

## A new species of *Thyrea* (Lichinales: Lichinaceae) from the Brazilian central-west region

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### Resumen

*Una nueva especie de Thyrea (Lichinales: Lichinaceae) de la región centro occidental de Brasil*

En el centro-oeste de Brasil, la región de la Serra da Bodoquena comprende bosques ribereños, caducifolios estacionales y semidecuiduos, donde se conocen unas 400 especies de líquenes. El área está sufriendo los impactos de la agricultura y los incendios intencionales, que amenazan la biodiversidad local. Estudiando los hongos formadores de líquenes de la Serra da Bodoquena a través de análisis integrativos, se describe una nueva especie, *Thyrea pulverulenta* (Lichinales, Lichinaceae). Además, se generaron secuencias de las regiones nuITS y mtSSU, y se infirió la posición del género dentro de los Lichinomyces, confirmando su estrecha relación filogenética con *Watsoniomyces*. También demostramos que la región aún necesita ser estudiada, siendo un posible punto crítico de biodiversidad con especies endémicas.

**Palabras clave:** Bodoquena; Cianolíquenes; Diversidad; Áreas nativas; Afloramientos rocosos.

### Abstract

In central-west Brazil, the Serra da Bodoquena region comprises riparian, seasonal deciduous, and semideciduous forests, where ca. 400 lichens species are known. The area is suffering the impacts of agriculture and intentional fires, which threaten the local biodiversity. Studying the lichen-forming fungi from the Serra da Bodoquena through integrative analyses, a new species was discovered, *Thyrea pulverulenta* (Lichinales, Lichinaceae). In addition, sequences of nuITS and mtSSU regions were generated, and the position of the genus within the Lichinomyces was inferred, confirming its close phylogenetic relationship with *Watsoniomyces*. We also demonstrated that the region still needs to be studied, being a possible biodiversity hotspot with endemic species.

**Key words:** Bodoquena; Cyanolichen; Diversity; Native areas; Rock outcrops.

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## Introduction

The Brazilian central-west region is mainly constituted by the Atlantic Forest, Amazon, Cerrado, and Pantanal biomes (Oliveira-Júnior 2020), which are distributed within an area of approximately 1.600.000 km<sup>2</sup> (IBGE 2020). The Serra da Bodoquena is localized at the southwestern portion of the region, within the Cerrado and near the Pantanal (Figs. 1A & 1B), where mountains up to 800 m of elevation are covered by forests and rocky outcrops partially protected by the Serra da Bodoquena National Park.

Despite the National Park, the region has been explored by ecotourism, and the natural areas have been negatively impacted by the advancement of agriculture, pasture, and fires (Zavala 2017, Cunha 2021). Therefore, brackish sawgrass marshes, riparian, and seasonal deciduous and semideciduous forests are threatened, and consequently the biodiversity (Baptista-Maria 2009, Zavala 2017, Scremin-Dias 2018).

Regarding the lichen-forming fungi diversity, Aptroot & Spielmann (2020) reported almost 400 species from the Serra da Bodoquena, of which 23 taxa are cyanolichens, including *Coccocarpia erythroxyli* (Spreng.) Swinscow & Krog, *Coccocarpia palmicola* (Spreng.) Arv. & D.J. Galloway, *Collema furfuraceum* (Schaer.) Du Rietz, *Collema glaucophthalmum* var. *implicatum* (Nyl.) Degel., *Collema leptaleum* Tuck., *Enchylium tenax* (Sw.) Gray, *Heppia despreauxii* (Mont.) Tuck., *Hondaria leptospora* (Malme) Kitaura, M.C. Scur & A.P. Lorenz, *Lathagrium cristatum* (L.) Otálora, P.M. Jørg. & Wedin, *Leptogium coralloideum* (Meyen & Flot.) Vain., *Leptogium corticola* (Taylor) Tuck., *Leptogium cyanescens* (Ach.) Körb., *Leptogium isidiosellum* (Riddle) Sierk, *Leptogium marginellum* (Sw.) Gray, *Leptogium milligranum* Sierk, *Leptogium phyllocarpum* (Pers.) Mont., *Metamelanea caesiella* (Th. Fr.) Henssen, *Paulia gibbosa* Henssen, *Paulia stipitata* Henssen, *Peltula euploca* (Ach.) Poelt, *Peltula obscurans* (Nyl.) Gyeln., *Synalissa matogrossensis* (Malme) Henssen, and *Thyrea confusa* Henssen.

*Thyrea confusa* was the second species of the genus reported in Brazil, after *Thyrea porphyrella* M. Schultz, Büdel & Porembski, from Espírito Santo state (Schultz 2001b). The *Thyrea* A.Massal. species are distributed worldwide, especially in the Northern Hemisphere, and nowadays are known ca. 15 species (Lücking 2017). The inte-

grative approaches have been used as a tool for the cyanolichen study, but for the genus, only three *Thyrea* genetic sequences are available in the GenBank database, one sequence of *T. confusa* from Czech Republic (GenBank accession code OL396742; Vondrák 2022), one sequence of *T. confusa* from Germany (GenBank accession code AF282915; Schultz 2001a), and *T. pachyphylla* (Müll. Arg.) Henssen (unpublished), all of the 18S region.

The present study aimed to study *Thyrea* specimens collected in the Serra da Bodoquena, using anatomical, morphological, and molecular approaches. In addition, a new species was described, the first sequences of the nuITS and mtSSU regions were deposited, and the confirmation of the species belonging in the Lichinales was inferred through phylogenetic analysis.

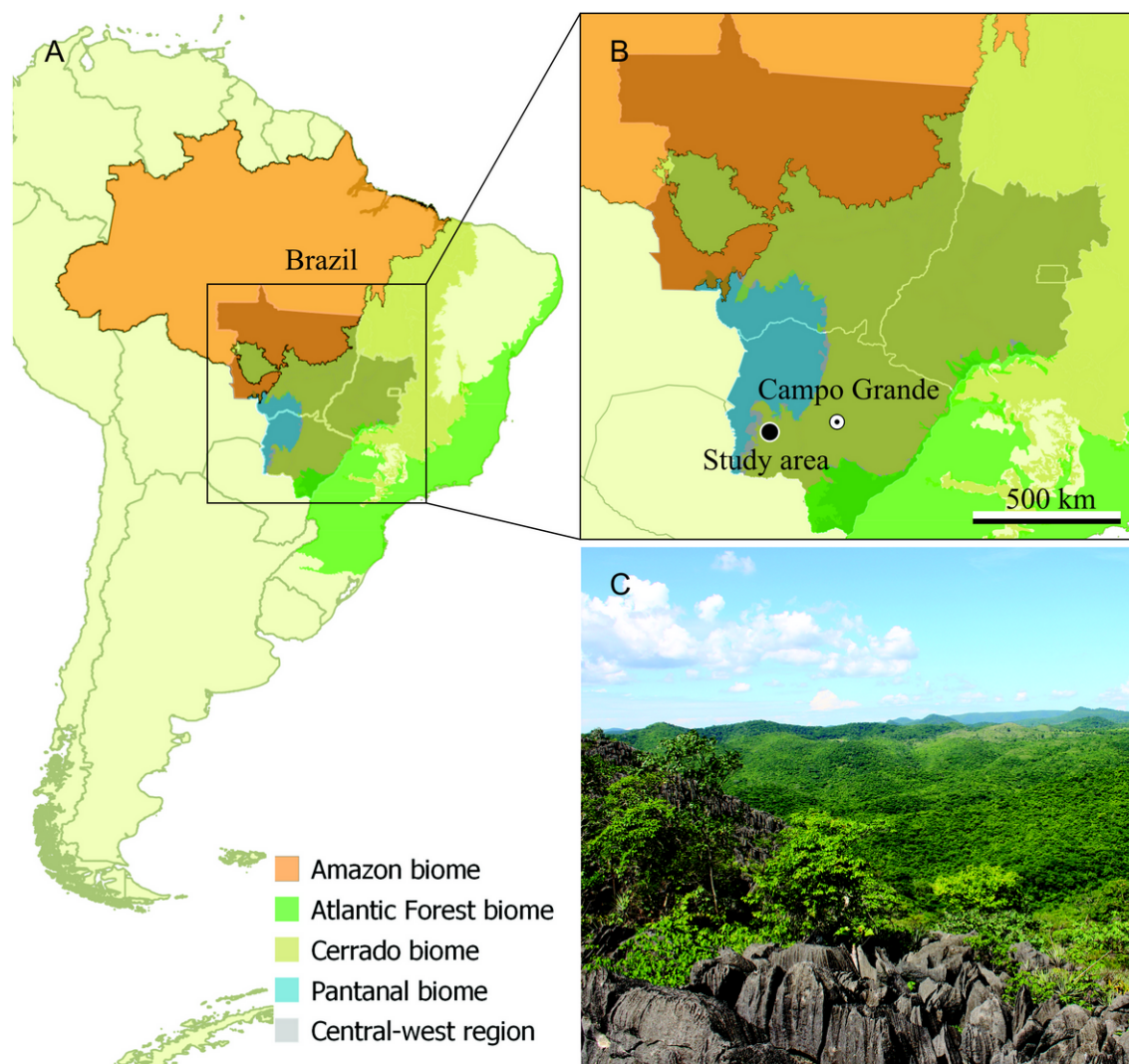
## Materials and methods

The field expeditions were realized in November 2018, during the Botany course offered by the Post-graduate Program in Plant Biology of the Federal University of Mato Grosso do Sul. The *Thyrea* specimens were collected on the rocky outcrops known as “Dente de Cão” in the Serra da Bodoquena region (Fig. 1C). All specimens were deposited in the CGMS herbarium (Campo Grande, Brazil), including the type material.

The material was observed under an Olympus stereomicroscope, where anatomical sections were made and observed in an Olympus CH30 light microscope. The images were generated with Canon REBEL3, and the sequences were produced through the FUNDECT/CNPq resources, Chamada 04/2019, destined to PDTCR post-doc program.

The DNA was extracted using a Wizard® Genomic DNA Purification kit (Promega), following the manufacturer's protocol. The PCRs were performed according to Kitaura (2018). The nuclear Internal Transcribed Spacer region (nuITS) and the mitochondrial Small Subunit (mtSSU) sequences were generated for three specimens, and the sequences were deposited in the GenBank (Table 1).

The sequences of both regions were used to perform phylogenetic analyses with the Lichinomycetes clade (Table 1). *Chaenotheca furfuracea* (L.) Tibell (GenBank accession code JX000121) and *Sclerophora farinacea* Chevall. (JX000130)



**Figura 1.** Área de estudio. **A:** Brasil, indicando los biomas de la región centro-oeste; **B:** Estado de Mato Grosso do Sul, indicando el área de estudio; **C:** Afloramiento rocoso donde se recolectó el material.

**Figure 1.** Study area. **A:** Brazil, highlighting the biomes of the central-west region; **B:** Mato Grosso do Sul state, highlighting the study area; **C:** Rock outcrop where the material was collected.

Species	GenBank Accession Code	Reference
<i>Heppia despreauxii</i> (Mont.) Tuck.	JN206650	Unpublished
<i>Lempholemma polyanthes</i> (Bernh.) Malme	AY584709	Lutzoni <i>et al.</i> (2004)
<i>Lichina confinis</i> (O.F. Müll.) C. Agardh	KX984072	Schultz (2017)
<i>Lichina pygmaea</i> (Lightf.) C. Agardh	KX984066	Schultz (2017)
<i>Lichinella nigrifella</i> (Lettau) P.P. Moreno & Egea	MF766342	Kauff <i>et al.</i> (2018)
<i>Peltula auriculata</i> Büdel, M. Schultz & Gröger	DQ922953	Miadlikowska <i>et al.</i> (2006)
<i>Peltula brasiliensis</i> (Zahlbr.) Büdel, Kauff & Bachran	MF766339	Kauff <i>et al.</i> (2018)
<i>Peltula corticola</i> Büdel & Sant.	MF766337	Kauff <i>et al.</i> (2018)
<i>Peltula steppae</i> (Kalb) Büdel, Kauff & Bachran	MF766338	Kauff <i>et al.</i> (2018)
<i>Phylliscum demangeonii</i> (Moug. & Mont.) Nyl.	AY853333	Wedin <i>et al.</i> (2005)
<b><i>Thyrea pulverulenta</i> (2)*</b>	<b>OQ986601</b>	<b>Present study MJK 4657</b>
<b><i>Thyrea pulverulenta</i> (3)*</b>	<b>OQ986600</b>	<b>Present study MJK 4658</b>
<b><i>Thyrea pulverulenta</i> (1)*</b>	<b>OQ986599</b>	<b>Present study MJK 4660</b>
<i>Watsoniomyces obsoletus</i> (Nyl.) D. Hawksw., M. Powell & T. Sprib. (1)	MW370265	Díaz-Escandón <i>et al.</i> (2021)
<i>Watsoniomyces obsoletus</i> (2)	MW370266	Díaz-Escandón <i>et al.</i> (2021)

\* Numbers in parenthesis

**Tabla 1.** Detalles de las especies utilizadas en el análisis filogenético de la región mtSSU. Las líneas en negrita corresponden a las secuencias generadas en el presente estudio.

**Table 1.** Details of species used in the phylogenetic analysis of the mtSSU region. Sequences generated in the present study are in bold.

were used as outgroups, considering the close phylogenetic relationships with Lichinomycetes (Prieto & Wedin 2013, Díaz-Escandon 2021). The intraspecific divergence among the *Thyrea* nuITS sequences were checked in the Geneious v9.1.2 (Kearse 2012).

The alignments were obtained in Geneious v9.1.2 (Kearse 2012) with the MAFFT v7.308 algorithm (Katoh 2002). Additionally, the Gblocks web server (<http://phylogeny.lirmm.fr/>) was used to exclude unreliably aligned sites (Dereeper 2008, Castresana 2000). The phylogenetic trees were estimated using Bayesian (BA) and Maximum Likelihood (ML) approaches in the CIPRES web server (<https://www.phylo.org>) (Miller 2010). Nucleotide substitution models were inferred following the Bayesian Inference Criterion in jModelTest2 (Darriba 2012, Guindon & Gascuel 2003).

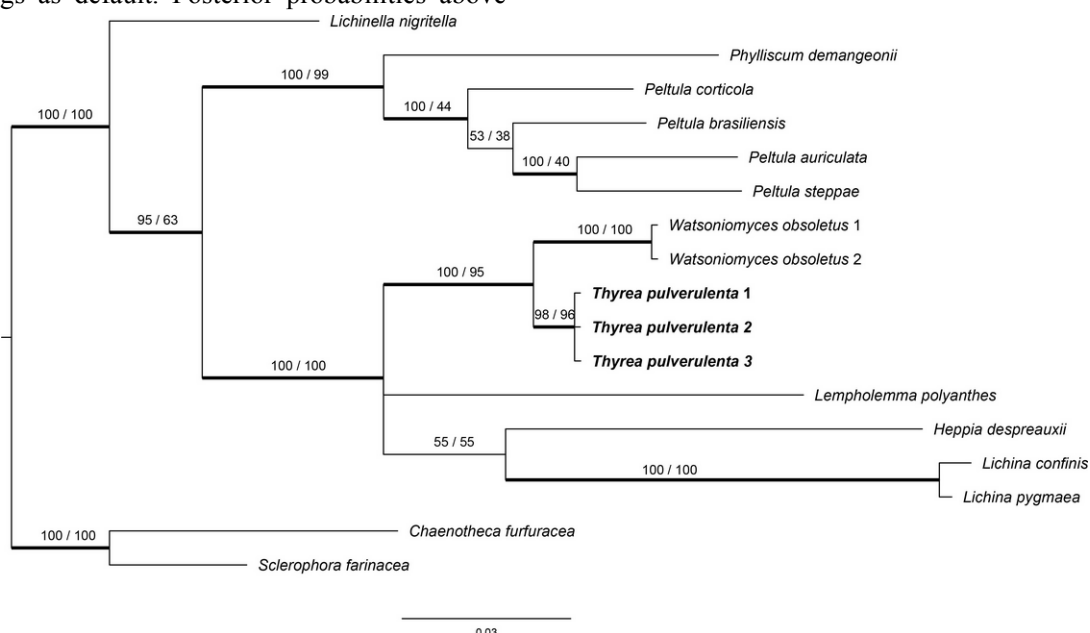
The BA was performed in MrBayes 3.2.7a (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using GTR+G substitution model to nuITS and TVM+G to mtSSU analyses, with a chain length of 10,000,000 generations sampled every 1,000 steps. The first 25% of the generated trees were discarded as burn-in, and the 50% majority-rule tree was built with the remaining trees sampled. The ML tree was built with the RaxML 8.2.12 (Stamatakis 2014) using the GTR+G substitution model for both regions, with 1,000 bootstrap replications and the remaining settings as default. Posterior probabilities above

0.95 and bootstrap values above 0.70 were considered significant support values for the hypotheses of phylogenetic relationships.

The specimens initially have been identified to genus level by Schultz & Büdel (2002), and were described according to the *T. porphyrella* description (Schultz 2001b). Periclinally arranged hyphae were observed at the central region of the thallus as well as *T. porphyrella*, but differed by the presence of photobiont cells, and the thallus was considered as homoiomerous. pycnoascocarps observed in *Thyrea* species are reported in the descriptions as apothecia.

## Results and discussion

The present study deposited the first sequences of the nuITS and mtSSU regions of the *Thyrea* genus in the public library GenBank. The variation among nuITS sequences of *T. pulverulenta* ranged from 98.4% to 99.7%. Due to the scarcity of nuITS sequences available, only the phylogeny constructed with mtSSU sequences is shown. The sequences of the mtSSU were analyzed with other taxa of the Lichinomycetes clade (Table 1). The phylogenetic trees obtained revealed that *Thyrea* is close to *Watsoniomyces obsoletus* (Nyl.) D. Hawksw., M. Powell & T. Sprib., clustering also with *Lichina* C.Agardh, *Heppia* Nägeli ex A.Masal. and *Lempholemma* Körb. (Fig. 2 & Table 1).



**Figura 2.** Hipótesis filogenética de la ubicación de *Thyrea* dentro del clado de Lichinomycetes según los enfoques Bayesiano y de Máxima Verosimilitud. Los valores por encima de las ramas corresponden al valor de *bootstrap* y las probabilidades posteriores, respectivamente.

**Figure 2.** Phylogenetic hypothesis of the *Thyrea* placement within the Lichinomycetes clade based on the Bayesian and Maximum Likelihood approaches. Values above branches correspond to posterior probabilities and bootstrap values, respectively.

Similar results were observed by Díaz-Escandón *et al.* (2021), using the 18S region, where *Thyrea confusa* (AF282915.1) and *T. pachyphylla* (AF336909.1) were close to *W. obsoletus*.

The phylogenetic analysis shown in the present study confirm the inclusion of *T. pulverulenta* in Lichinomycetes. However, extensive work is needed to confirm the species placement within Lichinomycetes and the internal relationships of *Thyrea*, especially regarding the scarcity of a complete genetic dataset, including the generation of genetic sequences of the other species that were registered in Brazil, *T. confusa* and *T. porphyrella*.

***Thyrea pulverulenta*** M.J. Kitaura, *sp. nov.* Fig. 3

MycoBank number: 848790

**Holotype:** Brazil, Mato Grosso do Sul, Bodoquena, Serra da Bodoquena, Santa Laura Farm, Morro Dente de Cão, calcareous rocks, on the top, saxicolous, -20.784611°, -56.750583°, 445 masl, 07-XI-2018. *M.J. Kitaura* 4660 (CGMS). GenBank codess: nuITS ([OQ975317](#)), mtSSU ([OQ986599](#))

**Etymology:** The thallus is covered by pruina and isidia, causing the pulverulent appearance.

**Description:** Thallus blackish under fluorescent light, whitish black under the stereomicroscope, 0.5-3.0 cm broad, matt, opaque, gelatinous when wet, foliose, initially monophyllous, irregularly branched and incised with the age. Lobes 3.0-8.0 mm wide, 5.0-12.0 mm long, overlapping, ascending, and centrally attached. Upper surface smooth at naked eye, smooth, rugulose and with irregular ridges at 10× magnification. Apical margin rounded, plane to ascending, smooth and thickened; lateral margin smooth and thickened, plane, undulated and sinuous. Lower side is blackish green to greenish gray, smooth under different magnifications. Pruina pale gray on the lamina. Isidia originating on the surfaces, 5.0-30.0 (-50.0) µm diameter, blackish to brownish, simple to grouped. Lobules absent. Thallus attached by rhizohyphae, grouped in the central part. Homoiomerous thallus structure, 250-400 µm thick, ecoriatic; thallus periphery with reticulate arranged hyphae, filled by photobiont cells, 25-100 µm thick at the upper side, 75-100 µm thick at the lower side; central hyphae thin, composed of few periclinally arranged hyphae, with some dispersed photobiont cells. The photobiont unicellular cyano-

nobacteria, 5.0-7.5 µm diam, spherical to elliptic; gelatinous sheath thin, up to 2.5 µm thick, brownish at the thallus periphery, hyalin in the thallus center.

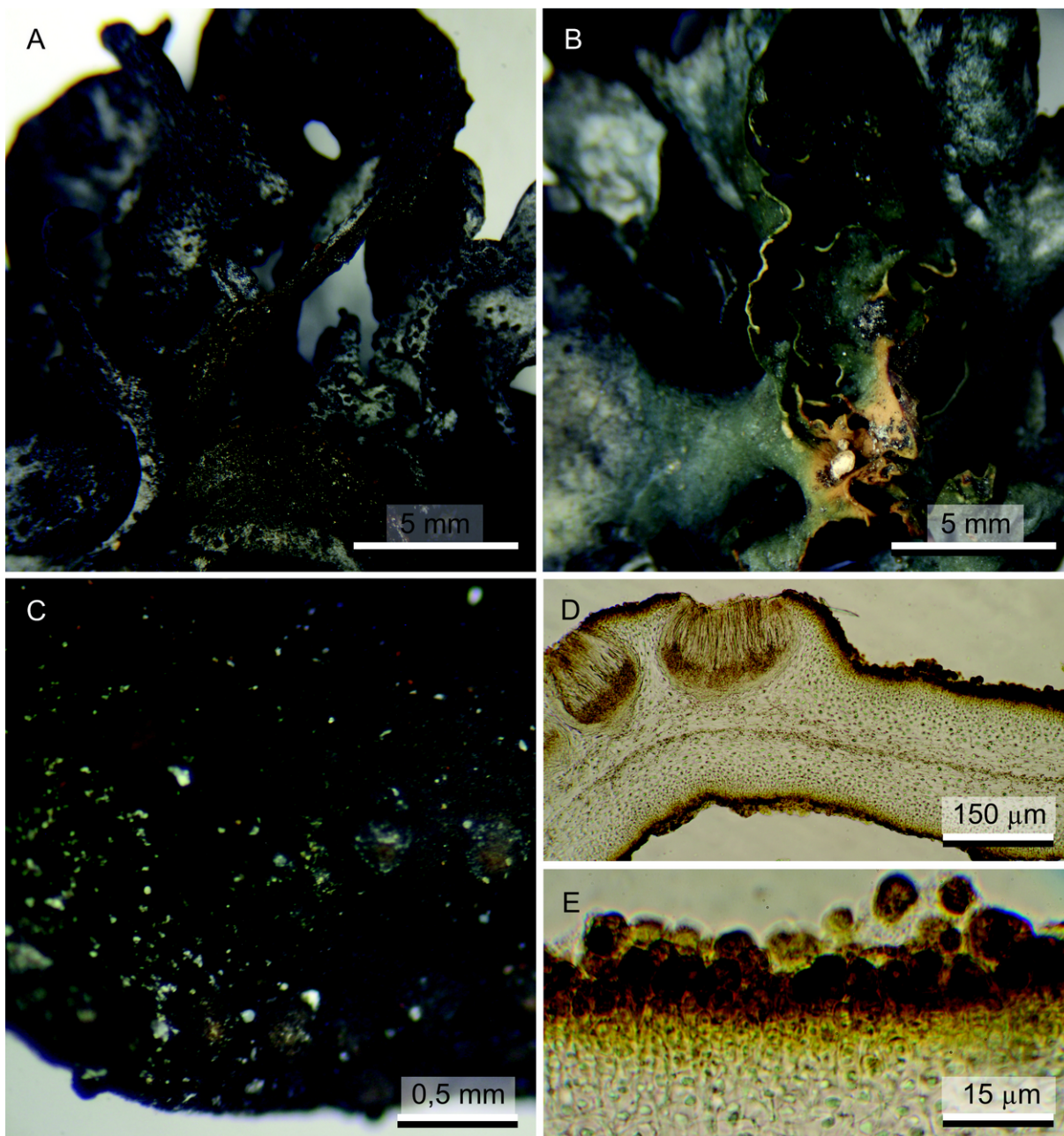
Apothecia abundant, 0.2-0.4 mm diam., usually laminal, semi-immersed; apothecial disc plane or concave, brownish to reddish, proper exciple yellowish around of the disc; amphithecium smooth, concolorous with the thallus, lacking ornaments and pruina. Epithyrium brownish, ca. 5 µm high. Hymenium 75-175 µm thick, I+ blue. Subhymenium ca. 75 µm thick, hyaline, I+ blue. Exciple proper 25.0-37.5 µm thick at the base, 12.5-25.0 µm thick at the lateral, hyaline. Ascospores simple, hyaline, globose to ellipsoid, 7.5-10.0 × 5.0-7.5 µm, spore wall distinctly thickened with age, up to 2.5 µm thick. Pycnidia immersed, globose, concolorous, ca. 0.2 mm diam., ostiole indistinct to yellowish. Conidia simple, bacilliform, ca. 3.0 × 1.0 µm.

**Additional material:** Brazil, Mato Grosso do Sul, Bodoquena, Serra da Bodoquena, Santa Laura Farm, Morro Dente de Cão, on the top, calcareous rocks, saxicolous, (DD) -20.784611°, -56.750583°, 445 masl, 07-XI-2018. *M.J. Kitaura* 4657 (nuITS [OQ975319](#)), 4658 (nuITS [OQ975318](#)), 4659 (paratypes – CGMS, [OQ986600](#)).

**Diagnosis:** *Thyrea pulverulenta* is characterized by the monophyllous thallus, lobes with pale gray pruina, and black inconspicuous isidia, covering the upper surface.

When compared with other isidiate *Thyrea* species, *T. confusa* is characterized by the presence of thallus polyphyllous, pruina bluish gray, lobes 1-3 mm wide, ± lingulate, isidia 7-20(-30) µm wide, and cyanobacteria with brownish sheaths (Henssen & Jørgensen 1990, Jørgensen 2007), which differs of *T. pulverulenta* that is constituted by monophyllous thallus, pruina pale gray, lobes 3.0-8.0 mm wide, rounded, isidia 5.0-30.0(-50.0) µm diam. and cyanobacteria with hyaline sheaths. *T. confusa* was initially described to France (Henssen & Jørgensen 1990) and reported to other localities, such as Norway, Spain, Socotra Island, mainly (northern) Mediterranean (Moreno & Egea 1992, Jørgensen 2007); Canada, USA (Nash III *et al.* 2002); and Australia (Allen *et al.* 2001). Recently, *T. confusa* was also reported to the Serra da Bodoquena (Aptroot & Spielmann 2020), and the specimen must be revised.

*Thyrea latissima* Asahina is characterized by



**Figura 3.** *Thyrea pulverulenta* (holotipo, MJK4660). **A:** Lado superior del talo; **B:** Lado inferior del talo; **C:** Detalle de la superficie superior en polvo; **D:** Sección transversal del talo y apotecio; **E:** Sección transversal del talo, detallando la cara superior y los isidios granulares.

**Figure 3.** *Thyrea pulverulenta* (holotype, MJK4660). **A:** Upper side of the thallus; **B:** Lower side of the thallus; **C:** Detail of the powdered upper surface; **D:** Transversal section of the thallus and apothecium; **E:** Transversal section of the thallus, detailing the upper surface and granular isidia.

monophyllous thallus, thallus up to 5 cm broad, lobes 300-400 µm thick, ecorticate, isidia 15-100 µm on the lower surface (Asahina 1958), differing of *T. pulverulenta* by thallus up to 3 cm broad, lobes 250-400 µm thick, ecorticate, isidia 5.0-30.0 (-50.0) µm diam. on the upper surface. Another isidiate species is *Thyrea asahinae* (Yoshim.) Yoshim., and is characterized by the thallus polyphyllous, epruinose, and with isidia globose ca. 135 µm (Yoshimura 1968), differing of *T. pul*

*verulenta* that has monophyllous thallus and pruina. *Thyrea indica* D.D. Awasthi & S.R. Singh has lobes 1.0-2.5 mm wide and ornaments on the thallus and apothecia (Awasthi & Singh 1979), whereas that *T. pulverulenta* has lobes 3.0-8.0 mm wide and isidia only on the thallus.

The non-isidiate species *Thyrea deusta* (Tuck.) Zahlbr. (Tuckerman 1872), *Thyrea leptophylla* (Tuck.) Zahlbr. (Tuckerman 1862), *Thyrea pachyphylla* (Müller Argoviensis 1881), and *Thyrea*

*girardii* (Durieu & Mont.) Bagl. & Carestia (Vincent & Maisonneuve 1846) were until now reported to North Hemisphere; differing of the isidate *T. pulverulenta*.

The other Brazilian *Thyrea* species, *Thyrea porphyrella* M. Schultz, Büdel & Porembski, has thallus polyphyllous with upper surface smooth to irregular, plicate to folded (Schultz *et al.* 2001b), and differs of *T. pulverulenta* that is characterized by the monophyllous thallus and isidia.

The hyphae parallelly arranged was observed at the medullar region of the thallus, which were reported as primitive heteromerous thallus by Moreno & Egea (1992). However, when the medullar region is lacking of photobiont cells, like *T. porphyrella* (Schultz *et al.* 2001b), the thallus is heteromerous; and when has some scattered photobiont cells, like *T. latissima* (Asahina 1958), the thallus is homoiomerous.

The diversity of cyanolichens of the Brazilian central-west region has revealed larger than previously known, and new species of *Leptogium* (Ach.) Gray (Kitaura *et al.* 2019), *Sticta* (Scherb.) Ach. (Torres *et al.* 2021), *Heterodermia* Trevs. (Souza *et al.* 2022) and new records, such as *H. despreauxii*, *M. caesiella*, *P. gibbosa* and *P. stipitata* (Aptroot & Spielmann 2020) were reported. Ultimately, the authors highlight the importance of the conservation of the Serra da Bodoquena natural areas, the maintenance of the ICMBio and Chico Mendes Institutes, and the necessity of more integrative lichenological studies in the Mato Grosso do Sul state, where frequently reveal new lineages to science.

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