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SCIENTIFIC NOTE

CONTRIBUTION TO THE KNOWLEDGE OF ASCOMYCETOUS GENERA *Biscogniauxia* (GRAPHOSTROMATACEAE), *Kretzschmaria* AND *Stilbohypoxylon* (XYLARIACEAE) FROM BAHIA, BRAZIL

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The xylariaceous fungi are among the most diverse group of fungi found on the Tropics, some genera reaching over 100 known species and colonizing different hosts and substrates. Although most of them are saprobes, some species present pathogenic behaviour, causing from root rot to canker. The present study brings the first report of *Biscogniauxia citriformis* var. *macrospora*, *B. uniapiculata*(Graphostromataceae) and *Stilbohypoxylon samuelsii* (Xylariaceae) from Brazil and *Kretzschmaria micropus* (Xylariaceae) from Bahia, contributing to the knowledge of the xylariaceous fungi and Brazilian Funga.

Key words: Fungal diversity, taxonomy, Xylariales.

Contribuição ao conhecimento dos gêneros de ascomicetos *Biscogniauxia* (Graphostromataceae), *Kretzschmaria* e *Stilbohypoxylon* (Xylariaceae) na Bahia, Brasil. Os fungos xylariáceos estão entre os grupos mais diversos encontrados nos Trópicos, alguns gêneros com mais de 100 espécies conhecidas, colonizando diferentes hospedeiros e substratos. Apesar de a maioria ser sapróbia, algumas espécies são conhecidas por demonstrar comportamento patogênico, causando desde podridão radicular a cancro. O presente estudo apresenta o primeiro relato de *Biscogniauxia citriformis* var. *macrospora*, *B. uniapiculata* (Graphostromataceae) e *Stilbohypoxylon samuelsii* (Xylariaceae) para o Brasil e *Kretzschmaria micropus* (Xylariaceae) para a Bahia, contribuindo com o conhecimento dos fungos xilariáceos e a Funga Brasileira.

Palavras-chave: Diversidade fúngica, taxonomia, Xylariales.

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The Xylariaceae was recently re-organized based on a polyphasic approach (Wendt et al., 2018), relocating some typical xylariaceous genera such as *Biscogniauxia* Kuntze and *Camillea* Fries to Graphostromataceae, a, previously, monotypic family represented by the genus *Graphostroma* Piroz. Despite obvious distinct morphological aspects of stromata, asci and ascospores among the genera cited above, the emendation proposed by Wend *et al*. (2018) has demonstrated their phylogenetic affinity.

The name *Biscogniauxia* was established by Kuntze (1891), since *Nummularia* was a name already in use. According to Ju et al. (1998), this genus is characterized by bipartite, usually carbonaceous stromata, no pigments released in KOH, ascospores generally brown to dark brown, in most cases with a conspicuous germ slit, asci short stipitate with iodine-positive apical ring broader than higher.

Some species of *Biscogniauxia* are well-known pathogens of Beech trees: *Biscogniauxia mediterranea* (De Not.) Kuntze and *B. nummularia* (Bull.) Kuntze in Europe (Nugent et al., 2005; Patejuk et al., 2021), *B. rosacearum* in grapevines in Iran (Balmani et al., 2021), *B. nothofagi* Whalley, L1«ssøe & Kile in Beech trees in Australia and *B. uniapiculata* (Penz. & Sacc.) Whalley & Lassøe in *Eucalyptus camaldulensis* Dehnh. (Nugent et al., 2005). Although most xylariaceous fungi usually present a saprophyte lifestyle, Ju et al. (1998) stated that *Biscogniauxia* species are probably pathogens and facultative saprophytes, since it is also found on dead material.

Among the remnant genera of the Xylariaceae *sensu stricto* known to occur in Brazil, *Stilbohypoxylon* Henn. and *Kretzschmaria* Fr. are interesting genera still poorly documented in Brazil, with 3 and 13 species reported, respectively. *Kretzschmaria deusta* (Hoffm.) P.M.D. Martin and *K. zonata* (Lev.) P.M.D. Martin, are known to cause disease in several plant species, including in Brazil (Cristini et al., 2022; Alfenas et al.; Cordin et al., 2021), while no reports of this type of behaviour are known from *Stilbohypoxylon*.

According to Rogers and Ju (1997) Sir et al. (2013), *Stilbohypoxylon* is characterized by spherical, gregarious stromata, with or without conical to acicular synnematal remnants borne on mature stromata, surface overlain with yellow, greenish-yellow or ochraceous scales at an early age. The ascospores are brown to dark brown, varying from 20 to 40 µm in length, with straight to sigmoid or spiral germ slit.

Kretzschmaria presents more variable morphology, with stromata clavate, turbinate, obconical, discoid to effused-pulvinate, densely aggregated, some species bearing umboes or spines on top, short to long stipitate, internal tissue usually darkening and disintegrating with age. Ascospores brown to blackish brown, ellipsoid to fusoid, inequilateral, reaching almost 100 µm, with straight to sigmoid germ slit. (Rogers and Ju, 1998).

The lack of studies focused on this group leaves a gap in how truly diverse they are in Brazil, a huge country known for high levels of biodiversity. Hence, the present study aims to contribute to the knowledge of xylariaceous fungi from Atlantic Rainforest protected areas in Bahia, Brazil.

Stromata of *Biscogniauxia* were found on dead trees of *Psidium guajava* L. (common guava) in the Reserva Particular do Patrimonio Natural (RPPN) Serra do Teimoso, Jussari County, (15°08’00.0"S 39°31’00.0"W); specimens of *Stilbohypoxylon samuelsii* were collected in RPPN Serra do Teimoso, Jussari (15°09’39" S 39°31’26" W) in an excursion back in 2013 by Jadergudson Pereira and *Kretzschmaria micropus* in RPPN Espinita, Igrapiúna County (13°52’31" S 39°09’09" W) and Parque Estadual Serra do Conduru, Uruçuca County (14°26’53" S 39°05’36" W).

After rehydration of dry material in distilled water, stromatal aspects such as shape, color, dimensions (length × width), consistency, perithecia (diameter when spherical or so; height × broad when tubular or obovoid); ascal dimensions (full length, spore part and stipe, if not degraded) and amyloid reaction (I^+) of apical plug in lugol, ascospore color, dimensions, shape and germ slit were measured with Piximètre Piximetre 5.10 (http://ach.log.free.fr/Piximetre/). Photomacrographs were taken with a smartphone directly into the eyepiece of a stereomicroscope Leica EZ4 for higher magnifications. Photomicrographs were taken as above on a Leica DM500 (100x) equipped with a camera ICC50 HD connected to a laptop and performed with Leica Application Suite EZ 3.4. Specimens were treated and deposited at the TFB/UESC Fungarium. Attempts to obtain the asexual stage were unsuccessful.

Biscogniauxia citriformis (Whalley, Hammelev & Talig.) Van der Gucht & Whalley var. *macrospora* Van der Gucht & Whalley, Mycol. Res. 96(10): 896 (1992) (Figure 1).

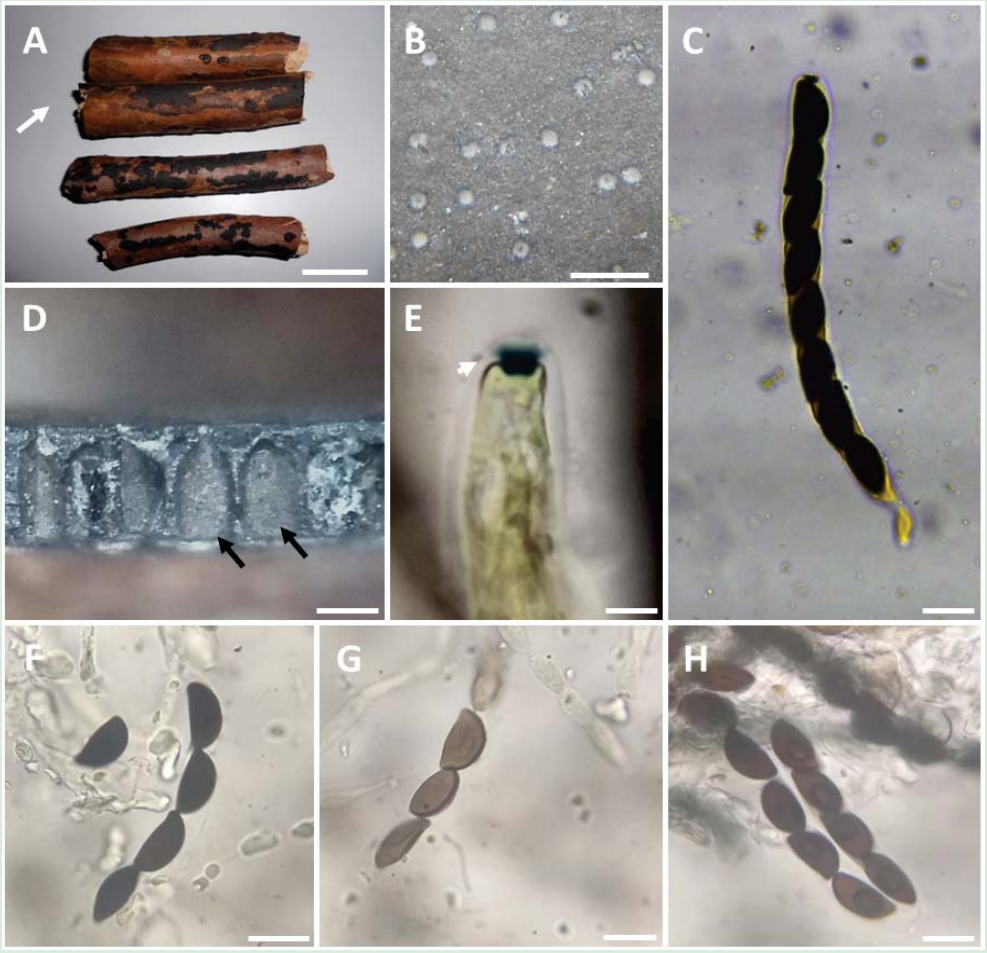

Figure 1 – *Biscogniauxia citriformis* var. *macrospora*. A – Stromata of *B. citriformis* (arrow) and *B. uniapiculata* (below) sharing the same host; B – Stromatal surface in close-up evidencing ostioles covered with white substance; C – Asci with mature ascospores; D – Stroma in vertical section showing perithecia (arrows); E – Immature ascus in Lugol with I^+ apical apparatus (arrow); F-H – Ascospores. Bars: A= 3 cm; B= 0.5 mm; C= 10 µm; D= 0.5 mm; E= µm; F-H= 20 µm.

Stromata: applanate, orbicular to irregularly ellipsoid-lobed, surface black, carbonaceous tissue between and beneath perithecia, ostioles punctate, evenly distributed, plugged with whitish substance; perithecia short-cylindrical to tubular, 0.5-0.7 × 0.3-0.5 mm; asci cylindrical, with 8 overlapping uniseriate ascospores, short-stipitate, the spore-bearing parts 121–145 × 10-11 µm, stipes 16–24 µm, apical apparatus 2–3 × 3.5–5 µm, bluing in Lugol, top larger than the base. Ascospores dark brown to blackish brown, ellipsoid-inequilateral with unequally pinched ends, (17-) 18-20 (-21) × 8-10 µm, inconspicuous straight germ slit spore-length or nearly so, on the less convex side.

Comments: the stromatal characters of our specimen conform with *Biscogniauxia citriformis* var. *macrospora* of Fournier et al. (2017), although the ascus stipe and ascospores are slightly longer in the specimen presented herein. These variations are acceptable, since when comparing the ascus spore-bearing parts and short stipes, it matches well with their description of this species, as well as the morphology of ascospores, with the pinched ends observed. The specimens were curiously found to share the same host with *B. uniapiculata*.

Biscogniauxia uniapiculata (Penz. & Sacc.) Whalley & Læssøe, Mycol. Res. 94(2): 239 (1990) (Figure 2).

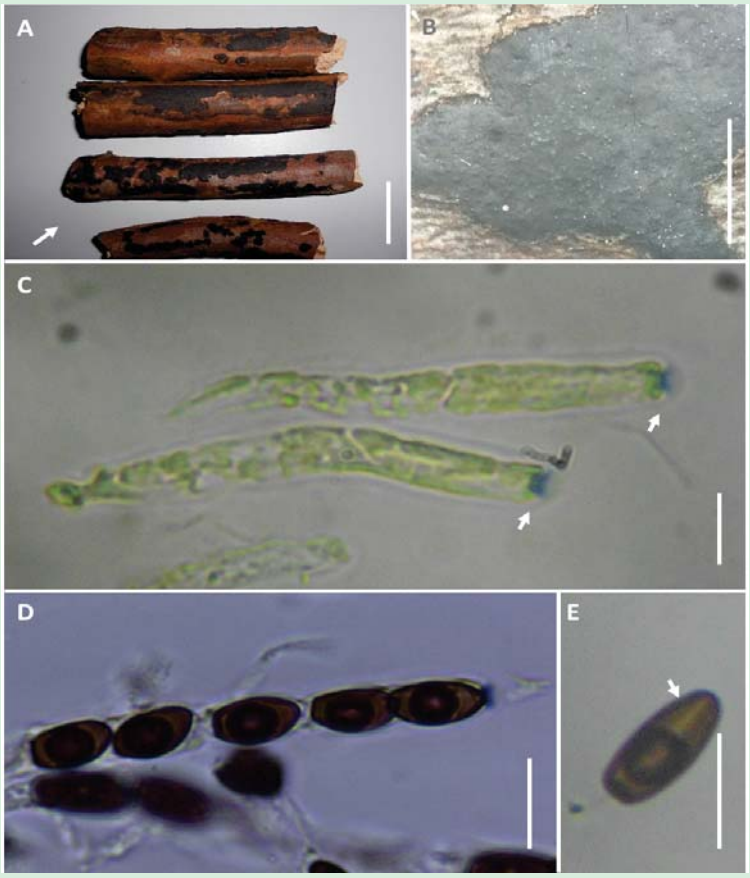

Figure 2 – *Biscogniauxia uniapiculata*. A – *B. citriformis* var. *macrospora* and *B. uniapiculata* (arrow); B. Upper view of stroma of *B. uniapiculata* showing surface and ostioles (greyish dots); C. Young asci with I^+ apical apparatus (arrows) in Lugol; D. Ascospores with a blunted end indicating loss of appendage; E – Ascospore with a straight germ slit (arrow). Bars: A= 3 cm; B= 5 mm; C= 6 µm; D, E= 10 µm.

Stromata: applanate, irregular to ellipsoid-lobed, sometimes coalescing into a longer stroma, (1.2–) 2–5.7 × (0.5–) 0.6–1.0 cm, 0.4–0.5 mm thick, outer dehiscing layer dark brown to black, carbonaceous immediately under the surface and between perithecia; tissue between perithecia inconspicuous, ostioles mostly inconspicuous, at the same level or lower than stromatal surface, with openings punctuate, some overlain with white to greyish substance. Perithecia obovoid to oblong, (0.3–) 0.4–0.5 × 0.15–0.25 mm. Asci cylindrical, uniseriate, 80–97 total length, 5–6 µm broad, the spore bearing-part 74–82 µm long, stipes 6–15 µm long, apical apparatus, discoid to slight trapezoid, 1–2 × 2–2.5 µm, bluing in Lugol; ascospores brown, ellipsoid, nearly equilateral, with one end broadly rounded and the other truncate bearing a small appendage, mostly collapsed or absent, (8–) 9–10 × 4–5 µm without the appendage, germ slit almost spore-length on either side.

Comments: *Biscogniauxia uniapiculata* is an interesting species, presenting appendaged ascospores or a truncate end evidencing the loss of the appendage (Ju et al., 1998). Fournier et al. (2017) collected this species in Guadeloupe and Martinique. This is not the first report of *B. uniapiculata* on *Psidium guajava* or a host of the Family Myrtaceae, this species is well-known in East Asia and Hawaii (Rogers & Ju, 2012). It was reported by Nugent et al. (2005) as the causative agent of Canker on *Eucalyptus*, although we are not certain if it is pathogenic to *P. guajava* and further studies should be done to prove its pathogenicity to this host.

Stilbohypoxylon samuelsii J.D. Rogers & Y.M. Ju, Mycol. Res. **101**(2): 137 (1997) (Figure 3).

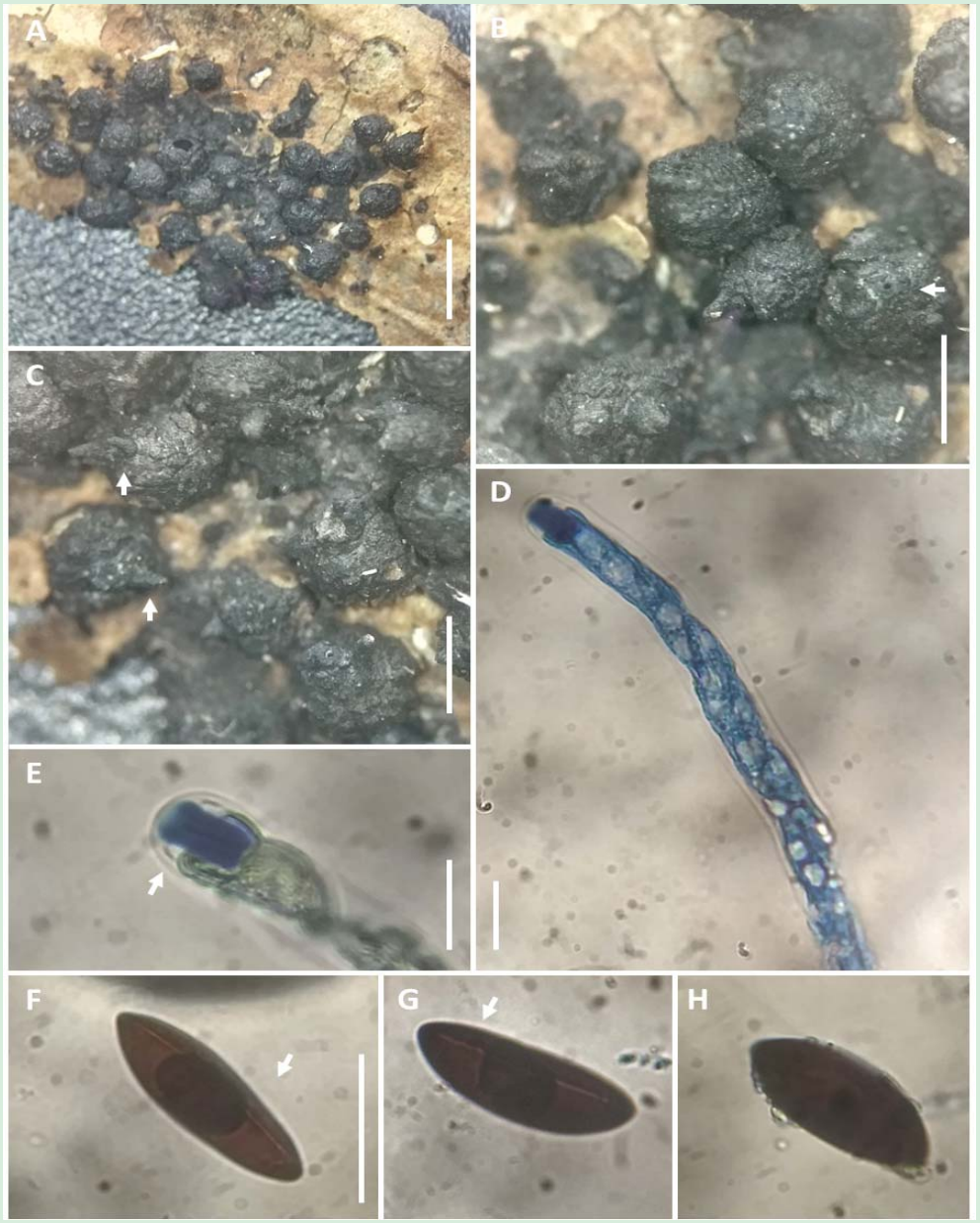

Figure 3 – *Stilbohypoxylon samuelsii*. A. Stromata on the substrate; B. Stromata in close-up showing irregular surface and ostioles (arrow); C. Stromata in close-up showing synnematal remnants (arrows); D. Ascus with immature spores in lugol and cotton blue; E. Ascus in Lugol with apical apparatus I^+ (arrow); F-H. Ascospores (arrows= germ slit). Scale Bars: F= A= 1.5 mm; B, C= 1 mm; D, E= 10 µm; 30 µm.

Stromata: perithecioid, subglobose to spherical, gregarious, 0.5–1.3 mm diam., usually bearing, or not, a conical to acicular synnematal remnant; surface black, rugulose, sometimes overlain with ochraceous scales becoming black in mature stromata, interior black, texture hard, carbonaceous; perithecia: spherical, 0.4–1 mm diam; ostioles: papillate, some encircled with a vaguely flattened area. Asci seldomly 210 to 300 µm in total length × 12–15 µm broad, with apical ring bluing in Lugol, cylindrical, 9.7–12.5 µm high × 5.1–6.3 µm broad. Ascospores: dark brown, unicellular, ellipsoid-inequilateral, with narrowly

rounded ends, (31.9–) 32.5–38.3 (–39) × (10–) 10.4–12.7 (–14.7) µm, with straight germ slit nearly spore-length, surface smooth.

Material examined: Brazil. Bahia, Jussari: RPPN Serra do Teimoso, wood, 13.III.2013, *leg.* Jad. Pereira, Det. C. Silva & Jad. Pereira.

Comments: *Stilbohypoxylon samuelsii* was unknown from Brazil until now. The ascospores measuring up to 30–40 µm in length with a straight germ slit slightly less than spore length are very characteristic of this species. Petrini (2004) stated that *S. samuelsii* is a synonym of *S. immundum* (Berk. & M.A. Curtis) L.E. Petrini, although both are distinct species on Index Fungorum and Mycobank databases. Despite that, they are, indeed, quite similar, as shown in table 1 below.

As seen in Table 1, both species indeed share similar stromatal features and overlapping ascospore and ascal plug dimensions, thus, probably Petrini (2004) is correct about placing *S. samuelsii* as a synonym of *S. immundum*, which could be confirmed with phylogenetic analysis. Still, neither of these names was mentioned from Brazil before.

Table 1. Comparison of *Stilbohypoxylon samuelsii* and *S. immundum*

	Stromata	Ascospore dimensions	Ascal plug
S. samuelsii (present study)	Subglobose to spherical, bearing a conical to acicular synnematal remnants	(31.9-) 32.5-38.3 (-39) × (10-10.4-12.7 (-14.7) µm	Cylindrical, 9.7-12.5 µm high × 5.1-6.3 µm broad
S. samuelsii (Rogers & Ju, 1997)	Spherical, bearing a conical to acicular synnematal remnants	(27.5-) 30-36 (-40) × 8.5-11 (-13) µm	Somewhat coffin-shaped, 8-13 µm high × 5.5-7 µm broad
S. immundum (Petrini, 2004)	globose, subglobose, obovate to cupulate, cylindrical synnematal growing out from the upper part	(27.0-) 32.0 ± 3 (-39.0) × (8.5-)11 ± 1.5 (-14.0)	Cylindrical, 6-11 µm high × 4-7 µm broad.

Kretzschmaria micropus (Fr.) Sacc., Sylloge Fungorum (Abellini) 1: 389 (1882) (Figure 4).

Stromata: obconical to convex, surface blackish brown, usually containing one or two perithecia per stroma, 2.5–4 mm diam, densely aggregate, bearing at least one spine on top, attached to the substrate by a narrow connective, carbonaceous immediately beneath the surface, tissue between and beneath perithecia coriaceous to woody, brownish to black, disintegrating with age; ostioles: conical; perithecia: spherical to obovoid, 0.5–1.5 × 1.2–1.8 mm; asci: fragmented; ascospores brown to dark brown, ellipsoid, inequilateral, broadly round ends, 35–36.5 × 8–10 µm, straight germ slit less than spore length, surface smooth.

Material examined: Brasil. Bahia, Igrapiúna; RPPN Espinita, on bark, coll. C. Silva, det. C. Silva & Jad. Pereira, 17.II.2023; Serra do Conduru State Park, on rotten branches, coll. and det. C. Silva 01.IV.2023.

Comments: until the present study was finished, only one specimen identified as *Kretzschmaria micropus* was found to occur in Bahia. This specimen is deposited at the CEPEC-Fungi collection (CEPEC-Fungi 2405), although it was not studied, since no access to this collection was granted, hence there is no confirmation if it is the mentioned species. *Kretzschmaria micropus* is known to occur in Amazonas, Pernambuco, São Paulo and Rio Grande do Sul under the name *K. spinifera*, which is but a synonym of *K. micropus* (Dennis, 1957; Rogers et al. 1998).

Table 2, below, presents *Biscogniauxia*, *Kretzschmaria* and *Stilbohypoxylon* species known to occur in Brazil, and clearly, the latter is indeed poorly documented since the 3 only species known to occur within the country were reported from Bahia, Santa Catarina, Pernambuco, Rio Grande do Sul and São Paulo (Flora e Funga do Brasil; specieslink, 2024), with *S. quisquiliarum* the most common.

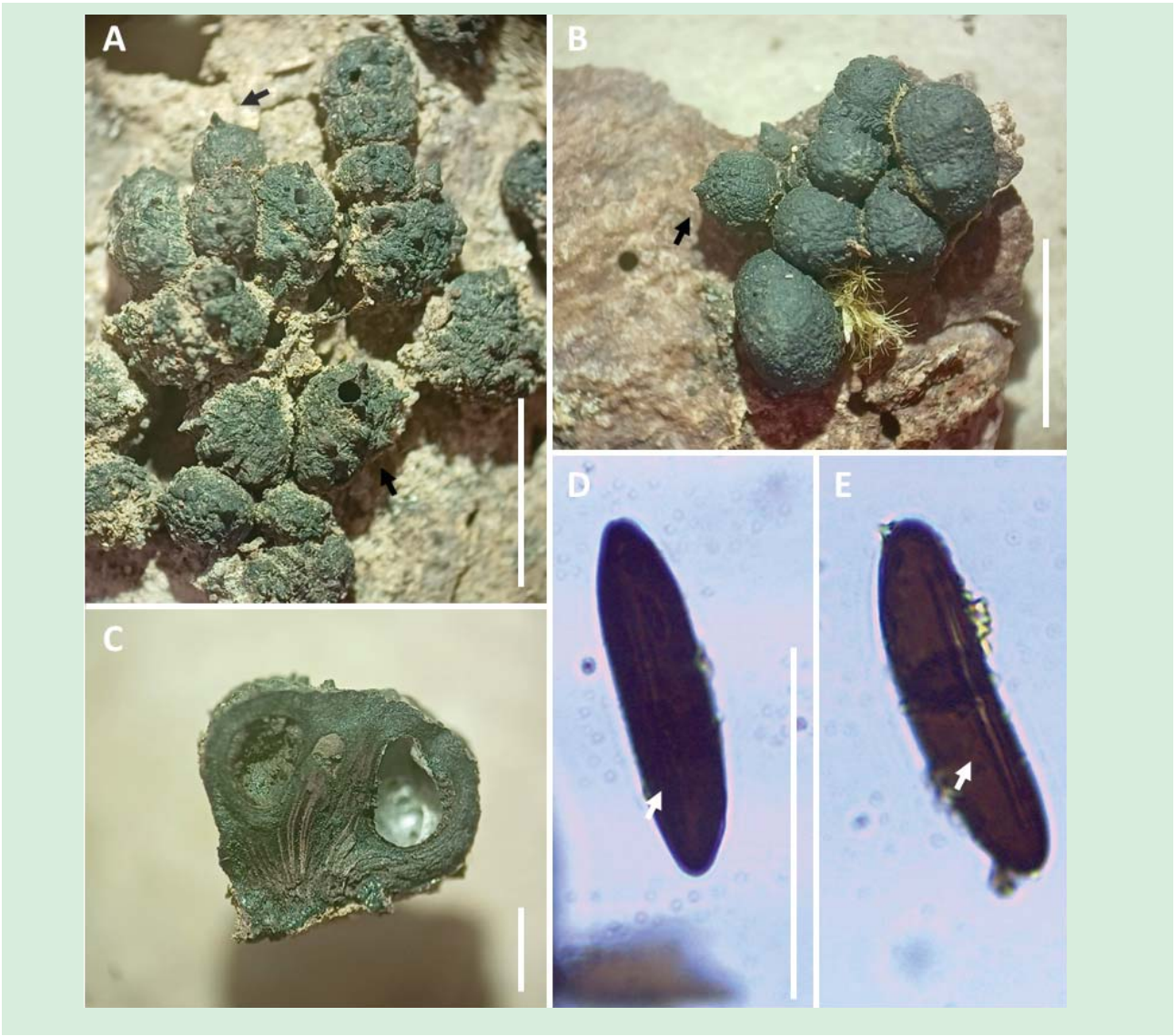

Figure 4 – *Kretzschmaria micropus*. A, B. Stromata with conspicuous obtuse projections (arrows); C. Vertical section of stroma evidencing perithecia and fibrous interior; D. Ascospore with faint germ slit (arrow); E. Ascospore with visible germ slit. Scale: A, B= 5 mm; C= 1.5 mm; D= 35 µm.

It is not certain if both or one of the species of *Biscogniauxia* presented herein caused the decay of the Guava trees or just colonized them after that, although the first possibility would not be a surprise, since *B. uniapiculata*, as mentioned previously, is already known as a causative agent of canker in *Eucalyptus*, which is from the same family of *P. guajava*. No mention of *B. citriformis* var. *macrospora* causing disease has been found so far. Studies focusing on the pathogenic behaviour of xylariaceous fungi are not common in Brazil, with *Rosellinia*/*Dematophora* the most frequently mentioned (Oliveira et al., 2008; Kleina et al., 2018; Araújo et al., 2023). *Biscogniauxia citriformis* var. *macrospora* and *B. uniapiculata* were previously unknown from Brazil, which emphasizes the need for more studies on the xylariaceous fungi in the country.

Kretzschmaria seemingly has a wide distribution when compared to *Stilbohypoxylon*, being reported from 17 states, while the latter is known only from 5 states, which does not exactly mean the latter is rarer, but probably poorly known since there are just a few

Table 2. Species of *Biscogniauxia*, *Kretzschmaria* and *Stilbohypoxylon* known to occur in Brazil (according to Flora e Funga do Brazil and specieslink, 2024). AC= Acre, Am= Amazonas, BA= Bahia, CE= Ceará, ES= Espírito Santo, MT= Mato Grosso, PA= Pará, PB= Paraíba, PE= Pernambuco, PR= Paraná, RJ= Rio de Janeiro, RO= Rondônia, RS= Rio Grande do Sul, SC= Santa Catarina, SP= São Paulo

Species	Known occurrence
B. capnodes (Berk.) Y.M. Ju & J.D. Rogers	AM, BA, PE, MG, RS, SC
B. capnodes var. *capnodes* (Berk.) Y.M. Ju & J.D. Rogers	MG, RS
B. capnodes var. *rumpens* (Cooke) Y.M. Ju & J.D. Rogers	RS
B. capnodes var. *theissenii* (Syd. & P.Syd.) Y.-M. Ju & J.D. Rogers	RS
B. cinereolilacina (J.H. Miller) Pouzar	SP
B. communapertura Y.-M. Ju & J.D. Rogers	RS
B. divergens (Theiss.) Whalley & Laessøe	RS
B. mediterranea (De Not.) Kuntze	PE, RS
B. nummularia (Bull.) Kuntze	RS
B. repanda (Fr.) Kuntze	A M
B. sinuosa (Theiss.) Y.-M. Ju & J.D. Rogers	RS
B. waitpela Van der Gucht	BA
K. aspinifera Jad. Pereira, J.D. Rogers & J.L. Bezerra	BA, PE
K. berkeleyana (Cooke) Berl. & Voglino	PE, RS
K. cetrarioides (Welw.) Sacc.	AC, AM, BA, CE, MT, PB, PE, RJ, RO, RS, SP
K. clavus (Fr.) Sacc.	AC, AM, BA, ES, MT, PA, PB, PE, PR, RJ, RO, RS, SP,
K. curvirima J.D. Rogers & Y.M. Ju	AM, PE
K. deusta (Hoffm.) P.M.D. Martin	SC
K. guyanensis J.D. Rogers & Y.M. Ju	A M
K. lucidula (Mont.) Dennis	AM, RS
K. micropus (Fr.) Saccardo	AM, PA, PB, PE, RS, SP, BA (this study)
K. neocaledonica (Harr. & Pat.) J.D. Rogers & Y.M. Ju	PE
K. pavimentosa (Ces.) P.M.D. Martin	AM, BA, PR, RO
K. sandvicensis (Reichardt) J.D. Rogers & Y.M. Ju	PR
K. sigmoidirima A.I. Hladki & A.I. Romero	SC
S. moelleri Henn.	SC
S. samuelsii	Brazil (this study)
S. quisquiliarum (Mont.) J.D. Rogers & Y.M. Ju	BA, CE, PE, SP, RS
S. quisquiliarum var. *quisquiliarum* (Mont.) J.D. Rogers & Y.M. Ju	BA, CE
S. quisquiliarum var. *microsporum* Jad. Pereira, J.D. Rogers & J.L. Bezerra	BA

mycologists dedicated to the Xylariaceae in Brazil. Pereira *et al*. (2009) described *K. aspinifera* from Bahia and, regardless of the nomenclatural similarity with the name *K. spinifera* (= *K. micropus*), the morphology of both species is completely different, with the former presenting stipitate stromata lacking spines or projections on the surface (thus [a]*spinifera*) and the latter presenting stromata with conspicuous conical projections on the surface, non-stipitate, more attached to the substrate, usually with a narrow basal connective.

Serra do Conduru Park and RPPNs Serra do Teimoso and Espinita are interesting areas of the Atlantic Forest for exploring fungal diversity and should be given more attention, since, they had shown quite considerable diversity of fungal species collected on expeditions that resulted in the present study and others yet to be published. Despite Ascomycota being the

main group studied by researchers from TFB Fungarium and Phytopathology and Nematology Laboratory at UESC, Basidiomycota has shown to be diverse as well, and both groups deserve more attention within these areas, highlighting the importance of the RPPNs and other protected areas in the conservation of diversity.

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