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Rediscovery of *Scleroderma anomalosporum* Baseia, B.D.B. Silva & M.P. Martín (Boletales, Basidiomycota) in the Brazilian Amazon: is the species now safe?

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Abstract

The year 2019 was marked by several fires in the Amazon Domain. Rondônia, a northern Brazilian state, is part of the Amazon Domain and is the country's third most deforested state. Anthropic action has been reported in several cases as the main source of biological diversity reduction. Among gasteroid fungi, *Scleroderma camassuense* and *S. anomalosporum* were thought to be extinct, since the only known locality was submerged due to the construction of the Belo Monte Hydroelectric Power Plant. The present work registers the presence of *S. anomalosporum* in Porto Velho in Rondônia, providing taxonomic data, photographs and discussion about the rediscovery and its unfolding.

Key words – Biodiversity – Earthball – Gasteroid fungi – Neotropics –Taxonomy

Introduction

The Amazon Domain, composed of several biomes, is characterized by its huge size, covering almost 50% of the national territory. It is the home of the hydrographic basin that holds the largest volume of fresh water in the world and that concentrates 1/5 of all fresh water on earth (MMA 2007). According INPE (2019), between August 2018 to July 2019, the estimated value for the deforestation rate is 9,762 km². This value represents an increase of 29.54% in relation to the previous period, which was 7,536 km². Fonseca et al. (2020) registered 529 km² of deforestation recorded in the Legal Amazon only in April 2020, an increase of 171% in relation to April 2019, which represents the highest value in ten years.

According to the National Institute for Space Research (INPE), in 2019, the main outbreaks of fire in the Brazilian Amazon occurred between January and August, 83% higher than in the same period of the previous year, with over 72,000 outbreaks of fire. Mato Grosso do Sul (+ 256% outbreaks), Pará (+ 199%), Acre (+ 196%) and Rondônia (+ 190%) were the most affected states (INPE 2019).

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A note published by the Amazon Environmental Research Institute (IPAM 2019), shows the relationship between burning and deforestation in the Amazon Domain, where Porto Velho in Rondônia state (RO) is listed as the third most deforested municipality, with 280 hectares. It is listed as the city with the fourth greatest number of outbreaks of fires, accounting for 3110 outbreaks from January to August 2019. It is thus the only state capital in the Amazon region to be among the 10 cities listed by INPE.

The Amazon is considered one of the largest reserves of biodiversity on the planet (MMA 2007). Approximately 44,000 species have already been recorded for the Amazon, including plants, mammals, birds, fishes, and fungi, most of which are concentrated in the Brazilian Amazon, according to The Nature Conservancy (TNC 2019). In recent years, several studies have contributed to new records and new species of fungi for the Amazon (Trierveiler-Pereira et al. 2009, Henkel et al. 2010, Alfredo et al. 2012a, b, 2017, Cabral et al. 2014, 2017, 2019, Smith et al. 2015, Baseia et al. 2016, Accioly et al. 2018, 2019, Assis et al. 2019, Jobim et al. 2019). However, for the Amazon, data on fungal diversity are still scarce and insufficient to estimate the biodiversity of this group. These data are important in order to develop protection and preservation measures.

The genus *Scleroderma* was described by Persoon in 1801 and has so far been recorded for tropical, subtropical and temperate regions, forming ectomycorrhizal associations (Guzmán et al. 2004, Kumla et al. 2013). This genus is currently allocated in the Boletales E.-J. Gilbert order (Hibbett et al. 2014) and divided into three sections based on basidiospore ornamentation and the presence or absence of connecting clamps: (1) *Reticulatae*, characterized by reticulated basidiospores, (2) *Scleroderma*, with echinulated basidiospores, (3) *Sclerangium*, presenting sub-reticulated basidiospores (Guzmán et al. 2013).

Eighteen *Scleroderma* species have been reported from Brazil, most of which have been observed in ectomycorrhizal associations with exotic plants such as *Pinus* spp. and *Eucalyptus* spp. (Hennings 1904, Viégas 1945, Rick 1961, Guzmán 1970, Bononi et al. 1981, Baseia & Milanez 2000, Giachini et al. 2000, Sobestiansky 2005, Meijer 2006, Drechsler-Santos et al. 2008, Gurgel et al. 2008, Cortez et al. 2011). However, *Scleroderma dunensis* B.D.B. Silva, Sulzbacher, Grebenc, Baseia & M.P. Martín, *S. minutisporum* Baseia, Alfredo & Cortez, *S. duckei* B.D.B. Silva, M.P. Martín & Baseia, *S. camassuense* M.P. Martín, Baseia & B.D.B. Silva and *S. anomalosporum* Baseia, B.D.B. Silva & M.P. Martín were found in native vegetation of the Amazon rainforest, the last latter two being considered extinct to date (Alfredo et al. 2012b, Baseia et al. 2016, Crous et al. 2016). Baseia et al. (2016) reported the likely extinction of *S. camassuense* and *S. anomalosporum*, as the collection site was submerged due to the construction of the Belo Monte Hydroelectric Power Plant and these species are not registered in other locations.

Amazonian biodiversity is threatened, largely due to the growth of agribusiness, illegal logging and high deforestation rates for these purposes or others (INPE 2019). Vast forested areas with potential for large amounts of biological diversity disappear every year, many of which were still unexplored by science. Thus, the present study aimed to record the rediscovery of a species of *Scleroderma* genus considered "extinct" in the Brazilian Amazon in an area not yet studied.

Materials & Methods

Study area

The study collection area was the Parque Natural Municipal de Porto Velho – RO (8°41'10.8"S 63°52'05.4"W). The Parque Natural is a preservation area located in the northern region of Porto Velho municipality, 15 km from the center of the capital. In the area, open ombrophilous forests predominate, with palm trees mainly of the species *Mauritia limnophila* Barb. Rodr., popularly known as "caranaí". The area is cut by several streams and has trails that allow access to the interior of the forest (SMMM/PMPV 2012). The climate of the region is predominantly rainy, with the rainy season extending from October to April, followed by a well-defined dry season, with the driest months being June, July and August. The average temperature during the year is 24 to 26°C, with highs and lows between 17 and 36°C.

The collection area map (Fig. 1) was constructed in QGis 3.4, Geographic Coordinate Systems: Datum SIRGAS, 2000, with IBGE, 2017 (https://www.ibge.gov.br/geociencias/downloads-geociencias.html) and TerraBrasilis (http://terrabrasilis.dpi.inpe.br/downloads/) cartographic bases.

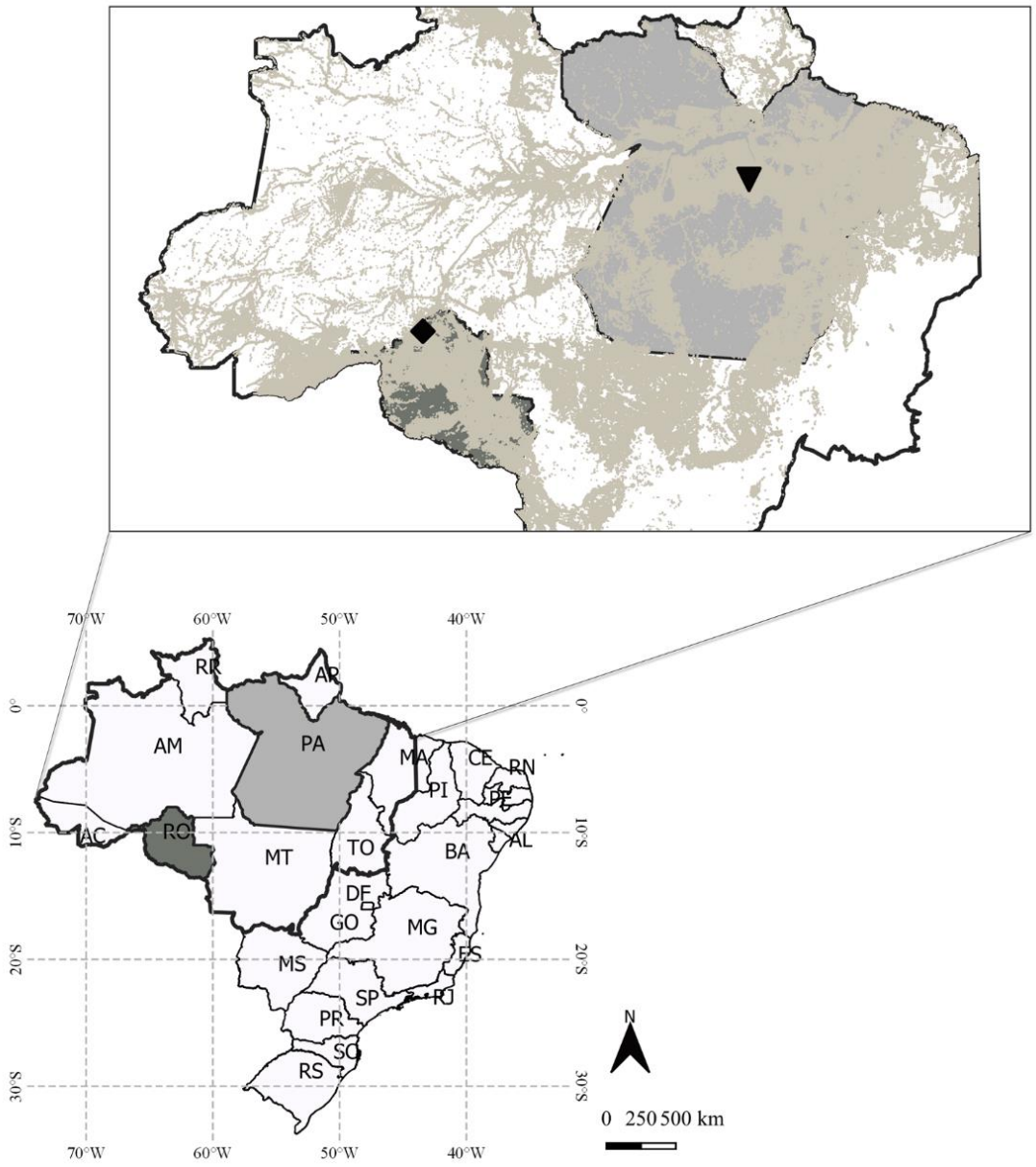

Fig. 1 – Re-collection area (rhombus) and initial collection (inverted triangle) of *Scleroderma anomalosporum*. The Brazilian Legal Amazon is outlined in a darker line and the collection states are filled in. Gray traces indicate deforestation from 1998 to 2019.

Collection and herborization

The collection was performed following pre-existing trails and entering the forest whenever necessary, following Baseia et al. (2014). The basidiome was photographed with a scale ruler, georeferenced and collected manually with the help of a penknife (used to remove the basidiome). The basidiome removed from the substrate was stored in a paper bag; the date, locality, collectors, substrate, habitat, geographic coordinates, and collector number were noted. The basidiome was sun-dried and deposited in UFRN-Fungos located in Natal, Brazil.

Morphological analysis

Macro and microscopic analysis of the dehydrated specimen followed the methodology of

Baseia et al. (2016), carried out at the Fungus Biology Laboratory of the Federal University of Rio Grande do Norte (UFRN). The macroscopic characteristics (color, dimensions and characteristics of the peridium, gleba and rhizomorphs) were observed with a Nikon SMZ 1500 stereoscopic microscope with a Nikon DS-Ri1 attached camera, and the color chart (Kornerup & Wanscher 1978) was used to determine the color.

Microscopic observations were performed using a Nikon DS-Ri1 optical microscope with an attached camera Nikon Eclipse Ni. Slides of the peridium layers and gleba were mounted in 5% KOH aqueous solution. The basidiospore statistics followed Bates (2004) and were written as follows: length (min) – length (max) × width (min) – width (max) [x = mean length ± standard deviation × mean width ± standard deviation; Qm = mean quotient of basidiospore length to width; n = number of randomly measured basidiospores].

Results

Taxonomy

Scleroderma anomalosporum Baseia, B.D.B. Silva & M.P. Martín Fig. 2a–d

Mycobank number: MB 818095

Basidiomata epigeous, sessile, 25 mm height × 50–70 mm diam, with stellate dehiscence forming 7 irregular rays (Fig. 2a). Surface cracked, with small scales, reddish brown (8F6) to dark brown (8F4) with aggregate soil particles, especially in the basal portion (Fig. 2b). Basal mycelium forming aggregated rhizomorphs. Peridium 0.9–1.3 mm thickness, composed of three layers (Fig. 2b1, d). Gleba when mature grayish brown (6E3), powdery, protected by grayish yellowish endoperidium (3C4–4C5).

Exoperidium composed of entangled, branched, septate, cylindrical hyphae, hyaline, 5.7–7.2 μm diam, thin walls (<1.0 μm thickness). Mesoperidium consisting of cylindrical hyphae, with rounded ends in some hyphae, branched, hyaline, 7.5–15.7 μm diam, thin walls (<1.0 μm thickness). Endoperidium consisting of intertwined, cylindrical, branched, hyaline to yellowish hyphae 3.4–7.9 μm diam, thick walls (>1.0 μm thickness). Basidiospores 4.7–6.4 × 4.3–6.2 μm (5.4 ± 0.5 × 5.1 ± 0.5; Qm= 1.05 ± 0.03; n = 30), globose to subglobose, hyaline to yellowish, smooth under light microscope (Fig. 2c).

Known distribution – Amazon rainforest.

Material examined – Brazil, Rondônia, Porto Velho, Parque Natural Municipal S8°41'46.0" W63°51'56.5", on clay soil covered by litter, 25 Fev 2019, S.O. Almeida, UFRN–Fungos 3275.

Notes – *Scleroderma anomalosporum* is characterized mainly by globose to subglobose, smooth basidiospores in light microscopy and peridium formed by three layers (Baseia et al. 2016). According to Guzmán (1970), smooth basidiospores occur only in immature basidiomata, with basidiospores becoming reticulated, subreticulate or equinulate at maturity. In the species discussed here, even in the developed basidiome the basidiospores were smooth, a characteristic not observed in other species of the genus.

Characteristics such as the peridium formed by three layers, size and absence of ornamentation of the basidiospores give support of the identification as *S. anomalosporum*, however the specimen described here has smaller expanded basidiome. These small variations in the dimensions of basidiome are acceptable, as a result of environmental physico-chemical conditions (humidity, availability of nutrients, shadow, species of plants, among others) that vary from one location to another.

The species *S. anomalosporum* is easily distinguished, based on morphological data, from the other species of *Scleroderma* that occur in Brazil, as we can see in Table 1. *Scleroderma tuberoideum* Speg. and *S. floridanum* Guzmán are not present in the table because they are not described morphologically in the works that registered them for Brazil, Bononi et al. (1981) and Giachini et al. (2000), respectively.

Table 1 Comparison of morphological characters used to delimit *Scleroderma anomalosporum* with other species of *Scleroderma* that occur in Brazil

Characters	Basidioma (diam. in mm)	Exoperidium (ornamentation)	Basidiospores (diam. in µm)	Basidiospores (ornamentation)	References
S. anomalosporum	Up to 115	Cracked, with small scales	4.7–6.4	Smooth	Present study
S. albidum Pat. & Trab.	Up to 39	Smooth, cracked, to squamulous	8–17	Echinulate	Montagner et al. (2015)
S. areolatum Ehrenb.	(5–)10–40(–55)	Squamous	(10–)11–17 (–18)	Echinulate	Guzmán (1970)
S. citrinum Pers.	25–67	Smooth	9–12	Reticulate	Montagner et al. (2015)
S. verrucosum (Bull.) Pers.	(5–)10–30(–40)	Squamous	(7–) 8–11 (–23.7)	Thorns	Guzmán (1970)
S. camassuense M.P. Martín, Baseia & B.D.B. Silva	Up to 14	Scaly to squamous	6.4–8.0	Irregular reticulate	Baseia et al. (2016)
S. cepa Pers.	30–60	Squamous (large and irregular)	(7.5–) 8.8–12 (–13.6)	Echinulate	Guzmán (1970)
S. stellatum Berk.	25–45	Finely squamous	(5–) 6.4–7.2 (–8)	Finely squamous and subreticulate	Guzmán (1970)
S. duckei B.D.B. Silva, M.P. Martín & Baseia	Up to 20	Verrucose	5.7–7.1	Regularly grouped warts	Baseia et al. (2016)
S. bougheri Trappe, Castellano & Giachini	7–28	Felty to roughened	7.5–9(–11)	Thorns	Giachini et al. (2000)
S. bovista Fr. ≡ *S. verrucosum* subsp. *bovista* (Fr.) Šebek ≡ *S. fuscum* (Corda) E. Fisch.	20–30	Smooth	9–11	Echinulate and reticulate	Gurgel et al. (2008)
S. nitidum Berk.	15–30	Verrucose	11–12	Echinulate	Gurgel et al. (2008)
S. polyrhizum (J.F. Gmel.) Pers. ≡ *S. geaster* Fr.	20–40	Squamous	6–7	Echinulate	Baseia & Milanez (2000)
S. tenerum Berk. & M.A. Curtis	–	Squamous	12	Verrucosus	Rick (1961)
S. uruguayense (Guzmán) Guzmán ≡*S. citrinum* var. *uruguayense* Guzmán	60–75	smooth to finely warty or finely scaly	(9.5–)11–14 (–16)	Echinulate and reticulate	Guzmán (1970)

Discussion

The collection analysed in the present study has the same characters as *Scleroderma anomalosporum*, which was recently described as new and possibly extinct because of human activities (Baseia et al. 2016). It was originally collected on March 28, 2015 on Camassú Island, Pará, at an approximately distance of 1,750 km from the Parque Natural Municipal de Porto Velho, where it was rediscovered on February 25, 2019 (Fig. 1). Despite being in different states, both sites belong to the Amazon Domain, which may indicate the wide dispersal area of the species, with the Madeira and Amazon Rivers as possible dispersing agents.

Rondônia state, as well as its capital Porto Velho, suffered in the year 2019 with severe fires, which are directly linked to the deforestation promoted by unbridled agricultural development. Figure 3 shows “thermal anomalies” and fires in the Porto Velho area, represented by red dots on NASA

images. Thermal anomalies indicate a fire outbreak or “any significant heat source”. Fig. 3a was taken on August 4, 2019 and Fig. 3b ten days later on August 14 (BBC 2019).

Although alarming, the work performed by Baseia et al. (2016) highlighted the importance of continued scientific investigation of the Amazon rainforest to identify new species records. The rediscovery of the species encourages to pay more attention to these environments, as they still are under heavy human pressure.

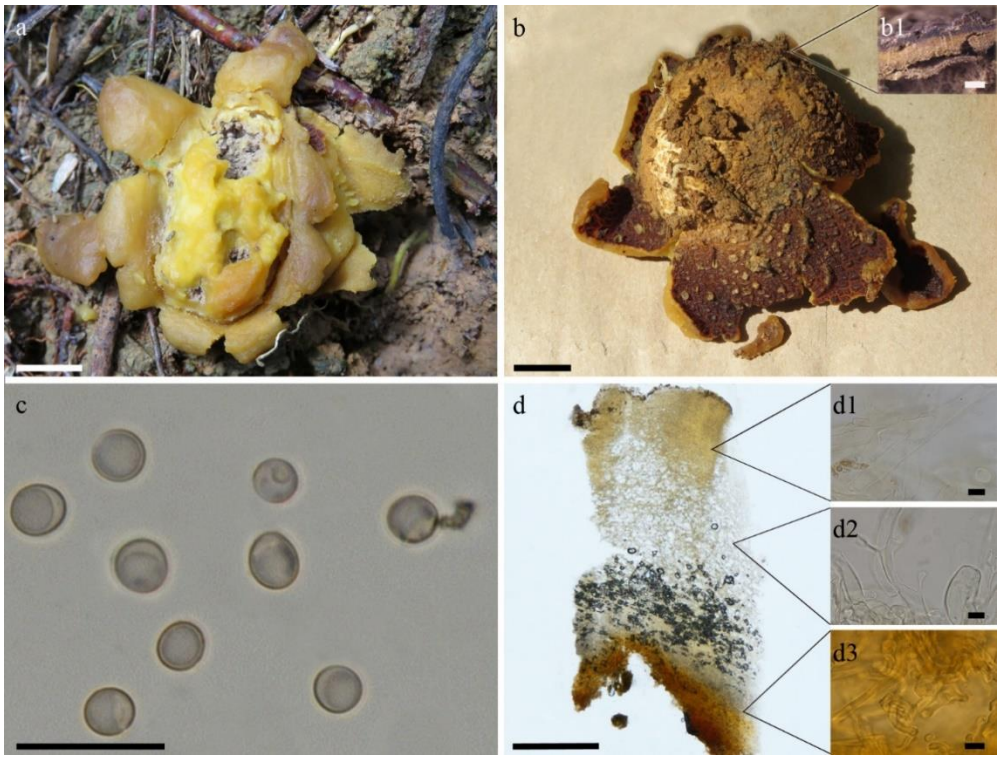

Fig. 2 – *Scleroderma anomalosporum*. a Basidioma in the field. b Overturned basidioma illustrating the exoperidium surface. b1 Basidioma section illustrating the peridium layers. c Basidiospores in optical microscope. d Peridium layers in optical microscope: exoperidium, mesoperidium and endoperidium from the outermost to the innermost layer (from top to bottom). d1 Exoperidium. d2 Mesoperidium. d3 Endoperidium. Scale Bars: a, b = 10mm, b1 = 0.5 mm, c, d1, d2, d3 = 10µm, d = 500 µm.

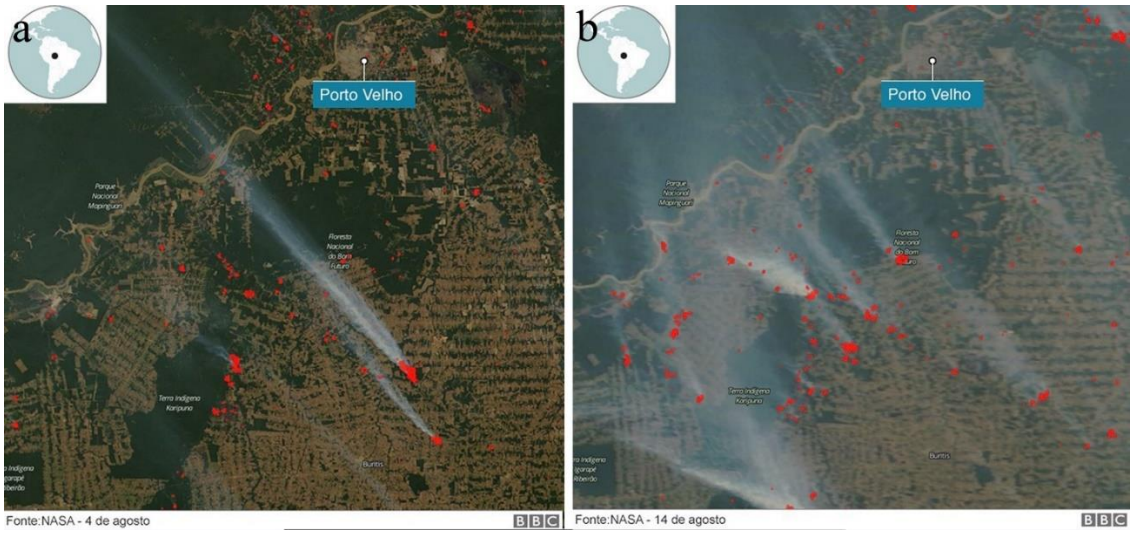

Fig. 3 – Map of fire outbreaks in the Porto Velho region. a August 4, 2019. b August 14, 2019. Source: BBC (2019).

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