



## *Mycenastrum catimbauense* (Agaricales, Basidiomycota), a new puffball species from the Brazilian semi–arid region

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### Abstract

*Mycenastrum catimbauense* is found growing in the xerophytic biome named “Caatinga” in the Catimbau National Park, Pernambuco State, Brazil. Basidiomata are found solitary in an open place and exposed to sunlight. The morphological analyses of macro- and microstructures followed the specific literature of the group. The fundamental diagnostic characteristics for this new taxon are the dark-colored peridium and gleba, tomentose peridium and spiny capillitium with brittle thorns. Full description, color photographs of the basidiomata and microstructures of this new species, and a discussion are provided.

**Key words** – Biodiversity – Gasteromycetes – Lycoperdales – Neotropics – Taxonomy

### Introduction

*Mycenastrum* is an unusual puffball genus, easily recognized by its branched capillitium and covered in numerous thorn-like spines. Desvaux (1842) established this interesting genus based on *Lycoperdon corium* Guers., which was combined as *Mycenastrum corium* (Guers.) Desv., becoming the type species of the genus. Over time, some other species (17), subspecies (2) and varietal ranks (5) (Index Fungorum 2017) were proposed. However, some authors consider *Mycenastrum corium* as a single cosmopolitan species (Hollo’s 1904, Cunningham 1944, Bottomley 1948, Pila’t 1958, Smith & Smith 1973, Miller & Miller 1988, Pegler et al. 1995, Grgurinovic 1997, Hansen & Knudsen 1997).

The Catimbau National Park is an important conservation area in the Caatinga, an exclusively Brazilian biome. This unique National Park is the second largest archaeological site in Brazil, with a surface area of 62,300 hectares and altitude range of 900–1000 m. It is located in the semi-arid region of Pernambuco State in the transition between distinct vegetation zones, forming a mosaic of landscapes with high biodiversity, including endemic species (Andrade et al. 2004, Gomes et al. 2006).

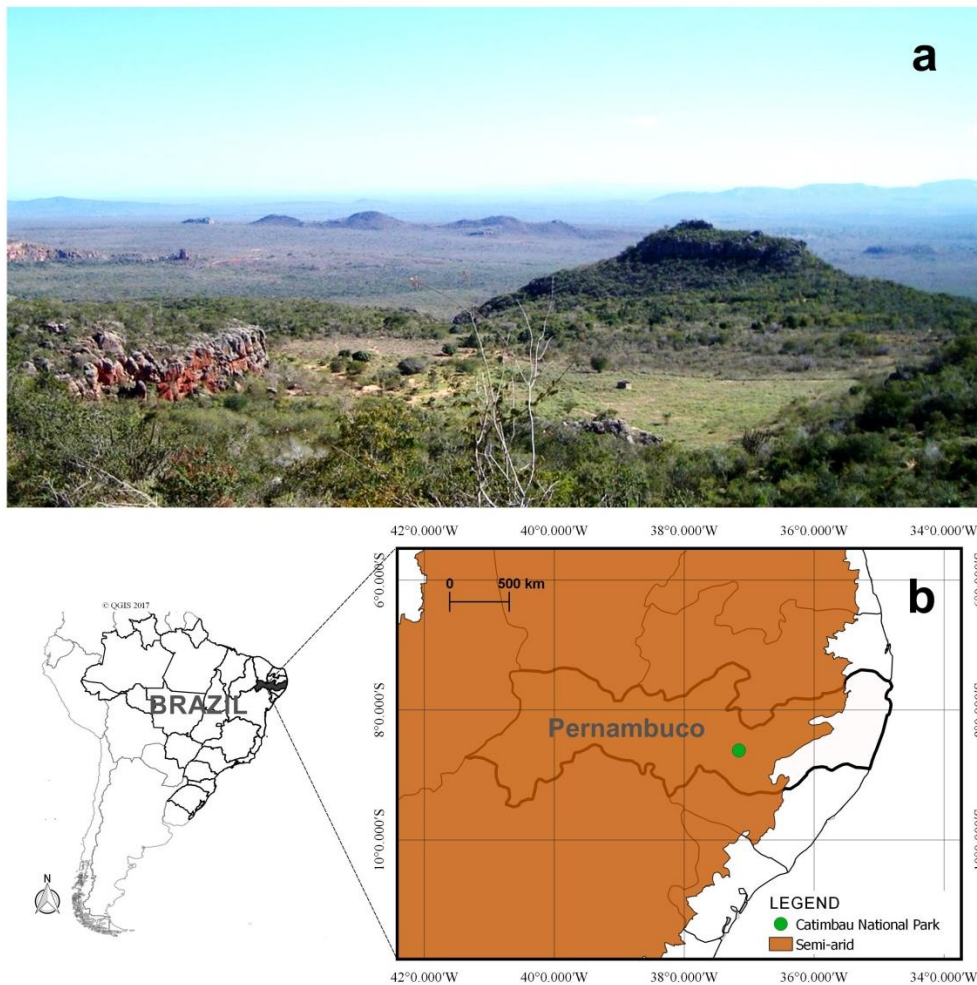
There is a need for a revision study of the genus *Mycenastrum* using integrative taxonomy in order to evaluate the real distribution of *M. corium* and to establish the informative characteristics

for species delimitation within the genus. This work aims to broaden the knowledge of this genus through the description of a new species.

## Materials & Methods

### Collection site details

Two specimens were collected in the rainy season of 2006 in Catimbau National Park (Fig. 1a). This park is situated in Buique, Pernambuco state, Brazil and its geographical coordinates are  $-8^{\circ}37'0.01''$  S –  $37^{\circ}08'60.00''$  W (Fig. 1b).



**Fig. 1** – Collection site in semi-arid region, Brazil. a. Landscape images from Catimbau National Park. b. Map of Pernambuco State and type locality.

### Collection methods

The specimens were collected following a methodology proposed by Baseia et al. (2014), in which the basidiomata were photographed with scales, georeferenced and removed from the substrate with a pocketknife. In the Biology of Fungi laboratory at the Federal University of Rio Grande do Norte the basidiomata were dehydrated in an electric dehydrator and packaged in a ziplock bag; these were then placed together with an information sheet from the collection.

### Morphological observations

The morphological analysis was conducted according to Demoulin (1968) and Calonge & Demoulin (1975). Macroscopic characters are based on observations of the dehydrated basidiomas,

including characteristics of the peridium and gleba. The size, shape and color of these characters were analyzed. Microscopic observation was performed using an optical Nikon Eclipse Ni (LM) microscope with Nikon DS–Ri1 camera attached and all the measurements were carried out using the NIS–Elements AR v.4.51.00 software. Slides for microscopic analysis were mounted in cotton blue, Melzer's reagent and KOH 5% containing portions of the gleba and peridium. For each specimen, 30 basidiospores were randomly selected and measured, including ornamentation, 20 hyphae of the peridium and gleba and 40 process spines. The statistical measurements used in basidiospores were performed according to Sousa et al. (2014), where measurements were conducted of 30 spores (including ornamentation), which determined the variation of the length ( $H_{Min}$  and  $H_{Max}$ ) and width ( $W_{Min}$  and  $W_{Max}$ ), the mean of the two dimensions ( $x_H$  and  $x_W$ ), the standard deviation of these (+/- SD) and the height (h) by the width (w) quotient represented by Qm ( $H_{Min}–H_{Max} \times W_{Min}–W_{Max}$  [ $x = x_H \pm SD_H \times x_W \pm SD_W$ ]) always observed on 100× objective. Scanning electronic microscope (SEM) photography was performed according to Cortez et al. (2008). The color was coded following Kornerup & Wanscher (1967). The herbarium specimens were deposited in the Fungus Collection of the Federal University of Rio Grande do Norte (UFRN), Natal, Brazil.

## Results

### Taxonomy

*Mycenastrum catimbauense* Baseia, Gurgel, Melanda, Ferreira R.J. & Alfredo, sp. nov. Figs 2–4  
MycoBank: MB821075; Facesoffungi number: FoF03658.

Etymology – In reference to the type locality, Catimbau National Park, Pernambuco State, Brazil.

Holotype – Brazil, Pernambuco, Buíque, Catimbau National Park (–8°37'0.01" S – 37°08'60.00" W) on soil, 3 Aug 2006, T. Ottoni (UFRN Fungos – 2847, holotype! UFRN Fungos – 2848).

Diagnosis – Basidiomata 40 mm wide; peridium tomentose, 1.4–2.1 mm thick, grayish brown to dark brown; dehiscence by fragmentation of endoperidium; gleba dark brown; spiny capillitium, brittle thorns; basidiospores globose, 11.7–14.5 µm diam., pedicelate.

Description – Basidiomata at maturity epigeous, 40 mm wide, globose to subglobose (Fig. 2); Subgleba absent; Peridium double 1.4–2.1 mm thick divided into exo– and endo–peridium; Exoperidium persistent, papery, 0.14–0.21 mm thick, tomentose, 0.04–0.08 mm length, dark brown (6F4), pruinose surface and amorphous substance forming flakes; Endoperidium persistent, coriaceous, 1.15–1.77 mm thick, darkish (6F2) to dark brown (6F4), delimited by outer layer 1.1–1.7 mm thick and inner layer 0.02–0.06 mm thick; Dehiscence by rupture of the apical portion of the peridium in irregular pointed rays; Gleba dark brown (6F4), powder releasing easily from peridium. Rhizomorphs not visualized.

Exoperidium composed of interwoven hyphae, 2.6–3.7 [ $3.13 \pm 0.34$ ] µm diam., wall 0.6–1.3 [ $0.91 \pm 0.20$ ] µm thick and tortuous, aseptate, clamp connections absent, reduced lumen, dextrinoid, cyanophilous, yellowish in 5% KOH; Endoperidium composed of outer and inner layer: outer layer, with hyphae (2.4)3.3–5.7 [ $4.35 \pm 0.60$ ] µm diam., wall 0.3–0.7 [ $0.54 \pm 0.08$ ] µm thick, aseptate, clamp connections absent, dextrinoid, yellowish in 5% KOH; inner layer with hyphae 3.2–4.9(6.0) [ $4.12 \pm 0.62$ ] µm diam., wall 0.6–1.3 [ $0.93 \pm 0.15$ ] µm thick, aseptate, clamp connection absent, dextrinoid, cyanophilous, yellowish in 5% KOH; Capillitium 7–15.7 [ $10.5 \pm 1.3$ ] µm diam., aseptate, branched, wall 1.1–2.2 [ $1.64 \pm 0.24$ ] µm thick, spiny capillitium, spines abundant at level of the terminal conical portion, weakly dextrinoid, light brown in 5% KOH, acyanophilous; Spines of capillitium 3.0–15.9 [ $7 \pm 2.8$ ] µm × 1.3–7.3 [ $2.8 \pm 1.4$ ] µm, brittle, branched; Basidiospores globose to subglobose, 13.3–15.0 × 11.7–14.5 [ $14.0 \pm 0.7 \times 13.2 \pm 0.7$ , Qm = 1.1] µm, dextrinoid, acyanophilous, reddish brown in 5% KOH, reticulate and pedicelate.

Habitat – in open soil of 'Caatinga' biome (dry forest).

Habit – solitary, not gregarious

Remarks – *Mycenastrum catimbauense* is a typical species of the genus *Mycenastrum*. It has a double and thick peridium with the dehiscence occurring from the upper hemisphere, subgleba absent, gleba powdery, spiny capillitium and ornamented basidiospores (Desvaux 1842, Seynes 1869, Long 1930, Bottomley 1948). This species is characterized mainly by its dark tomentose exoperidium, dark brown endoperidium, dark brown gleba (Fig. 3a–d), capillitium with brittle spines (Figs 4a, 4c) and bigger basidiospores measuring 13.3–15.0  $\mu\text{m}$  in diameter (Figs 4b, 4d). These characteristics are closely related to *M. corium* and *M. corium* var. *diabolicum*.

*Mycenastrum corium* differs from *M. catimbauense* by having a smooth exoperidium that falls off at maturity, gleba umber to purple–brown and basidiospores smaller (8.7–13.6  $\mu\text{m}$  diam.) (Desvaux 1842, Bottomley 1948, Demoulin 1968, Homrich & Wright 1973). Moreover, the exo- and endo-peridium in *M. catimbauense* have no clamp connection, while in *M. corium* the exoperidium is composed of hyphae with clamp connections and is bigger than in *M. catimbauense* (2.5–7.5  $\mu\text{m}$  diam. vs. 2.6–3.7  $\mu\text{m}$  diam.) (Demoulin 1968, Homrich & Wright 1973). According to the authors of the species, in *M. corium* the endoperidium is composed of a well-defined double layer with the closest thicknesses of 0.2–0.5 mm. In our analyses, the endoperidium of *M. catimbauense* is composed of an outer layer that is thicker than the inner layer (1.1–1.7 vs. 0.02–0.06 mm) (Fig. 3c).

*Mycenastrum corium* var. *diabolicum* differs from *M. catimbauense* by having a dark reddish brown peridium, extremely purplish black gleba and smaller pedicelate basidiospores (9–10  $\mu\text{m}$  diam.) (Homrich & Wright 1973). Moreover, in our morphological analyses, the spines of the capillitium are brittle and longer (Fig. 4c) than in *M. corium* var. *diabolicum* (15.9 vs. 10.8  $\mu\text{m}$  length).

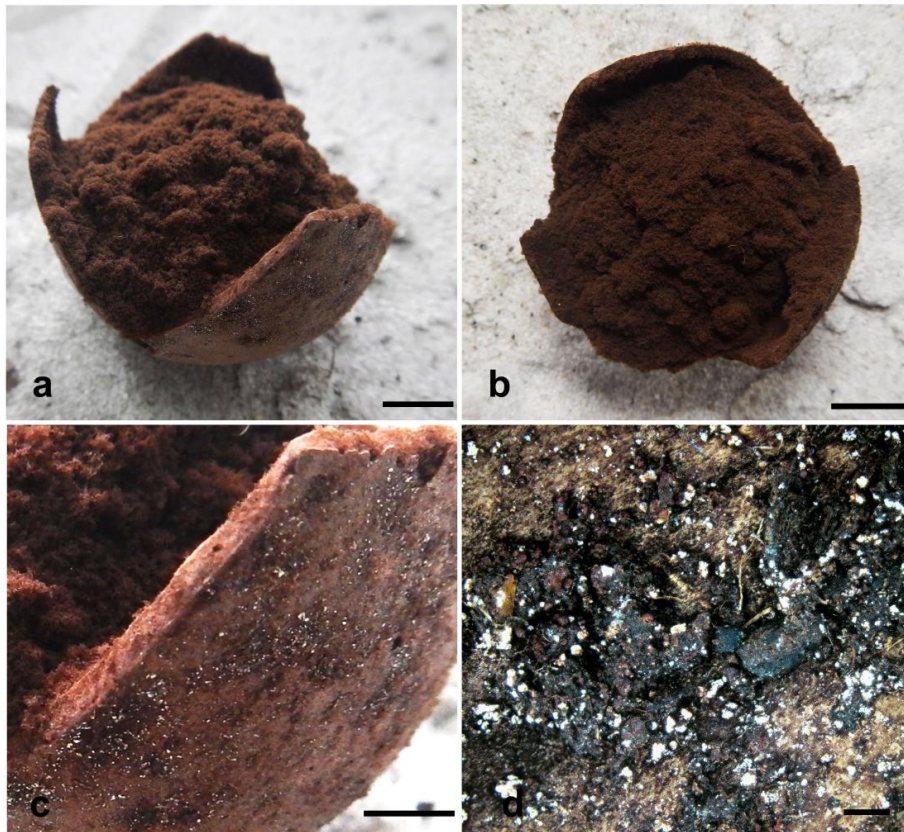


**Fig. 2** – *Mycenastrum catimbauense*. Illustration of the immature fruiting body and with the gleba exposed. Scale bars: 20 mm.

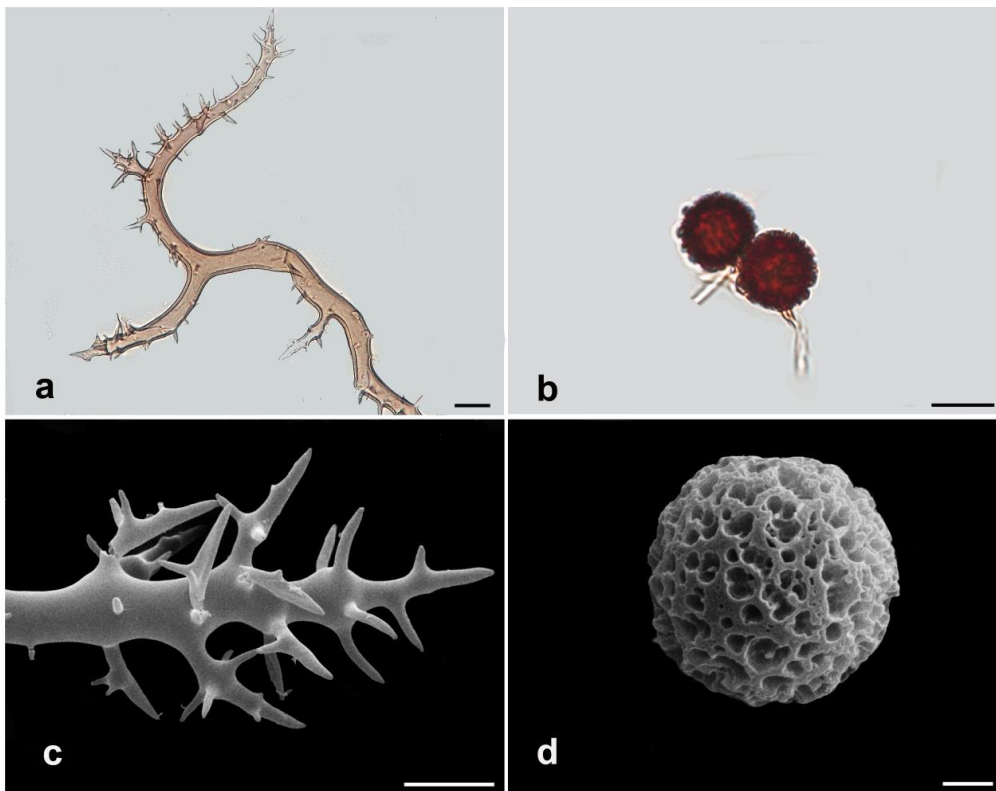
The other *Mycenastrum* species are not morphologically closed, since they have small spores with other ornamentation, such as echinulate (*M. radicum* Durieu & Montagne), smooth (*M. beccarii* Passerini, *M. dugesii* De Seynes, *M. leiospermum* Montagne, *M. leptodermeum* Durieu & Montagne, *M. martinicense* Patouillard, *M. ohienne* Ellis & Morgan and *M. oregonense* Ellis & Everh.), minutely verrucose (*M. spinulosum* Peck) or verrucose (*M. phaeotrichum* Berk.) (Berkeley 1843, Fries & Montagne 1847, Passerini 1875, Peck 1881, Seynes 1886, Cooke & Masee 1887 and Patouillard 1902). *M. olivaceum* Cooke & Masee has spores with approximately the same diameter as *M. catimbauense*; however, it has asperulate ornamentation (Cooke & Masee 1887).

Another contribution to defining the new species is the Caatinga biome. This is the only large natural region of Brazil whose limits are entirely restricted to the national territory, and it deserves great attention (Silva et al. 2004). The semi-arid climate, which is typical of the biome, is strongly seasonal, with less than 1000 mm of rain per year. It presents high potential evapotranspiration, from 1500 to 2000 mm per year (Velloso et al. 2002). There is rich biodiversity and the biome is very distinct from other habitats. It is a region that has high potential to develop new species.





**Fig. 3** – Basidiomata in situ. a–b. Mature basidiomata with the gleba exposed. c–d. Detailed tomentose exoperidium. Scale bars: a–b = 10 mm, c = 5 mm, d = 0.5 mm



**Fig. 4** – Microstructures. a. Capillitium with brittle spines under LM. b. Basidiospores pedicelate under LM. c. Capillitium with brittle thorns under SEM. d. Basidiospores reticulate under SEM. Scale bars: a= 20  $\mu$ m, b–c = 10  $\mu$ m, d = 2  $\mu$ m.

In recent years, the belief that some gasteroid genera were monotypic, such as *Astraeus* Morg., *Myriostoma* Desv. and *Pisolithus* Alb. & Schwein., has been revealed by molecular analyses to be wrong. This is mainly due to the universally adopted DNA barcode region for fungi (Schoch et al. 2012), which has unmasked hidden taxa. Distinct species have now been described within these genera and several more await formal naming and description (Phosri et al. 2012, 2013, 2014, Martín et al. 2013, Sousa et al. 2017). In the near future, review studies using integrative taxonomy will probably demonstrate similar results for the genus *Mycenastrum*.

The Index Fungorum (2017) gives 14 species of *Mycenastrum*. However, many authors have considered the genus as monospecific and widely distributed (Cunningham 1944, Bottomley 1948, Grgurinovic 1997, Kirk et al. 2008), although new varieties have been proposed (Homrich & Wright 1973, Miller et al. 2005). Thus, more studies are needed, allying morphological and molecular analyses to clarify the position of the species and variety in *Mycenastrum*. Unfortunately, despite many attempts, it has so far proved impossible to extract DNA from our specimens, and at times the amplification was contaminated with *Aspergillus* sp. Thus, in this work we have used a set of macro and micro morphological characteristics to propose *Mycenastrum catimbauense* as a distinct and reliable species.

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