NEW RECORDS OF *CYATHUS* SPECIES (NIDULARIACEAE, BASIDIOMYCOTA) FROM A BIOLOGICAL RESERVE IN ALAGOAS, BRAZIL

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ABSTRACT

In the present work, three new records for the state of Alagoas of *Cyathus* species were found in a Brazilian Biological Reserve: *C. microsporus, C. morelensis* and *C. limbatus*. Macro and microscopical analysis methodologies previously proposed for the genus were used. *Cyathus morelensis* is firstly reported for the Atlantic Rainforest. Species identifications were based on the analyses of morphological features. Detailed description, photographs, taxonomical notes, and a distribution map with the studied area are provided.
Keywords: Biodiversity, gasteroid fungi, taxonomy, funga, bird's nest fungi

RESUMO

No presente trabalho, foram identificados três novos registros de espécies de *Cyathus* para o estado de Alagoas, em uma Reserva Biológica Brasileira: *C. microsporus*, *C. morelensis* e *C. limbatus*. As metodologias de análises macro e microscópica previamente propostas para o gênero foram utilizadas. *C. morelensis* é reportado pela primeira vez para a Mata Atlântica. As identificações das espécies foram baseadas em análises das características morfológicas. São fornecidas descrições, fotografias, notas taxonômicas e um mapa de distribuição com a localidade de coleta.
Palavras-chave: Biodiversidade, fungos gasteroides, taxonomia, funga, fungos ninho-de-pássaro

INTRODUCTION

The genus *Cyathus* Haller was established by Albrecht von Haller in 1768 (White, 1902) and is characterized by basidiomata with a conical or inverted bell-shaped peridium with small discs in the interior. This arrangement resembles small eggs inside a bird's nest,

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responsible for the etymology of its popular name “bird’s nest fungi”. These internal structures are called peridioles and have a dispersion and protection function (Brodie, 1975). Most *Cyathus* species grow on decaying wood, but they can also occur on soil, manure, or seeds (Brodie, 1975; Blenis & Chow, 2005).

According to Brodie’s classification, the genus was divided into 7 major groups in accordance with morphological features: *olla*, *pallidus*, *triplex*, *gracilis*, *stercoreus*, *poeppigii* and *striatus*. Later, Zhao et al. (2007) categorized the genus into 3 distinct groups, based mainly on the basidiospore size: *pallidum*, *ollum* and *striatum*. Currently, the genus is located in the family Agaricaceae, but in an uncertain position (He et al., 2019).

The Pedra Talhada Biological Reserve is a large remnant of the Atlantic rainforest situated in the Brazilian Northeastern region, marked by a rainfall season from March to August (Nusbaumer et al., 2015). The reserve has an essential role in maintaining the local flora and fauna, including some endemic species (ICMBio, 2021). Until now, only one *Cyathus* species has been reported in Alagoas state, from the Usina Serra Grande Forest Reserve, identified as *C. striatus* (Trierveiler-Pereira & Baseia, 2009).

Studies on the genus *Cyathus* and its Brazilian distribution are still needed. Due to the lack of morphological standardization, several species are incorrectly identified, making it hard to develop new studies involving the distribution and biodiversity of these organisms. Therefore, this work aims to expand the knowledge on the genus by reporting the occurrence of three new records of *Cyathus* species from the state of Alagoas. Moreover, one species is reported for the first time from the Atlantic Rainforest.

MATERIALS AND METHODS

The specimens were collected in 2019 at Pedra Talhada Biological Reserve (9°13’43.8” S, 36°25’44.0” W) (Figure 1). The reserve is located on the border between Alagoas (AL) and Pernambuco (PE) states, present in the municipalities of Chã Preta (AL), Quebrângulo (AL) and Lagoa do Ouro (PE), covering an area of approximately 4.382,37 hectares of Atlantic rainforest (ICMBio, 2021). The samples were deposited in the UFRN-Fungi collection, located at the Federal University of Rio Grande do Norte, Natal, Brazil.

The methodology for the taxonomical studies of Nidulariaceae proposed by Brodie (1975) was used. The size and shape of the basidiomata were measured, as well as the tomentum size in the exoperidium, presence of internal and external striae, and the emplacement diameter and texture. For the peridioles, the shape, texture, surface, and cortex type were observed. The Color Chart “Methuen Handbook of Color” (Kornerup & Wanscher, 1978) was used for color standardization. The macroscopic analyses were performed using a Nikon SMZ1500 stereomicroscope (Nikon Corporation, Tokyo, Japan).

The microscopic analysis was performed on a Nikon Eclipse Ni-*U* optical microscope (Nikon Corporation, Tokyo, Japan). The microscopic slides were made from the macerated peridioles in 5% KOH with the aid of a steel blade (Cruz, 2017). The size and length of 30 basidiospores were measured, as well as the average length (L), average width (W), the ratio of width to length of each spore (Q), and the average of this ratio (Qm) (Zhao et al., 2008). To identify the spore shape, the Qm values defined by Bas (1969) were used. For the basidiospores, coloration, shape, abundance of spores, presence of apicule and spore wall were examined. The species identification followed Brodie (1975) and Cruz (2017), and other studies recently published for Brazil (Trieveiler-Pereira & Baseia, 2009; Cruz & Baseia, 2014; Silva et al., 2016; Crous et al., 2017a; Crous et al., 2017b; Accioly et al., 2018; Góis et al., 2020).

RESULTS

Cyathus microsporus Tul. & C. Tul., Annales des Sciences Naturelles Botanique 1: 73 (1844) (Figure 2)
Etymology*:* In reference to the small basidiospores.
Peridium infundibuliform, 6.55–7.30 mm in height, 3.98–4.55 mm in width at the upper part, not expanded at the mouth or tapering abruptly at the base. Emplacement not observed. Exoperidium hirsute, brown (5E5), with 0.41–0.67 mm tomentum, arranged in irregular and flexible tufts. External wall smooth. Mouth slightly fimbriated in a continuous pattern, 0.20–0.32 mm in height, brown (5E5). Endoperidium brownish grey (8D2–8E2), inconspicuous, 0.38–0.43 mm between the folds, shiny, contrasting with the exterior. Stipe 0.80–1.10 mm, orange brown. Epiphragm not observed. Peridioles 1.79–2.03 × 1.70–2.11 mm in diameter, greyish brown (7F3), single-layered cortex. Circular to elliptical in shape at borders, surface smooth to slightly rugulose, 10 per basidioma. Tunica present, hyaline. Basidiospores smooth, hyaline, 6.06–8.67 × 5.28–7.20 µm (L= 7.29 µm; W= 6.18 µm; n= 30), subglobose to elogated, some ovoid (Q= 1.04–1.61), elliptical on average (Qm= 1.30). Apicule present in some spores and spore wall 1.01–1.89 µm thick.
Material examined: Brazil, Alagoas, Pedra Talhada Biological Reserve. *T.B. Gibertoni.* 01 May 2019. (UFRN-Fungos 3324).
Habit: Gregarious, on decaying wood.
Known distribution: Haiti, U.S.A, Costa Rica, Cuba, Jamaica, Brazil.
Notes: *C. microsporus* belongs to group I (*olla*) in Brodie's (1975) classification and is mainly characterized by the single-layered cortex (Figure 2C) and the small basidiospores (Figure 2D). This species is often confused with *C. berkeleyanus* but distinguished by the presence of smaller spores (7.68–13.97 × 5.08–8.89 µm in *C. berkeleyanus*), darker colored basidiomata (Figure 2A), and smaller peridioles (2–2.6 × 1.75–2.1 mm in *C. berkeleyanus*) (Tulasne & Tulasne, 1844; Brodie 1975). This species can also be confused with *C. hookeri* by the diminutive spores and single-layered cortex, but the presence of smaller basidiomata (10–14 mm in the original description of *C. hookeri*), campanulate peridium, smaller stipe size (2–3 mm in *C. hookeri*), and outer wall with a larger distance between the striae (0.63–0.72 mm in *C. hookeri*) differentiates *C. microsporus* from the latter (Berkeley, 1854; Brodie, 1975). Until now it is known only from the states of Rio de Janeiro (Berkeley & Cooke, 1876) and Bahia (Góis et al., 2020), and this is the first record of this species from Alagoas.

Cyathus morelensis C.L. Gómez & Pérez-Silva, Mycotaxon 33: 419 (1988) (Figure 3).
Etymology: In reference to the State of Morelos, Mexico.
Peridium infundibuliform, 7.44–9.15 mm in height, 5.73–8.15 mm in width at the upper part, not expanded at the mouth or tapering abruptly at the base. Emplacement 2.83–3.24 mm, conspicuous, brown (6F5). Exoperidium hirsute, brown to dark brown (6E7–7F7), with 0.65–0.88 mm tomentum, arranged in irregular and flexible tufts. External wall conspicuously plicated, with 0.40–0.53 mm between the folds. Mouth slightly fimbriated in a continuous pattern, shattering in the folds, 0.32–0.41 mm in height, dark brown (7F7). Endoperidium greyish brown (8E2), conspicuously plicated, 0.28–0.53 mm between the folds, shiny, contrasting with the exterior. Stipe 0.53–1.04 mm, brown (7F5). Epiphragm greyish brown and rigid. Peridioles 1.93–2.58 × 1.93–2.37 mm in diameter, greyish brown (7F3), double-layered, subhomogeneous cortex. Angular to elliptical in shape at borders, surface smooth, 8 per basidioma. Tunica indistinct. Basidiospores smooth, hyaline, 17.83–20.75 × 10.09–14.60 µm (L= 19.42 µm; W= 13.14 µm; n= 30), slightly elliptical to elliptical (Q= 1.27–1.72), elliptical on average (Qm= 1.48). Apicule absent and spore wall 0.9–1.85 µm thick.
Material examined: Brazil, Alagoas, Pedra Talhada Biological Reserve. *T.B. Gibertoni.* 01 May 2019. (UFRN-Fungos 3325).

Habit: Gregarious, on decaying wood.
Known distribution: Mexico and Brazil.
Notes: *C. morelensis* is a rare species that belongs to group VI (*poeppigii*) in Brodie's (1975) classification and is mainly characterized by the peridioles with subhomogeneous cortex and ovoid-shaped basidiospores (Gómez & Pérez-Silva, 1988). This species was originally described for Mexico and was reported from Brazil by Cruz et al. (2012). The specimen analyzed in this study differs from the original description by having slightly smaller peridioles, and slightly smaller spore width, but these features still fit the species delimitation proposed by Gómez & Pérez-Silva (1988) and Cruz et al. (2012). *Cyathus morelensis* is similar to *C. limbatus*, however, the spore shape (with many ovoid forms), type of cortical layer, and basidiomata that are more expanded at the upper portion differentiate *C. morelensis* from the latter. Previously known for Brazil in Amazon Rainforest, it is the first record of this species from the State of Alagoas state and from the Atlantic rainforest biome.

Cyathus limbatus Tul. & C. Tul., Annales des Sciences Naturelles Botanique 1: 78 (1844) (Figure 4).
Etymology: From Latin "*limbatus*", which means with edges.
Peridium infundibuliform, 6.42–9.43 mm in height, 4.11–6.92 mm in width at the upper part, not expanded at the mouth or tapering abruptly at the base. Emplacement 2.46–3.47 mm, conspicuous, brown (6F5–7E5). Exoperidium hirsute, brown to dark brown (6E7–7F7), with 0.65–0.88 mm tomentum, arranged in irregular and flexible tufts. External wall conspicuously plicated, with 0.58–0.72 mm between the folds. Mouth slightly fimbriated in a continuous pattern, shattering in the folds, 0.21–0.34 mm in height, dark brown (6E7). Endoperidium greyish brown (6D3–6E3), conspicuously plicated, 0.35–0.50 mm between the folds, shiny, contrasting with the exterior. Stipe 0.53–1.04 mm, black. Epiphragm not observed. Peridioles 1.86–2.63 × 1.66–2.28 mm in diameter, greyish brown (7F3), double-layered cortex, exocortex and endocortex black, mesocortex greyish brown with compact hyphae. Angular to elliptical in shape at borders, surface smooth, 8 per basidioma. Tunica present, hyaline. Basidiospores smooth, hyaline, 16.68–20.99 × 12.93–14.60 µm (L= 19.42 µm; W= 13.18 µm; n= 30), slightly elliptical to elliptical (Q= 1.26–1.70), elliptical on average (Qm= 1.51). Apicule absent and spore wall 1.01–2.59 µm thick.
Material examined: Brazil, Alagoas, Pedra Talhada Biological Reserve. *T.B. Gibertoni*. 01 May 2019. (UFRN-Fungos 3320, UFRN-Fungos 3322, UFRN-Fungos 3326).
Habit: Gregarious, on decaying wood.
Known distribution: South America, Hawaii, Africa, India, China, South and Central Pacific Islands.
Notes: *Cyathus limbatus* belongs to group VI (*poeppigii*) in Brodie's (1975) classification and is characterized by darker peridium (Figure 4A), peridioles with a predominantly angular shape (Figure 4B), double-layered cortex (Figure 4C) and elliptical-shaped basidiospores (Figure 4D) (Tulasne & Tulasne, 1844; Brodie, 1975). Despite the bad condition of the examined specimen, the characteristics and measurements fit the original description of *C. limbatus* published by Tulasne & Tulasne (1844). This species is similar to *C. montagnei* and *C. striatus* due to the peridioles larger than 2 mm, basidiomata with dark colors, and similar spore size; however, the presence of the single-layered cortex in these two species is enough to separate them from *C. limbatus* (Brodie, 1975). Well known from Brazil, Trierveiler-Pereira & Baseia (2009) and Góis et al. (2020) reported this species from the Northeastern Region of the country, and this is the first record from the State of Alagoas.

DISCUSSION

In this study, three new records of *Cyathus* were identified and described for the State of Alagoas, Northeastern Brazil. Our analyses corroborated the descriptions that have been published in recent years for Brazil and the original protologue of each of these species (Tulasne & Tulasne, 1844; Brodie, 1975; Gómez & Pérez-Silva, 1988; Trierveiler-Pereira & Baseia, 2009; Cruz et al., 2012; Góis et al., 2020). Although these species have been recorded before in Brazil, neither has been reported from Alagoas until now and no samples of the genus were previously found in the Pedra Talhada Biological Reserve.

Even without molecular data from these analyzed samples, the morphological features of each species fit the original description presented in literature. With these new records, the knowledge about the distribution and diversity of the genus has been updated, filling gaps about the occurrence of these organisms in the Brazilian territory and biomes. Studies with specific distribution indicators for biomes, regions, or states in Brazil, like this one, are extremely important due to the continental proportions of the country. Some Brazilian states are larger than entire countries, as is the case in the state of Alagoas, similar in size to Haiti. Knowing the biodiversity of such a broad environment should be the focus of other studies and an important objective of local investment in research.

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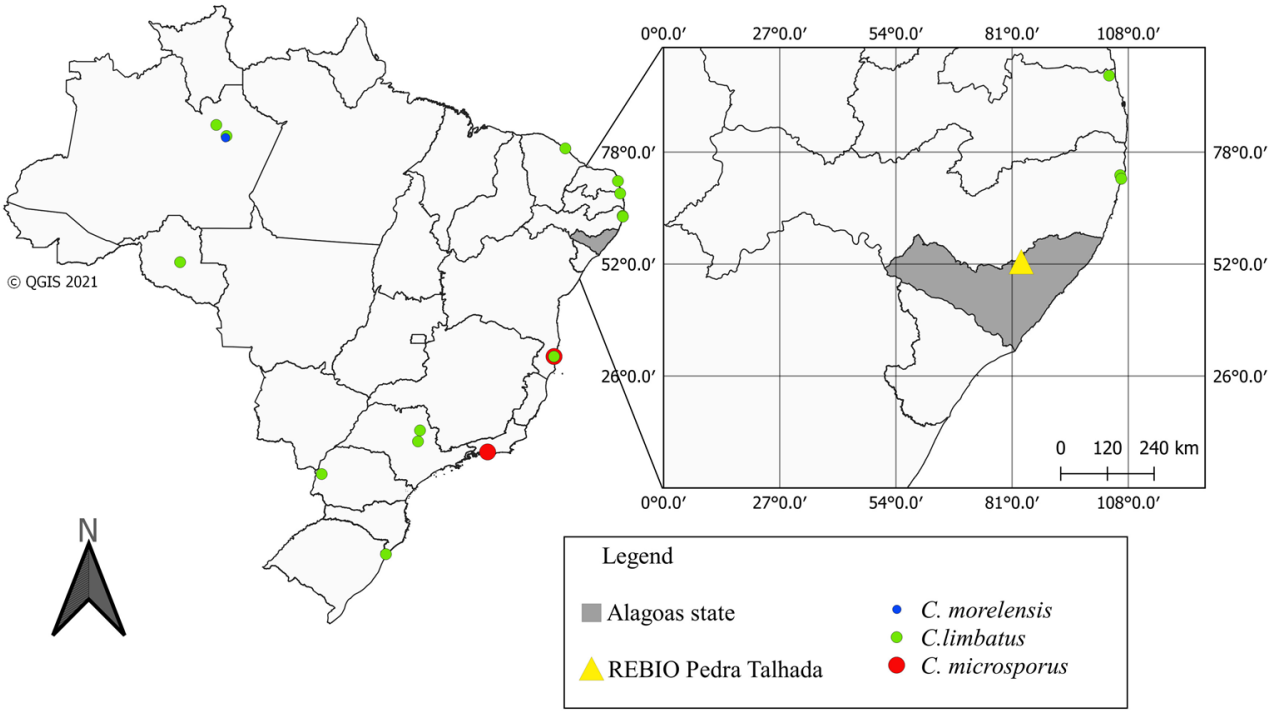

Figure 1. - Map of Brazil showing the collection site, Pedra Talhada Biological Reserve, Alagoas state. The colored dots represent the distribution records of *C. limbatus* (green), *C. microsporus* (red) and *C. morelensis* (blue).

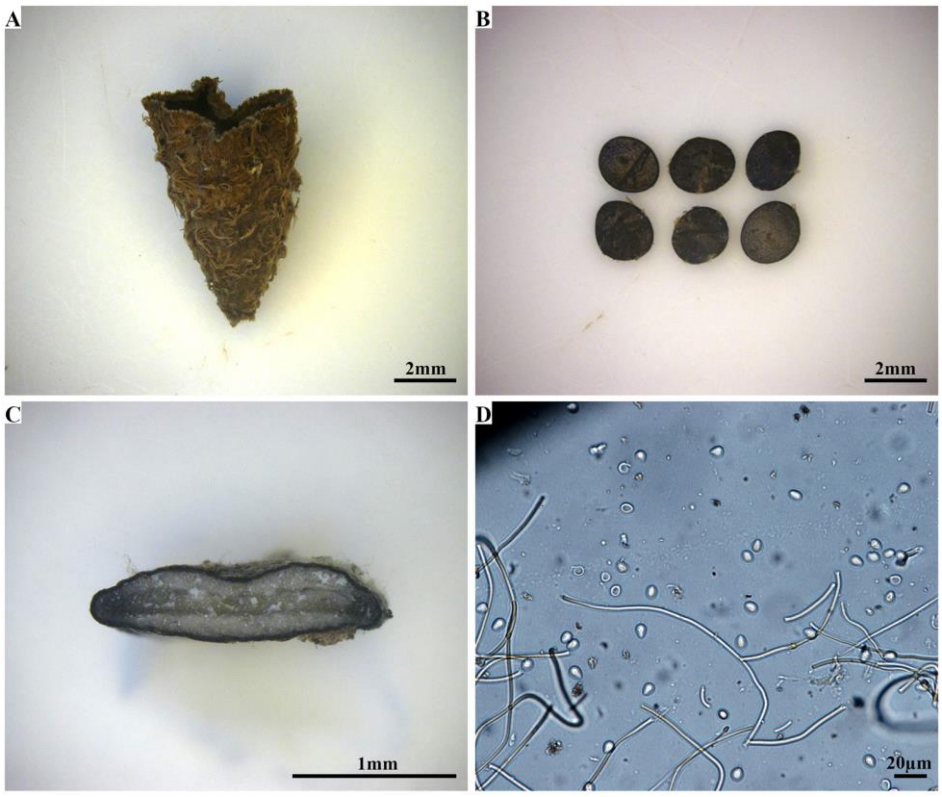

Figure 2. - *Cyathus microsporus* (UFRN-Fungos 3324). **A.** Basidiome with wooly exoperidium; **B.** Peridioles with slightly rugulose to smooth surface, and circular to elliptical shape. **C.** Transversal cut in the peridiole showing the single-layered cortex. **D.** Small basidiospores with elliptical shape, some of them ovoid.

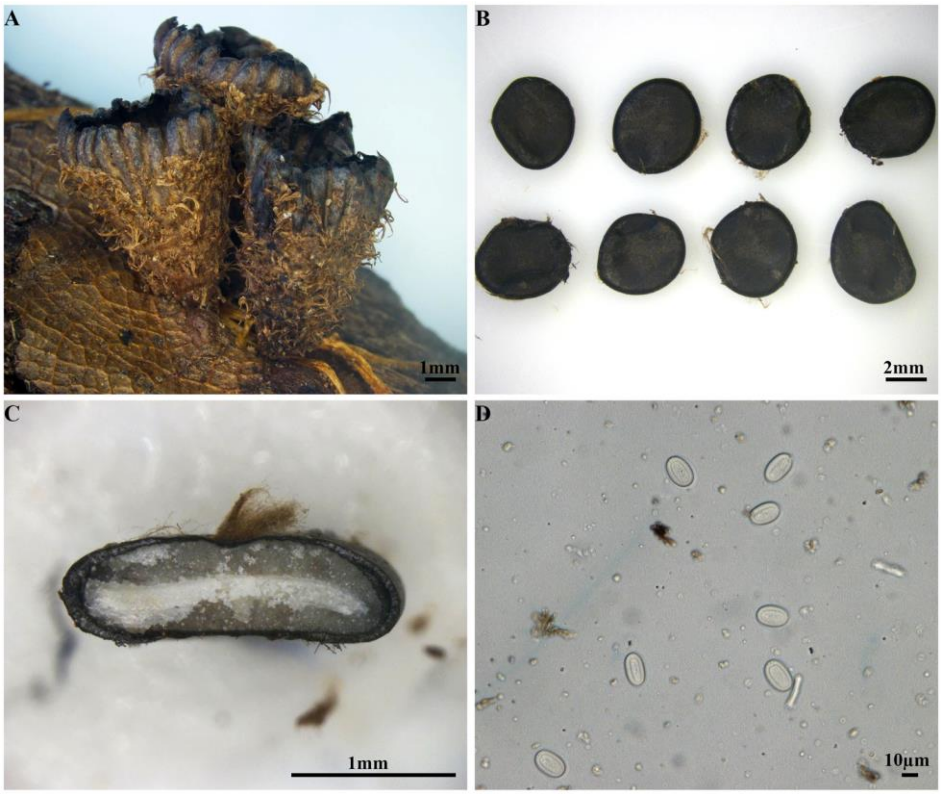

Figure 3. - *Cyathus morelensis* (UFRN-Fungos 3325). **A.** Basidiome with hirsute exoperidium. **B.** Peridioles with smooth surface, and angular to elliptical shape. **C.** Transversal cut in the peridiole showing the double-layered, subhomogeneous cortex. **D.** Basidiospores with elliptical shape.

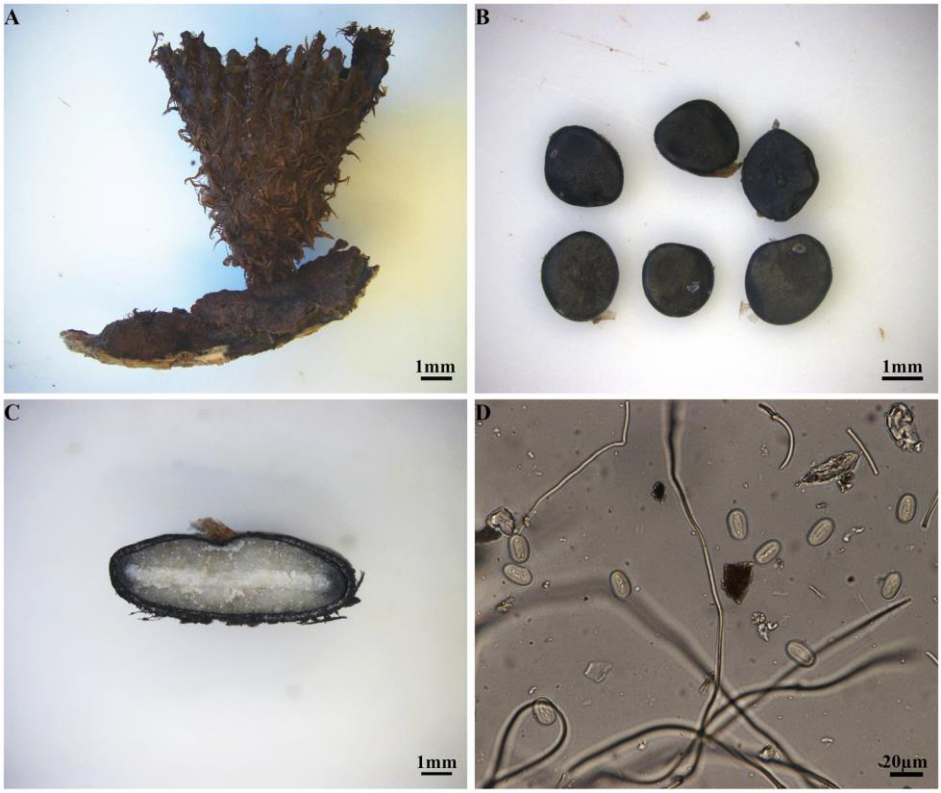

Figure 4. - *Cyathus limbatus* (UFRN-Fungos 3320). **A.** Basidiome with hirsute exoperidium. **B.** Peridioles with smooth surface, and angular to elliptical shape. **C.** Transversal cut in the peridiole showing the double-layered cortex. **D.** Basidiospores with elliptical shape.