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TWO NEW ARTHONIALES SPECIES (LICHENIZED ASCOMYCOTA) FROM BRAZIL

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ABSTRACT

Objective: The present work aimed to describe, comment, and illustrate two new lichenized species of Ascomycota.

Method: Morphological analyses of the samples were performed at the Laboratory in the State University of Bahia (UNEB), *Campus* VIII. The lichen thalli were observed using a stereomicroscope (Zeiss); chemical treatments were performed to observe the microscopic structures of the lichens after exposure to 10% KOH (potassium hydroxide), lactophenol (cotton blue), Melzer's reagent, chlorine, and viewed under UV light. The chemical compositions of the lichen were examined using thin layer chromatography (TLC) at the Biochemical Laboratory of the Federal University of Pernambuco.

Results and conclusion: *Stirtonia juaensis* and *Cryptothecia paramacrocephala* are two new lichenized species of Ascomycota found colonizing the plants *Spondias tuberosa* Arruda (Umbuzeiro) and *Piptadenia moniliformis* Benth. (Quipembe) in the Caatinga phytogeographical domain near the village of Juá in the municipality of Paulo Afonso, within the Raso da Catarina Ecoregion in northern Bahia State, Brazil.

Originality/value: Research related to lichen taxonomy in Brazil is still scarce, especially in the Caatinga. The new species of *Stirtonia* and *Cryptothecia* described and illustrated in this work expand the knowledge about fungi in Brazil.

Keywords: Caatinga, Lichen, Taxonomy, Stirtonia, Cryptothecia.

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DUAS NOVAS ESPÉCIES DE ARTHONIALES (ASCOMICOTA LIQUENIZADA) DO BRASIL

RESUMO

Objetivo: O presente trabalho teve como objetivo descrever, comentar e ilustrar duas novas espécies de Ascomycota liquenizados.

Método: No Laboratório da Universidade do Estado da Bahia (UNEB), Campus VIII, foram realizadas análises morfológicas. Os talos dos liquens foram observados em estereomicroscópio (Zeiss); tratamentos químicos foram realizados para observar as estruturas microscópicas dos liquens após exposição a KOH 10% (hidróxido de potássio), lactofenol (azul de algodão), reagente de Melzer, cloro e visualizados sob luz UV. As composições químicas dos liquens foram examinadas por cromatografia em camada delgada (TLC) no Laboratório de Bioquímica da Universidade Federal de Pernambuco.

Resultados e conclusão: Stirtonia juaensis e Cryptothecia paramacrocephala são duas novas espécies liquenizadas de Ascomycota encontradas colonizando as plantas Spondias tuberosa Arruda (Umbuzeiro) e Piptadenia moniliformis Benth. (Quipembe) no domínio fitogeográfico da Caatinga próximo ao povoado de Juá no município de Paulo Afonso, dentro da Ecorregião Raso da Catarina no norte do estado da Bahia, Brasil.

Originalidade/valor: As pesquisas relacionadas à taxonomia de líquens no Brasil ainda são escassas, especialmente na Caatinga. As novas espécies de Stirtonia e Cryptothecia descritas e ilustradas ampliam o conhecimento sobre fungos no Brasil.

Palavras-chave: Caatinga, Líquen, Taxonomia, Stirtonia, Cryptothecia.

1 INTRODUCTION

The Caatinga domain covers a large part of northeastern Brazil, with a xerophytic vegetation of differing phytophysiognomies (Lima & Coelho, 2018). The lichenized fungi associated with the plants in that region are dominated by crustose lichens, many of which belong to the order Arthoniales (Xavier-Leite et al., 2014).

Arthoniales is the third largest Order of lichenized Ascomycota, and one of the main representatives of lichenized fungi found in various forest types, especially in the tropics, with high numbers of corticolous and foliiocolous species (Ertz, 2009). The family Arthoniaceae comprises 717 species distributed among 20 genera, including Cryptothecia Stirt. (65 spp.) and Stirtonia A.L. Sm. (25 spp.) (Lücking et al., 2017).

Stirtonia is a genus of crustose lichens characterized by the absence of a true hamathecium and having asci found in ascigerous zones containing (generally) eight transversely septate ascospores with thick walls. The genus is very similar to Cryptothecia, but can be distinguished from it by having ascospores that are only transversely septate (Aptroot, 2009). Those two species can also be identified by other characteristics, such as their thallus structures, the presence of crystals and algae, the shapes of their ascigerous areas, and their staining by iodine (Aptroot, 2009; Aptroot et al., 2014).

Cryptothecia, in turn, is characterized by having a byssoid thallus, an irregular blue, I + medulla, a trentepohlia photobiont, asci clavate to globose within ascigerous areas, and muriform ascospores (Jagadeesh Ram & Sinha, 2016).

Research investigating lichen diversity and ecology in Brazil is still relatively rare, especially in areas distant from the more developed southern and southeastern regions (Cáceres, 2007). According to Menezes et al. (2011), that situation is related to the lack of specialized professionals and to the very irregular distribution of scientific production in that large country.

The new species of Stirtonia and Cryptothecia reported here were encountered colonizing the branches of Piptadenia moniliformis Benth. (Quipembe) and Spondias tuberosa Arruda (Umbuzeiro), respectively, in the Raso da Catarina ecoregion; they are described and illustrated here and compared to other species of those genera.

2 REFERENCIAL TEÓRICO

Ecorregião Raso da Catarina is one of the eight proposed areas for the Caatinga bioma and is located in the Northeast of Brazil, comprising an area of approximately 30, 800 km (DIAS; PAES, 2007). It is a sedimentary basin with a very flat relief, where in the southern part (state of Bahia) most of the soil is composed of sand and salt. While in the northern part (Pernambuco) predominate sandy soils. The availability of water is scarce, except in the canyon areas (VELLOSO et al., 2002). Its vegetation is composed of plants that appear to be open forests, with twisted, dry and whitened bushes for almost every year. (VELLOSO et al., 2002).

Among the native plants of the Caatinga, there is the Piptadenia moniliformis Benth, popularly known as catanduba, beetle branch, jurema-black, quipembé or angico-de-beetle, which is a species famous for supporting high temperatures, having a fast growth and producing annually a large number of seeds, being of great value for recovery of degraded areas. It can be easily found in the states of Bahia, Ceará, Piauí and Maranhão (AZERÊDO et al., 2010).

Piptadenia moniliformis is a tree that can measure between 4 and 9 meters in height, presenting a stem usually twisted, with a diameter between 20 and 30 cm, bark of whiteish color, thin and with little roughness and deciduous in the dry season. Their leaves are hairy, Korean, composite, bipinated, their flowers have the white-greenish shade when young, and yellow or browned in maturity, with inflorescences arranged in spikes, cylindrical, solitary or geminated. The fruit is vague, with an average size of 13 cm, the seeds are white, oval and compressed, the fruit and the seed are the most striking morphological characteristics. Their reproduction can occur both sexually and asexually, the latter being by means of stacks and rebirth of touches. (PEREIRA et al., 2003).

Because it has a heavy wood with a texture of medium mechanical strength and good natural durability it is used in the production of wood and coal for the low-income community, in addition to being highly employed in beekeeping and in the supply of excellent quality feed for cattle, goats and sheep (MAIA, 2004).

Another prominent plant for the region is the Spondias tuberosa Arruda, popularly known as umbu which, in tupi-guarani, means "tree that gives to drink", this because of its juicy fruits and its roots or tubers, which are able to store water and nutrients during the period of drought, being considered a symbol of the biodiversity of the Caatinga. (MELO; ANDRADE 2010).

The fruits of S. tuberosa are highly appreciated due to their exceptional taste and smell and have a high content of phenolic compounds, ensuring that the diet has a good supply of the antioxidants. These fruits are used both for human food and for the food supplement of goats and sheep, which constitute the predominant flocks of the Brazilian semi-arid. In addition, this species has another importance of populations of the semi-arid that is clear in the drought, when the rain is not enough for traditional corn and bean plantations, in this period the umbuzeiro supplies its fruits which are marketed by small farmers.

Plants, in general, to maintain or increase their adaptive value and prevent extinction, form continuous and diverse associations with other organisms, such as animals, bacteria, viruses and fungi (OCCHIPINTI, 2013).

Fungi are one of the most diverse groups of organisms that exist (Pansera et al., 2023; De Matos et al., 2023). They are characterized by being eukaryotic, heterotrophic by absorption and have a cell wall consisting of chitin and β-glucans, can be found in all ecosystems, both in terrestrial, aquatic environments, with high or low temperatures, as well as survive in places poor in nutrients, being the conditions of humidity and high temperature most suitable for the establishment and growth of these organisms. (SEIFERT et al., 2011; SPATAFORA et al., 2017). In addition, they are responsible for playing an extremely important role for the environment, the cycle of nutrients and carbon in terrestrial ecosystems.

In Bahia, few studies on the richness and composition of liquens have been developed (Aptroot & Cáceres, 2018). For the Ascomycota phylum, work has been intensified, especially with non-liquidated ascomycetes (VITÓRIA et al., 2008, 2010, 2011a,b, 2012, 2013, 2016a, 2019).

In areas of Ecorregião Raso da Catarina, research with fungi have revealed a high species richness of asexual, non-lichenized sexual and lichenized sexual species of Ascomycota (SILA & VITÓRIA, 2023; FORTES & VITORIA, 2022; FORTES et al., 2020; VITÓRIA et al., 2022, 2020, 2016; ROCHA & VITÓRIA 2020; ROCHA et al., 2023; SECUNDA & VITÓRIA 2020; BARBOSA & VITÓRIA 2019; SOUZA et al., 2021; SANTOS et al., 2020 a,b, 2019, 2016b, 2023).

Ascomycota is considered the largest phylum of the Fungi Kingdom. Ascomycota is a name derived from the Greek words askos (=leather bottle, bag or bladder) and mykes (=fungi). Its main characteristic is the production of ascospores in cells called asci (WEBSTER; WEBER, 2007). Most of its members develop their sexual stage in fruiting bodies called ascocarps or ascomas. These structures can be of distinct types: apothecium, cleistothecium, perithecium, and pseudothecium. Approximately, 30% of all Ascomycota are lichenized (Lücking et al., 2017).

Lichenization is the symbiotic association between fungi and algae or cyanobacteria that forms the biological unit called lichen (APTROOT et al., 2014). This association is made between representatives of distinct kingdoms and phyla, mostly by fungi of the phylum Ascomycota (98%), or Basidiomycota (2%), with algae (Protoctists), cyanobacteria (Monera) or both simultaneously (SPIELMANN; MARCELLI, 2006; MARQUES, 2008).

The lichen symbiosis is an advantage for the fungus, although the algae rely on the fungal tissue that surrounds it to protect it from light and dissection, making it possible for the photobiont to occur in otherwise inaccessible environments (MARQUES, 2008). A contribution to the argument of Marques (2008) is based on the fact that photobionts exist freely in nature, while about 20% of all known fungi are necessarily lichenized (MARCELLI, 1997).

The lichen thallus has characteristics from the mycobiont, presenting various morphological types. The most common type is thallus is called crustose, characterized by being completely attached to the substrate by the medulla (SPIELMANN; MARCELLI, 2006). There are also species with foliose thallus, which is laminated and dorsiventrally flattened structures fixed by small rhizinas or tomentos, presenting lobes (rounded divisions) or lacinias (long divisions); the fruticose thallus are cylindrical, attached to the substrate by a stype and form branches; those that are formed by tiny aggregated scales or swuamules are known as squamulouse; and the thin and interwoven threads are called filamentous (ALMEIDA, 2017).

In Brazil, most of the studies about the ecology of populations and communities of lichen species have been carried out in the states of the southern and southeastern regions

(LUCHETA; MARTINS, 2014), and also in the Amazon (ALMEIDA, 2017). For the Northeastern states, most of the studies were about the diversity and taxonomy of lichens for the two decades (Cáceres et al. (2007, 2011) in the biomas Caatinga and Mata Atlántica.

3 MATERIAL AND METHODS

Mycological expeditions to collect lichen specimens on Piptadenia moniliformis and Spondias tuberosa were undertaken in January/2018 in Juá village in the municipality of Paulo Afonso, Bahia State, Brazil. The collected specimens were held in paper bags and subsequently removed to the Mycology Laboratory at the State University of Bahia (UNEB), Campus VIII, where morphological analyses were performed. The lichen thalli were observed using a stereomicroscope (Zeiss); chemical treatments were performed to observe the microscopic structures of the lichens after exposure to 10% KOH (potassium hydroxide), lactophenol (cotton blue), Melzer's reagent, chlorine, and viewed under UV light. The chemical compositions of the lichen were examined using thin layer chromatography (TLC) (Orange et al., 2001) at the Biochemical Laboratory of the Federal University of Pernambuco.

4 RESULTS AND DISCUSSION

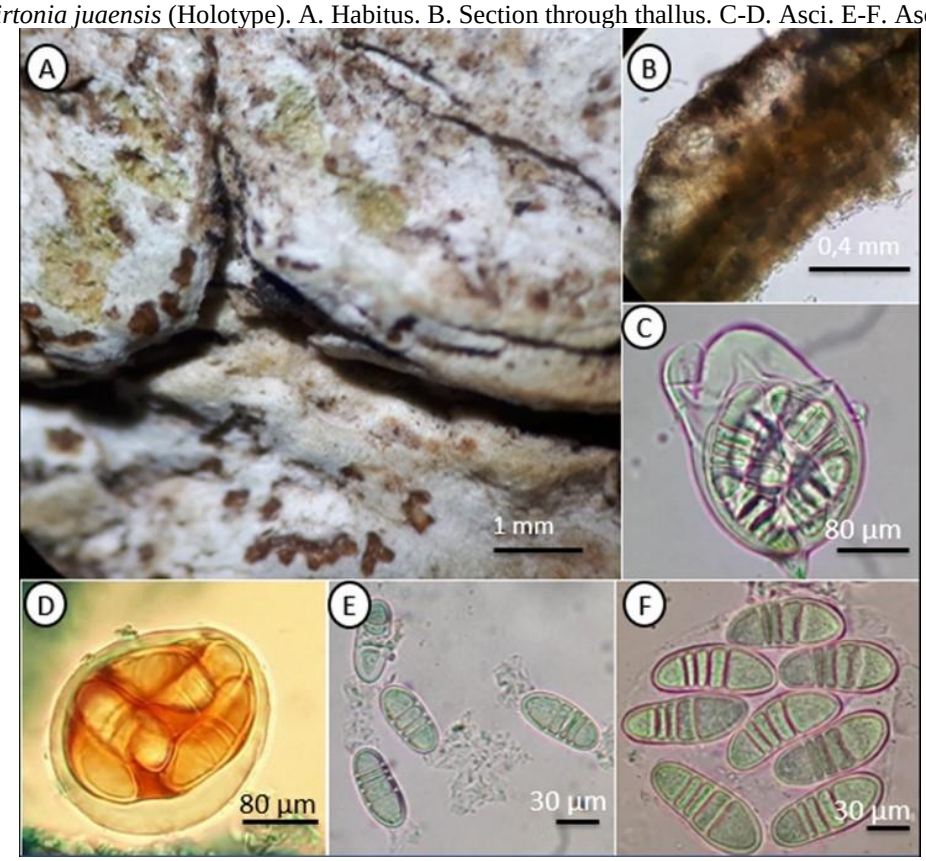
Taxonomy
Mycobank
MB#830939
Stirtonia juaensis T.E.F. Silva, M. Cáceres, Aptroot & N.S. Vitória sp. nov. Fig. 1
TYPE: BRAZIL, BAHIA: Paulo Afonso, Juá, Sítio Parença, alt. 704 m, 9º 24'03"S e 38º 12'54"O, in branch of P. moniliformis 24/01/2018. Col. T.E.F. Silva, voucher 0138 (holotype LABORATÓRIO DE MICOLOGIA UNEB-DEDC VIII).
Description: Thallus corticolous, crustose, whitish; ascigerous zones punctiform, up to 0.6–1 mm in diameter and 0.2–0.5 mm tall; light brown, with crystals, without algal cells. Interascal tissue not observed, amyloid, IKI+ blue; asci not abundant, globose to ovoid, not visible at the surface, with 8 ascospores, 70–80 × 50–62.5 µm. Ascospores hyaline, 30–40 × (10–)12.5–17.5 µm, with 3–4-septa, rarely with 2, upper cell oblong-ellipsoidal, notably enlarged (10–)12.5–15 × 10–12.5 µm; septa 1–1.5 µm thick. Corticoid; photobiont Trentepohlia. Pycnidia not observed.
Etymology: Latin, juaensis, referring to the locality where it was encountered.
Chemistry: C–, K –, KC –, UV–, TLC: unidentified substance.

Discussion: The new Stirtonia species is characterized by having a white thallus, punctiform ascigerous zones, 8 ascospores per ascus with 3–4 septa with an enlarged upper cell. Its morphology is very similar to S. alba Makhija & Patw, although S. alba has lirelliform ascigerous zones, slightly larger ascospores (38–60 × 14–22 µm), 4–5-septate, and contains perlatolic acid (Aptroot 2009). Other comparable species include: S. ghattensis Sumesh, which differs by having lirellate ascigerous zones, asci with 4–6 spores, 5-septate, and thallus UV+ white; and S. santessonii Makhija & Patw, which has a pale brown thallus, and asci with 8 ellipsoidal ascospores (35–50 × 13–18 µm), and 7 septa (Aptroot, 2009; Dudani et al., 2018) (Table 1).

Table 1. Comparisons of *Stirtonia* species showing ascospores with an enlarged upper cell.

Species	UV	Ascomata	N° of spores per ascus	Ascus size (µm)	Spores size (µm)	N° of septa	Chemistry
S. alba	-	Lireliform	8	-	38–60 × 14–22	4–5	Perlatolic acid
S. byssoidea	+	Lireliform	8	-	22–28 × 10–12	5–6	Perlatolic acid
S. ghattensis	+	Lireliform	4–6	67,37–112,29 × 51,23–70,75	58–63,6 × 22,1–26,7	5 (–7)	-
S. juaensis	-	Punctiform	8	70–80 × 50–62,5	30–40 × (10–) 12,5–17,5	(2–) 3–4	Unknown compound
S. microspora	-	Punctiform	8	30–39 × 25–33	23–27,5 × 9–10,5	4	-
S. santessoni	+	Lireliform	8	-	35–50 × 13–18	7	-

Figure 1. *Stirtonia juaensis* (Holotype). A. Habitus. B. Section through thallus. C-D. Asci. E-F. Ascospores.



Taxonomy

Mycobank

MB#830940

Cryptothecia paramacrocephala T.E.F. Silva, M. Cáceres, Aptroot & N.S. Vitória sp. nov. Fig. 2

TYPE: BRAZIL, BAHIA: Paulo Afonso, Juá, Sítio Parença, alt. 704 m, 9º 24’03"S e 38º 12’54” O, in branch of S. tuberosa 24/01/2018. Col. T.E.F. Silva, voucher 0139 (holotype LABORATÓRIO DE MICOLOGIA UNEB-DEDC VIII).

Description: Thallus crustose, whitish, fissured, corticolous, K+ yellow, greenish yellow; alga trentepohlioid. Ascigerous zones rounded and flattened, up to 0.7–1.8 mm in diameter and 360–400 µm tall, light brown, with crystals, without algae. Asci abundant, clavate to ovoid, with 8 ascospores, 60–120 × 45–55 µm, amyloid, IKI+ blue. Ascospores hyaline, muriform, with septum 5–7 × 1–3 µm, with an enlarged upper cell constituting 1/3 to 1/4 of the ascospore (macrocephalic), oblong-ellipsoidal, 30–42.5 × 15–17.5 µm. Pycnidia not detected.

Etymology: Latin, paramacrocephala, a reference to its similarity to Cryptothecia macrocephala

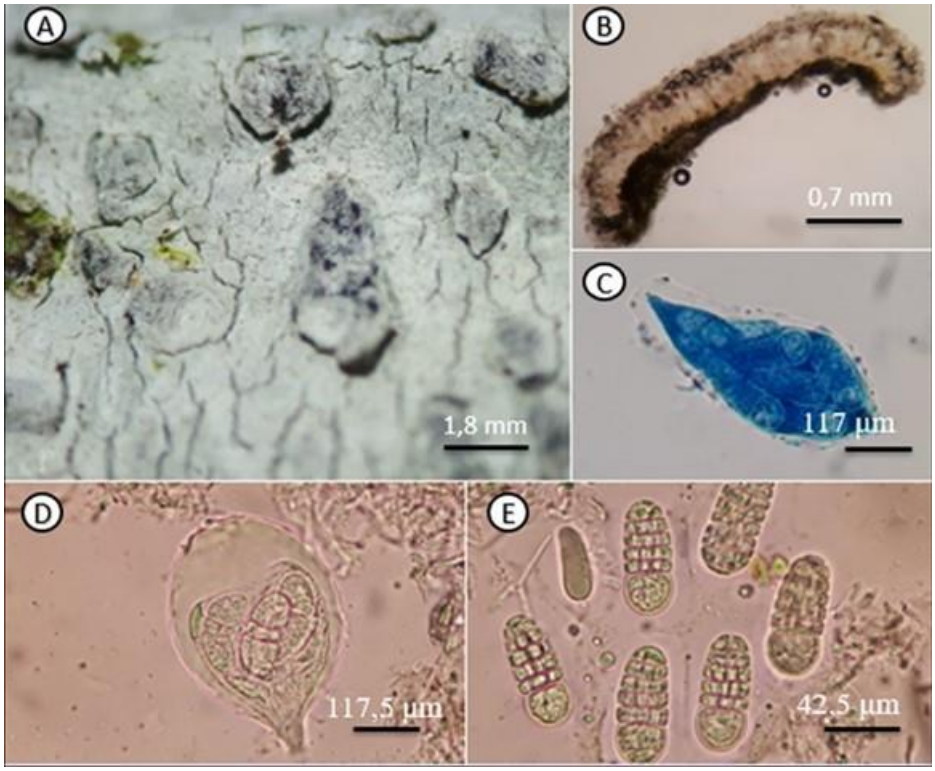
Chemistry: C–, K+ yllow, KC –, UV–, TLC: unidentified substance.

Discussion: The morphology of Cryptothecia paramacrocephala is very similar to that of Cryptothecia submacrocephala Seavey & J. Seavey, and to Cryptothecia macrocephala E.L. Lima, M. Cáceres & Aptroot, although C. macrocephala has a thallus surrounded by a brown line of the prothallus, ascigerous zones 0.3–0.6 mm in diameter, 7–9 transversally septate ascospores, and is K-negative (Menezes et al. 2013). Cryptothecia submacrocephala differs by having a greenish grey thallus, ascigerous zones 0.4–1.0 mm in diameter, small ascospores 50–55 µm, and is K-negative (Seavay et al. 2017) (Table 2).

Table 2. Comparisons of *Cryptothecia* species showing ascospores with an enlarged upper cell.

Taxa	UV	Ascoma size (mm)	N° of spores per ascus	Ascus size (µm)	Spore size (µm)	N° of septa	Chemestry
C. macrocephala	-	0,3–0,6	8	90–120 × 40–60	45–50 × 14–19	7–9 × 1–3	Psoromic acid
C. paramacrocephala	-	0,7–1,8	8	62,5–117,5 × 45–52,5	30–42,5 × 15–17,5	5–7 × 1–3	Not identified
C. submacrocephala	-	0,4– 1,0	8	45–55 × 50–55	25–29 × 10–11	5 (–7)	Psoromic acid

Figure 2. *Cryptothecia paramacrocephala* (Holotype). A. Habit. B. Section through the thallus. C-D. Asci. E. Ascospores.



5 CONCLUSIONS

This study adds two new species of Ascomycota fungi to the Brazilian and Caatinga mycobiota. Our finds increase the known Brazilian mycobiota, highlighting the need to explore new areas in the Caatinga region, where fungal diversity remains underestimated.

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