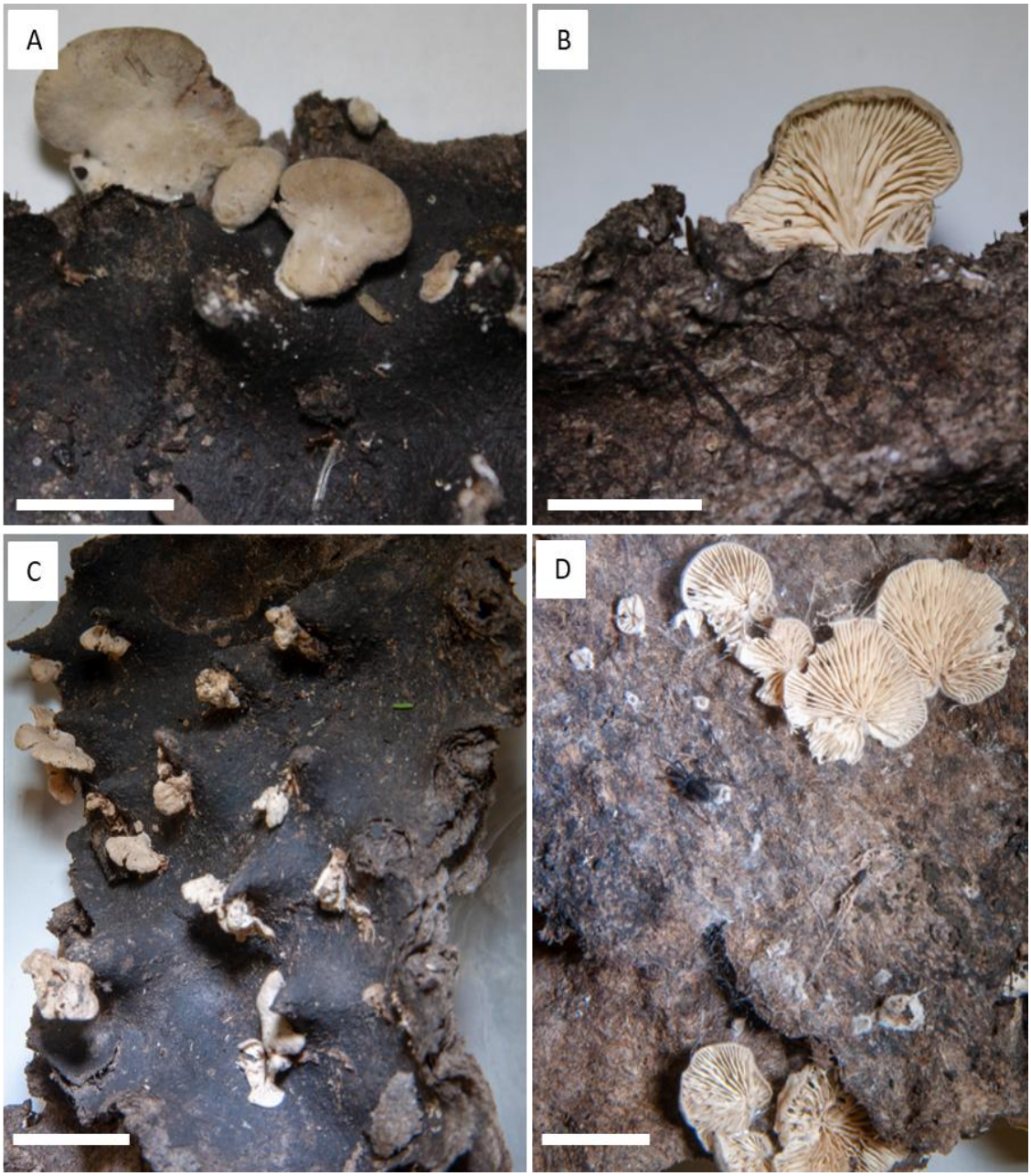

Figure 3. Basidiome of *Clitopilus argentinus* growing in the *Polybia* nest: A - View of the abhymenium; B, D – Hymenophore. Scale = 20 mm.

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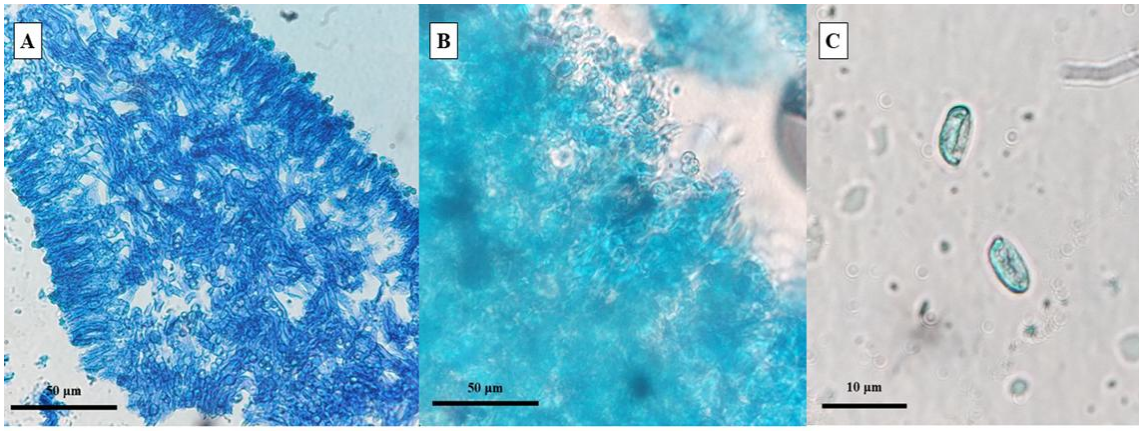

Figure 4. Microscopic features of *Clitopilus argentinus*. A- Hymenophore trama; B- Cortical layer; C- Spores.

Insects are reported in the literature not only to associate with fungi as a nutritional source, but also to benefit from the acquisition of digestive enzymes, which, when ingested through mycophagy, are integrated into the intestinal tract, acting in the degradation of complex polymers (ERTHAL et al., 2007). For example, in the study by Dayo-Owoyemi (2013) involving the fungal species *Auriculibuller* sp. and *Papiliotrema* sp. associated with the nest and intestinal tract of *Polybia ignobilis*, it was possible to verify enzymatic activities arising from mycophagic acquisition (Table 3). Scarce and poorly digestible compounds from the flowers of *Hibiscus* sp. were found in biochemical analyzes of the intestinal tract of the wasp species, and found only in individuals whose nest contained the fungi.

Polybia sp. has a diverse diet, foraging habits and trophallaxis behavior, which optimizes fungal dissemination in its combs (SOUSA, 2011). Many of the mentions described in the literature related to the wasp-fungi association for the genus are related to yeasts or filamentous fungi (Table 3). For *Clitopilus argentinus*, reported in this study associated with a nest of *Polybia* sp. the description of the association is unprecedented.

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CONFLITO DE INTERESSES

Os autores não declararam que o artigo tem conflito de interesses.

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