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A new occurrence of *Gyrodontium sacchari* (Spreng.) Hjortstam Pat. (Boletales, Coniophoraceae) expands the geographic distribution of the genus in Brazil

Lucas Leonardo-Silva^{1*}, Francisco J. Simões Calaça¹, Geovane Pereira-Silva¹, Carlos de Melo e Silva-Neto², Solange Xavier-Santos¹

1 Laboratório de Micologia Básica, Aplicada e Divulgação Científica – FungiLab, Universidade Estadual de Goiás, Anápolis, GO, Brazil • LL-S: lucasleo.bio@gmail.com
https://orcid.org/0000-0001-6298-4293 • FJSC: calacafjs@gmail.com
https://orcid.org/ 0000-0002-0623-8306 • GP-S: geovanep1995@gmail.com
https://orcid.org/0000-0001-9182-0562 • CMS-N: carloskoa@gmail.

com 🕑 https://orcid.org/0000-0001-8624-3836 • SX-S: solange.xavier@ueg.br 🕑 https://orcid.org/0000-0002-3397-0885

2 Instituto Federal de Goiás, Campus Cidade de Goiás, Goiás, GO, Brazil

* Corresponding author

Abstract. We report the first occurrence of the genus *Gyrodontium* Pat. from the Brazilian Cerrado based on our finding of *G. sacchari* (Spreng.) Hjortstam. This also represents the first record of the family Coniophoraceae from the Midwest Region of central Brazil. We identified the sample by morphological and phylogenetic analyses (ITS gene), and a voucher was deposited in the fungarium of the Universidade Estadual de Goiás (HUEG-Fungi). This record adds to the knowledge of the Cerrado's funga by providing new phylogenetic and biogeographic data for *G. sacchari* from a Neotropical region.

Keywords. Basidiomycota, Brazilian savanna, corticioid fungi, taxonomy, phylogeny

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Introduction

Gyrodontium Pat. is a small genus in Coniophoraceae (Boletales, Basidiomycota) with a worldwide distribution and only three currently accepted species: *G. arizonicum* (Ginns) Zmitr., Kalinovskaya & Myasnikov, *G. eberhardtii* Pat., and *G. sacchari* (Spreng.) Hjortstam (He et al. 2019). *Gyrodontium* was proposed by Patouillard (1900) to accommodate *Hydnum henningsii* Bres. (Hydnaceae) from Cameroon in Central Africa. Linnaeus (1753) proposed the genus *Hydnum* L. for *H. repandum* L. (the type species), which has a spinose hymenophore. *Hydnum* is, currently, a *nomen conservandum* which was also sanctioned by Elias Magnus Fries. Species of *Hydnum* have their spores produced in structures resembling teeth or spines (spinose hymenophores) rather than in the most common lamellate hymenium of Agaricomycetes. Probably due to this characteristic, when Sprengel described *Hydnum sacchari* Spreng., he included *Odontia* Pers. as a subgenus of *Hydnum*, which led to numerous synonyms and misidentifications involving *Hydnum*, *Odontia* and, consequently, *Tomentella* Pers. ex Pat. (Sprengel 1820). Hundreds of names in *Hydnum* have been allocated to other genera in recent years due to advances in molecular phylogenetics that have clarified cryptic species with spinose hymenophores and misclassified in this genus (MycoBank 2022).

Hydnum sacchari Spreng. was described from material collected on the decaying plant debris of Saccharum officinarum L. (Poaceae) from Guadeloupe in the Caribbean (Sprengel 1820). Later, based on morphological characteristics, Hjortstam (1995) synonymized

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H. henningsii, *H. sacchari*, and other names in *Hyd-num* with *Gyrodontium sacchari* (Spreng.) Hjortstam, now typified as the type species. *Gyrodontium sacchari* is a cosmopolitan species, with hotspots of occurrence mainly in pantropical areas of the world; it has been reported from all continents except Antarctica (Carlier et al. 2004; Bernicchia and Gorjón 2010; Robledo et al. 2014; He and Zhao 2022).

Although present in some regions of Asia (Bao et al. 2006; Park et al. 2015; Karun and Sridhar 2016), *G. sacchari* occurs mainly in tropical regions (Carlier et al. 2004) and shows a broad distribution in the Americas, where is it known from Argentina, Belize, Brazil, Costa Rica, Cuba, Ecuador, French Guiana, Guadeloupe, Mexico, Panama, and the USA (Hjortstam 1995; Carlier et al. 2004; Wright and Wright 2005; Læssøe and Petersen 2008; Valenzuela et al. 2012; Robledo et al. 2014; He et al. 2019).

In Brazil, the genus *Gyrodontium* is known only from records of *G. sacchari* in the Atlantic Forest, in the states of São Paulo (Southeast Region) and Sergipe (Northeast Region) (Hjortstam 1995; Robledo et al. 2014). In the Brazilian Cerrado, the second largest biome in Brazil and an important hotspot for biodiversity conservation, the family Coniophoraceae is represented by only two species in the genus *Coniophora* DC—*Coniophora arida* (Fr.) P. Karst., and *C. olivacea* (Fr.) P. Karst—from grassland and cerrado forest in Mogi Guaçu, São Paulo state (Gibertoni and Drechsler-Santos 2010; Strassburg et al. 2017). Here, we report the first occurrence of *G. sacchari* in the Brazilian Cerrado, which is also the first record of family Coniophoraceae in the Midwest Region of Brazil, the core area of the Cerrado biome.

Methods

The studied specimen was collected in the municipality of Goiânia, Goiás state, Brazil, in a domestic agroforestry yard. There was a guapuruvu, *Schizolobium parahyba* (Vell.) Blake (Fabaceae), a tree native to the Atlantic Forest. This tree had been planted about 11 years ago but had rotted due to termite attack and had to be cut down. The cut trunk was left exposed in this yard for about three years, when *G. sacchari* specimens first appeared.

The species identification followed both morphological and phylogenetic analyses. Microscopic characterization and measurements were performed from freehand cuts of dried material. The sections were rehydrated in 3% KOH and dyed with floxine, when necessary. Cotton blue and Melzer's reagent was used to check cyanophily and amyloidicity or dextrinoidicity, respectively. Slides were photographed by using an Olympus CX31 optical microscope and a digital camera. Color classification of basidiomata morphology were based on the Kornerup and Wanscher (1978) color chart. Microscopic structures were measured by using Piximètre v. 5.10 R 1541, where Q refers to the quotient between the length and width, Qm is the medium value of Q, and N is the number of measured spores (Henriot and Cheype 2017). Scanning electron microscopy was performed at the Center of Analysis, Innovation, and Technology (CAiTec, Universidade Estadual de Goiás), by using a Hitachi TM3030Plus scanning electron microscope (SEM) at 15.0 kV, with previously dehydrated material. The morphological species description was based on comparison with specific literature (Hjortstam 1995; Carlier et al. 2004). A voucher specimen was preserved and deposited in the fungarium of the Universidade Estadual de Goiás (HUEG-Fungi; Thiers 2022).

Total DNA was extracted from fragments of dried and cleaned basidioma macerated in liquid nitrogen, according to CTAB method (Goés-Neto et al. 2005; Hosaka et al. 2006). The extracted DNA was quantified by a spectrophotometer using Qubit (Invitrogen), and then the nuclear rDNA internal transcribed spacers (ITS) region of the DNA was amplified with the ITS5/ITS4 primer pairs (White et al. 1990). The amplification products were purified and sequenced with the same primers used in the amplification performed in an Applied Biosystems 3730xl DNA Analyzer (MacroGen Ltd., South Korea).

The DNA sequence obtained in this study were assembled and edited using Staden Package v. 2.0 (Staden et al. 1998). The consensus sequence was aligned with sequences of Coniophoraceae based on studies of Binder et al. (2010) and Zhao et al. (2018), and through a BLAST (http://blast.ncbi.nlm.nih.gov/) search database (Table 1) using MAFFT (Katoh and Standley 2013); these data were manually inspected using MEGA v.6 (Tamura et al. 2013). Athelia arachnoidea (Berk.) Jülich and A. epiphylla Pers. were used as outgroups in our phylogenetic analyses following Zhao et al. (2018). Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were performed in W-IQ-TREE (Kalayaanamoorthy et al. 2017) and MrBayes v. 3.2 (Ronquist and Huelsenbeck 2003), respectively, under the TIM2+F+I+G4 substitution model estimated based on the Akaike Information Criterion (AIC). ML was determined with branch support (BS) inferred by 1000 bootstrap replications and Ultrafast bootstrap (UB). BI was performed with 10 million generations, with convergence verified in TRACER v. 1.7.1 (Rambaut et al. 2018), and the first 25% of the resulting trees were discarded as burn-in; Bayesian posterior probabilities (PP) were calculated from the remaining sampled trees. Statistical support for branches was considered strong with BS and UB \geq 70% and PP \geq 0.95.

Results

Phylogenetic analyses. Based on the BLAST search of the GenBank nucleotide database, the highest matches were all sequences of Coniophoraceae. The ITS sequence had up to 98% identity with sequences from *Gyrodontium sacchari* in GenBank, while for other species of Boletales was below 92%.

In the phylogenetic analysis, sequence alignment had a length of 772 characters with gaps and included

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Таха	Voucher	Origin	ITS	Reference
Athelia arachnoidea	CBS: 41872	Netherlands	GU187504	Binder et al. 2010
A. epiphylla	CFMR: FP-100564	USA	GU187501	Binder et al. 2010
Coniophora arida	CFMR: FP-104367	USA	GU187510	Binder et al. 2010
C. cerebella	HK'8'	USA	GU187513	Binder et al. 2010
C. hanoiensis	He 5197	Vietnam	MG763873	Zhao et al. 2018
C. hanoiensis	He 5202	Vietnam	MG763874	Zhao et al. 2018
C. marmorata	MUCL: 31667	Belgium	GU187515	Binder et al. 2010
C. olivacea	CFMR: FP-104386	USA	GU187516	Binder et al. 2010
C. prasinoides	CFMR: FP-105969	USA	GU187519	Binder et al. 2010
C. puteana	MUCL: 1000	Germany	GU187521	Binder et al. 2010
C. puteana	He 2909	China	MG763876	Zhao et al. 2018
Coniophora sp.	Braz-6	Brazil	GU187517	Binder et al. 2010
Coniophoropsis bambusicola	He 5208	Vietnam	MG763877	Zhao et al. 2018
C. bambusicola	He 5210	Vietnam	MG763878	Zhao et al. 2018
Gyrodontium sacchari	HUEG14877	Brazil	ON819574	This study
G. sacchari	MEL: 2382749	Australia	KP012932	GenBank
G. sacchari	He 4157	Thailand	MG763879	Zhao et al. 2018
G. sacchari	CLZhao 4800	China	MK343540	GenBank
G. sacchari	UOC MINNP MK05	Sri Lanka	KR867661	GenBank
G. sacchari	CLZhao 4804	China	MK343541	GenBank
G. sacchari	MUCL: 42789	French Guiana	GU187522	Binder et al. 2010
Penttilamyces olivascens	UC 2022939	USA	KP814170	GenBank
P. olivascens	CFMR: HHB-11134	USA	GU187532	Binder et al. 2010
P. romellii	CFMR: T-547	Canada	GU187529	Binder et al. 2010

Table 1. Specimens along with GenBank accession numbers used in the phylogenetic analysis. The sequences obtained in this study are marked in bold.

25 Coniophoraceae specimens. Of these, 351 characters were constant, 403 were variable, and 348 were parsimony informative. BI analysis resulted in an average standard deviation of split frequencies of 0.004012. The tree topology generated by ML was identical to that generated by BI analyses and was therefore used as the basal tree (Fig. 1). Based on phylogenetic analysis four supported clades were formed and *Gyrodontium* species recovered in this study were grouped into a clade with strong support values (BS = 100%, UB = 100%, PP = 1).

Gyrodontium sacchari (Spreng.) Hjortstam,

Mycotaxon 54: 186 (1995)

≡ Hydnum sacchari Spreng., K. svenska Vetensk-Akad. Handl., ser. 3 41: 51 (1820)

Figure 2

New record. BRAZIL – GOIÁS • Goiânia, in a domestic agroforestry yard; 16°35'15.9"S, 049°17'33.2"W; 749 m a.s.l.; 11.III.2021; C. M. Silva-Neto (FJSC107); on *Schizolobium parahyba* (Vell.) Blake (Fabaceae) dead trunks; GenBank ON819574; HUEG14877.

Identification. Basidiomata annual, resupinate, effused-reflexed to pileate, imbricate, fleshy to spongy, to 6×10 cm. Pileus applanate, ressupinate in some parts and others forming conchate that coalesce to form complex basidiomata (effused-reflexed). Pilear surface white (1A1), pale grey (1B1) to greyish yellow (1B4), slightly zonate, velvety. Margin white (1A1) to pale grey (1B1)

when freshly, sterile, entire, obtuse to irregular, thick, spongy to cottony. Context concolorous to pilear surface, homogeneous, to 6 mm thick, soft to spongy. Hymenial surface yellowish white (1A2) to pale yellow (1A3), smooth to tuberculate when young, light yellow (3A5) to olive yellow (3C6), aculeolate to hydnoid with maturity, gradually paler towards the margin. Spines 2–4 mm long, elongate to cylindric, with tip subulate, flattened to angular. Rhizomorphs white (1A1) and cottony.

Hyphal system monomitic; generative hyphae simple septate, clampless, hyaline to yellowish in KOH, inamyloid, thin to thick-walled, in the context 3.0–7.0 μ m in diameter, often branched and mostly thin-walled, in the hymenium 2.2–7.0 μ m in diameter simple to slightly branched, parallel arranged and thin to thickwalled. Cystidia not seen. Basidia clavate, hyaline in KOH, 4-sterigmate, 10.8–16.2 × 3.5–5.0 μ m; sterigmata to 5.6 μ m long. Basidiospores ellipsoid to elongate, smooth, yellowish brown in KOH, cyanophilic, dextrinoid, thick-walled, (4) 4.2–5.3 (5.6) × (2.2) 2.5–3.0 (3.2) μ m [Q = (1.5) 1.6–1.9 (2.1); Qm = 1.8; N = 50].

Distribution in Brazil. Known to occur in the states of São Paulo and Sergipe in the Atlantic Forest biome (Hjortstam 1995; Robledo et al. 2014), Bahia and Rio Grande do Sul (ecosystem data not available) (Maas Gesteranus 1966; Bononi 1988), and Goiás (present study) in the Cerrado biome (Fig. 3).

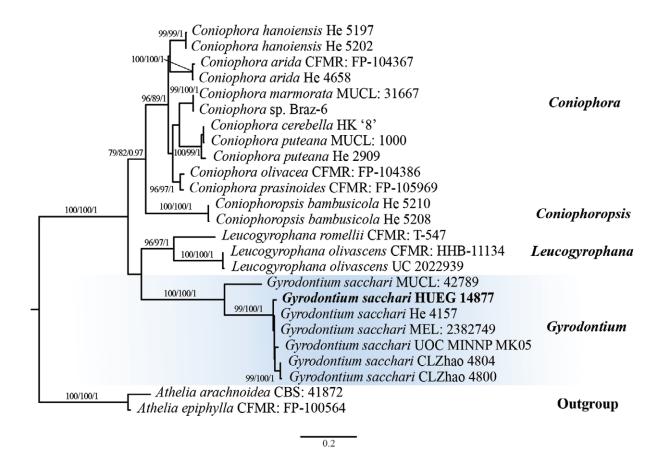


Figure 1. Phylogenetic tree obtained using ITS gene. The tree topology is from an ML analysis and *Gyrodontium sacchari* specimen reported in this study is indicated in bold. Numbers at branches indicate the values of bootstrap frequency (BS)/ultrafast bootstrap (UB)/Bayesian posterior probability (PP).

Discussion

Gyrodontium sacchari is a peculiar species and is easily identified in the field due to the resupinate to effuse-reflexed basidiomata and yellow to olive-yellow hydnoid hymenial surface. Microscopically, the monomitic hyphal system with its simple septate generative hyphae and thick-walled smooth yellowish basidiospores is distinctive in the identification of the species. These features differ from other Coniophoraceae, such as Coniophora, Coniophoropsis Hjortstam & Ryvarden and Penttilamyces Zmitr., Kalinovskaya & Myasnikov, which are characterized by smooth to merulioid hymenophores and resupinate basidiomata (Zmitrovich et al. 2019). Gyrodontium sacchari is a saprotrophic fungal species, and in many cases, it has been reported as a brown-rot-causing agent in tropical rainforests, as observed in some other members of Coniophoraceae; its occurrence in the rotting hollow of woody plant species is common (Carlier et al. 2004; Dai et al. 2007; Robledo et al. 2014; Joshi et al. 2021).

Unlike *Hydnum* species, which are described as ectomycorrhizal, *G. sacchari* has a saprophytic habit, as we and others have observed (Feng et al. 2016; Swenie et al. 2018; Chen et al. 2019; this study). In studies of hydnoid species, this habit should be considered, together with the phylogenetic data to avoid misidentifications and unnecessary synonymization. In addition to our present study, only two other studies on this species' distribution have present phylogenetic data (Carlier et al. 2004; Zhao et al. 2018). We recommend that studies based on both morphological and multigene phylogeny must be carried out, including on type material and sequences from Neotropical species, to best clarify the taxonomic status of species with spinose hymenophores, such as *Hydnum* and *Hydnum*-like fungi.

Fidalgo et al. (1965) presented the first survey of macroscopic fungal diversity in the Brazilian Cerrado, and since then, some areas of this ecosystem have been explored further (Gibertoni and Drechsler-Santos 2010; Alvarenga et al. 2015, 2017; Calaça et al. 2018, 2020; Leonardo-Silva et al. 2020, 2021). Here, with our discovery of *G. sacchari*, we report the first occurrence of the genus Gyrodontium in the Brazilian Cerrado, as well as the first record of the family Coniophoraceae in the Midwest Region of the country. This new record adds to our knowledge of the Cerrado's funga and provides new phylogenetic and biogeographic data for this species from a tropical region. Considering that the Cerrado is a biodiversity hotspot and that there is the lack of records of the family Coniophoraceae from this biome, it is supposed that this species is threatened by habitat loss in the Cerrado (Dahlberg and Mueller

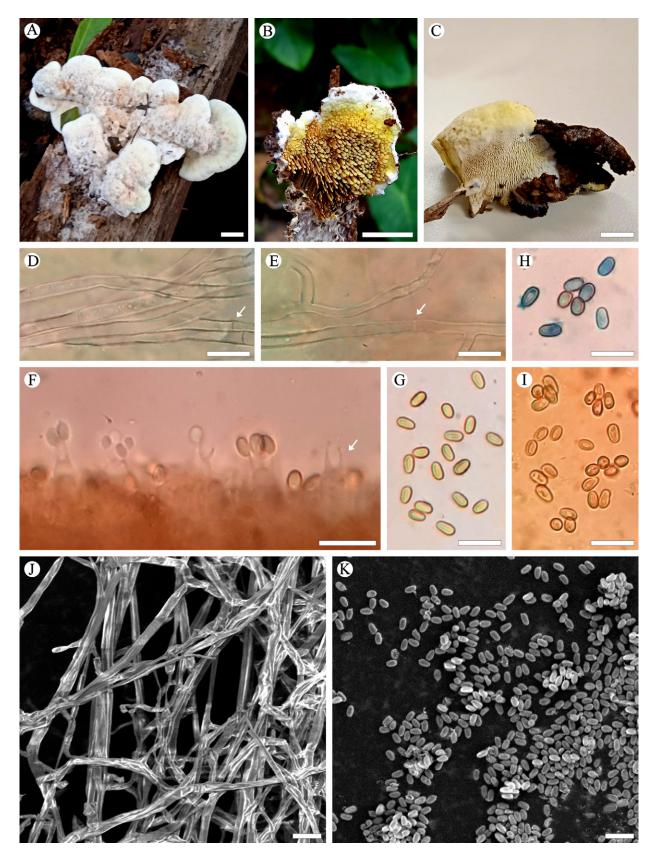


Figure 2. *Gyrodontium sacchari.* **A.** Pilear surface of mature basidioma on a guapuruvu trunk. **B, C.** Details of the mature and young hydnoid hymenial surface. **D, E.** Generative hyphae simple septate (arrow) in context and hymenium. **F.** Basidia with immature basidiospores and evident sterigma (arrow). **F–I.** Basidiospores in KOH 3% and dyed with cotton blue and Melzer's reagent. **J.** Generative hyphae viewed in Scanning Electron Micrograph (SEM). **K.** Basidiospores viewed in SEM. Scale bars: A-C = 1 cm; $D-I = 5 \mu \text{m}$; J, K = 10 μm .

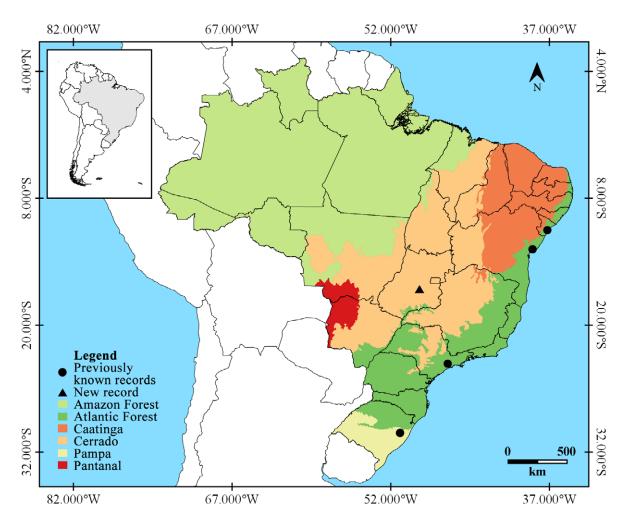


Figure 3. Sampled area and geographic distribution of Gyrodontium sacchari in Brazil.

2011; Strassburg et al. 2017). We recommend that public policies give attention to the conservation of fungi of the Cerrado, especially due to the important function that fungi have in terrestrial ecosystems. We highlight the need for additional studies on the taxonomic diversity of the Cerrado's funga.

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Author Contributions

Conceptualization: FJSC, SXS, CMSN, LLS. Data curation: LLS, FJSC, GPS. Formal analysis: LLS, FJSC. Funding acquisition: SXS, CMSN. Methodology: FJSC, CMSN, LLS, GPS. Supervision: SXS. Writing – original draft: FJSC, LLS. Writing – review and editing: CMSN, SXS.

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