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Original Paper

New species and new records of *Peltula* (*Lichinales, Ascomycota* lichenized) from Mato Grosso do Sul, Brazil

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Abstract

Peltula species occur in arid and semi-arid environments, with new species being regularly described worldwide. Lichen diversity is being studied in the diverse but poorly known Midwest region of Brazil, and new species and records are being proposed. In this paper, *Peltula anthracina* is proposed as new to science, and *P. leptophylla* and *P. lingulata* are reported as new records from Mato Grosso do Sul state. As it is a region highly threatened by the expansion of agricultural activities, the authors highlight the importance of the conservation of native flora to preserve lichens' hidden diversity.

Key words: arid habitat, cyanolichen, diversity, fire forest, new species.

Resumo

Espécies de *Peltula* ocorrem em ambientes áridos e semiáridos e espécies novas têm sido constantemente descritas em todo o mundo. Neste mesmo sentido, a diversidade de liquens está sendo estudada na diversa, porém pouco conhecida, região centro-oeste do Brasil, e espécies e registros novos têm sido descobertos. Como parte do resultado, *Peltula anthracina* é proposta como nova para a ciência, e *P. leptophylla* e *P. lingulata* como novos registros para o estado de Mato Grosso do Sul. Como a região é altamente ameaçada pela expansão das atividades agrícolas, os autores destacam a importância da conservação da vegetação natural para preservar a diversidade oculta dos liquens.

Palavras-chave: habitat árido, cianoliquens, diversidade, incêndios florestais, espécie nova.

Introduction

Peltula Nyl. is a worldwide genus of lichenized fungi with cyanobacteria as photobiont, found in arid and semi-arid environments; and arid microclimate islands within humid areas (Wetmore 1971; Büdel 1987, 1995; Büdel *et al.* 2000; Schultz *et al.* 2000; Marques *et al.* 2013; Makryi 2016, 2017). Nowadays, new species are constantly reported, and the number of *Peltula* species is still uncertain. For example, the number of *Peltula* was estimated at 40 species in a generic study of lichen classification (Lücking *et al.* 2017). One year later, 50 species were estimated for the genus in a phylogenetic study of *Peltulaceae* (Kauff *et al.* 2018), but more than 60 taxa are accepted for the genus according to Index Fungorum and MycoBank websites.

Regarding the photobiont partner, information about the cyanobacteria is practically absent. The name of the lichens is related to mycobiont partner, and the cyanobacteria identification has been neglected within of the thallus. In this way, the photobiont are practically unknown to lichens and only the *Chroococcidiopsis* genus is

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reported to *Peltula* (Nübel *et al.* 1997; Komárek & Anagnostidis 1999).

In Brazil, 16 species of *Peltula* have already being reported, and most of the records were collected in states near the Atlantic Ocean (Tab. 1). In the Midwest region, only *Peltula euploca* (Ach.) Poelt, *P. obscurans* (Nyl.) Gyeln., and *P. tortuosa* (Ach.) Wetmore have been reported (Fleig & Riquelme 1991; Aptroot & Spielmann 2020).

This paper aims to contribute to the knowledge of *Peltula* biodiversity in Mato Grosso do Sul, Brazil. One new species and two new records are proposed using an integrative approach, utilizing morphological, anatomical, and phylogenetic analyses.

Table 1 – Species of *Peltula* reported in Brazil. In bold, the species reported to Mato Grosso do Sul state.

Species	States	Reference
Peltula auriculata Büdel, M. Schultz & Gröger	Roraima	Schultz & Aptroot (2008)
Peltula brasiliensis (Zahlbr.) Büdel, Kauff & Bachran	São Paulo	Zahlbruckner (1909)
Peltula bolanderi (Tuck.) Wetm.	Rio de Janeiro, Rio Grande do Sul, Santa Catarina	Vainio (1890), Spielmann (2006), Aptroot *et al*. (2017b)
Peltula boletiformis (Hue) A. Henssen & Büdel	Bahia	Schultz & Aptroot (2008)
Peltula clavata (Kremp.) Wetm.	Rio de Janeiro, Rio Grande do Sul, Santa Catarina	Vainio (1890), Spielmann (2006), Aptroot *et al*. (2017b)
Peltula congregata (Nyl.) Swinscow & Krog	Espirito Santo, Minas Gerais, Pernambuco, Rio Grande do Sul	Schultz & Aptroot (2008), Spielmann (2006)
***Peltula euploca* (Ach.) Poelt *ex* Ozenda & Clauzade**	**Bahia, Ceará, Mato Grosso do Sul, Pernambuco, Rio de Janeiro, Rio Grande do Sul**	**Spielmann (2006), Schultz & Aptroot (2008), Cáceres *et al.* (2017a), Aptroot & Spielmann (2020)**
Peltula farinosa Büdel	Bahia	Aptroot & Cáceres (2018)
Peltula impressa (Vain.) Swinscow & Krog	Bahia, Ceará	Cáceres *et al.* 2017b, Schultz & Aptroot 2008
Peltula koflerae A. Henss. & Büdel	Rio Grande do Sul	Spielmann (2006)
Peltula leptophylla (Vain.) Büdel & M. Schultz	Rio de Janeiro	Vainio (1890)
Peltula lingulata (Vain.) Swinscow & Krog	Rio Grande do Sul	Spielmann (2006)
***Peltula obscurans* (Nyl.) Gyeln.**	**Ceará, Mato Grosso do Sul, Sergipe, Rio Grande do Sul, Tocantins**	**Schultz & Aptroot 2008, Cáceres *et al.* 2014, Aptroot *et al.* 2017a, Cáceres *et al.* 2017a,b, Aptroot & Spielmann 2020**
Peltula placodizans (Zahlbr.) Wetm.	Pernambuco	Schultz & Aptroot 2008
Peltula tenuis Büdel & Henss.	Rio Grande do Sul	Spielmann 2006
***Peltula tortuosa* (Nees) Wetm.**	**Roraima, Pernambuco, Mato Grosso do Sul, Minas Gerais, Espírito Santo, Rio de Janeiro, Rio Grande do Sul**	Vainio 1890, Fleig & Riquelme 1991, Spielmann 2006, Schultz & Aptroot 2008

Material & Methods

The lichen collection deposited at the Universidade Federal de Mato Grande do Sul Herbarium (CGMS) was revised, and the *Peltula* species were selected for study. Students from the Federal University of Mato Grosso do Sul collected most of the specimens during expeditions carried out in the Corumbá and Ladário municipalities on the geographical boundaries between Brazil and Bolivia. Both municipalities are located in the Pantanal biome, a seasonally floodable tropical savannah characterized by 25.1 °C of mean annual temperature and 1,070.0 mm of mean annual precipitation (Soriano 1997), and a climate type Aw (Peel *et al.* 2007).

Initially, anatomical and morphological studies were performed and the fungal barcode sequences, the Internal Transcribed Spacer (nuITS) region, were generated for all specimens, according to Kitaura *et al.* (2018). The obtained sequences were compared to those deposited at GenBank. The dataset was initially constituted of sequences of the *Peltula* and *Lichinella* from Kauff *et al.* (2018), including other species available in GenBank, as: *P. africana* (Jatta) Swinscow & Krog (MN103150), *P. auriculata* Büdel, M. Schultz & A. Gröger (DQ832329), *P. euploca* (MK811928), *P. radicata* Nyl. (MN103151, and MN103152), and *P. umbilicata* (Vain.) Swinscow & Krog (DQ832333). After the preliminary analyses, the dataset was reduced to the *Peltula* clades in which the analyzed species were positioned (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.19300175.v1>). *Trichoglossum hirsutum* (Pers.) Boud. (AY789314) was settled as outgroup.

The alignments were performed in Geneious v9.1.2 (Kearse *et al.* 2012) with the MAFFT v7.308 algorithm (Katoh *et al.* 2002). Additionally, the Gblocks web server (<http://molevol.cmima.csic.es/castresana/Gblocks_server.html>) was used to exclude unreliably aligned sites. Phylogenetic trees were estimated using the Bayesian (BA), and Maximum Likelihood (ML) approaches. The nucleotide substitution and site heterogeneity models were inferred following the Bayesian Inference Criterion in jModelTest2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). The BA was performed in Beast v.1.8.0 (Drummond *et al.* 2012) using GTR+I+G model, and the Yule speciation process was set prior to one run with a chain length of 10,000,000 generations sampled every 1,000 steps. The first 25% of the generated trees was discarded as burn-in, and maximum clade credibility trees were built with treeannotator. All values of effective sample sizes were checked for >200 in tracer (Rambaut *et al.* 2018). ML trees were built with the RaxML v.7.2.8 (Stamatakis 2014) plugin in Geneious v9.1.2, with 1,000 bootstrap replications and the remaining settings as default. FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) was used to edit the trees and check for incongruences between the trees produced by the BA and ML methods. Support values of BA above 0.95 and bootstrap values of ML above 70 were considered significant for the hypotheses of phylogenetic relationships between the species examined.

The species descriptions included most of the characteristics used by Wetmore (1971), and the anatomical sections were made through free hand (Kitaura *et al.* 2018). Furthermore, we added details for the apothecia tissues when present, like subhymenium, hypothecium, parahymenium tissue, proprium and thalline exciples (Kitaura *et al.* 2018), but chemical tests were not made. The lichen substances are usually lacking in *Peltulaceae* (Kauff *et al.* 2018).

Results

Phylogenetic analyses

In the present study, seven nuITS sequences were generated: five from *P. anthracina*, one from *P. leptophylla* and one from *P. lingulata*. The generated sequences were compared with other *Peltula* sequences available in GenBank (James *et al.* 2006; Kauff *et al.* 2018; Marthinsen *et al.* 2019), resulting in one prior dataset constituted of 55 sequences and 789 bp (base pairs), and the final dataset with 30 sequences and 685 bp (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.19300175.v1>).

The phylogenetic trees of BA and ML approaches were congruent and retained the main phylogenetic relationships revealed by the six-locus analysis of Kauff *et al.* (2018) (Fig. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.19300175.v1>; Fig. 1). The new species, *Peltula anthracina*, is phylogenetically close to *P. clavata* (Kremp.) Wetmore (MF766348) and *P. lingulata* (Vain.) Swinscow & Krog (MF766360 and LSC3364).

The *P. leptophylla* sequence from Mato Grosso do Sul clustered with the other *P. leptophylla*

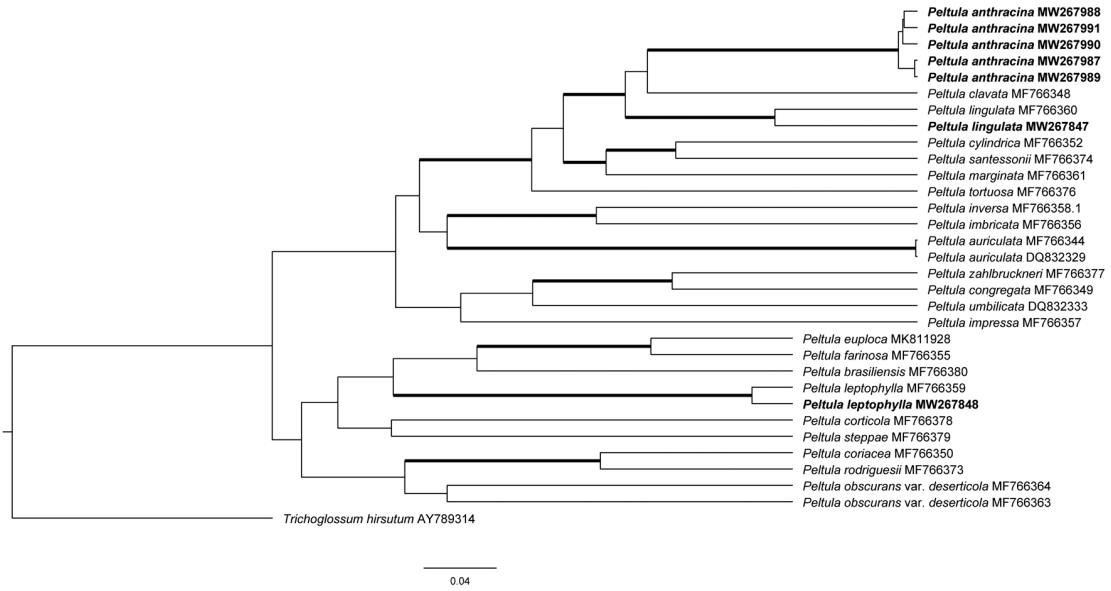

Figure 1 – Bayesian Maximum Clade Credibility tree based on the nuITS dataset showing the phylogenetic relationships among *Peltula* species. Branches with posterior probabilities (PP) > 0·95 are in bold. Sequences obtained in this study are in bold.

sequence from Mexico, and the *P. lingulata* sequence from Mato Grosso do Sul clustered with the *P. lingulata* sequence from South Africa (Kauff *et al.* 2018).

New species

Peltula anthracina Kitaura, *sp. nov.* Fig. 2a-d Type: BRAZIL, MATO GROSSO DO SUL STATE: Corumbá Municipality, Pantanal from Paraguay subregion, RPPN Rumo ao Oeste, Gaiba Bay, 17°44'18.10"S, 57°41'27.80"W, 91 m alt. 28.XI.2010. *T.H. Stephanello et al. 384* (holotype CGMS). MycoBank: 839130

The epithet refers to the color of charcoal, or blackish as the charcoal. The color of the thallus is black as most of the *Peltula* species. We highlighted the importance of forest conservation, and we repudiate the destruction of the biomes from Mato Grosso do Sul through fire, which has literally transformed the native vegetation into charcoal.

Thallus peltate to squamulose, black under fluorescent light, matt, opaque, greenish-black under the stereomicroscope. Squamules 0.3–0.6 mm, simple or overlapping, 1–5(–8) squamules, simple to irregular branched, plane to concave, upper cortex smooth to the naked eye and 20x magnification; apices rounded to irregular, plane, without ornamentation; lateral margins irregular, plane to ascending, without ornamentation. Isidia and lobules absent. Thallus attached by canaliculus, adpressed on substrata, constituted cylindrical hyphae and cyanobacteria, cyanobacteria between the squamules. Apothecia immersed to adnate, 0.3–0.6 mm diam., laminal, one per squamule, disc concave, brownish; margin of apothecia and amphithecia concolorous with thallus, without ornaments. Pedicel absent. Pycnidia absent.

Anatomy. Thallus 110–225 µm, cortex subparaplectenchymatous, 15–25 µm thick, 3–5 (–7) layers of irregular cells; cells 2.5–5.0 µm diam. Medullar layer with clusters of cyanobacteria, usually near the upper cortex, green, spherical, up to 7.5 µm diam., central part hollow, scattered hyphae with some cyanobacteria. Apothecia with hymenium 75 µm thick; subhymenium and hypothecium indistinct, 25 µm thick; proper exciple absent; thalline exciple with subparaplectenchymatous cortex and cyanobacteria, apothecia cortex like the thallus cortex. Asci 45–50 × 10 µm, clavate, numerous ascospores per ascus, apices acute. Ascospores spherical, 2.5–4.0 µm diam., simple, hyaline.

Examined material: Corumbá municipality, Pantanal from Paraguay subregion, RPPN Rumo ao Oeste, Gaiba Bay, 17°44'18.10"S, 57°41'27.80"W, 91 m alt. 28.XI.2010. Leg. *T.H. Stephanello et al. 382, 383, 386, 385* (paratypes CGMS).

Peltula anthracina is characterized by the thallus with simple to overlapping squamules,

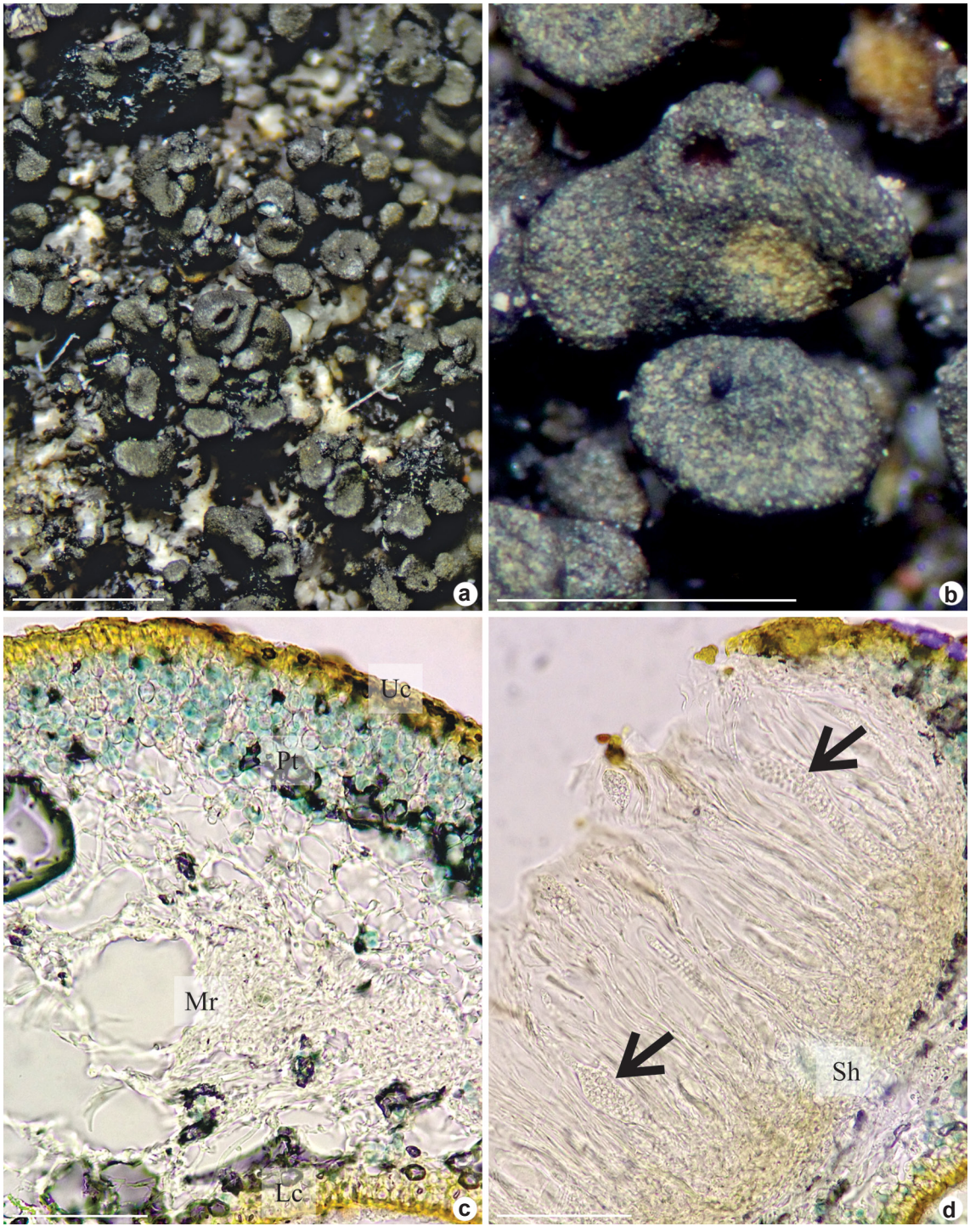

Figure 2 – a-d. *Peltula anthracina* – a. distribution of the *P. anthracina* squamules; b. detail of the squamule with immerse apothecia; c. transversal section of the thallus, with upper cortex (Uc), photobiont (Pt), medullar region (Mr), and lower cortex (Lc); d. diametral section of the apothecia, highlighting the subhymenia (Sh) and asci with innumerous ascospores (arrow). (a-b. stereomicroscope images; c-d. light microscope images).

subparaplectenchymatous cortex, constituted with 3–5 layers of isodiametric cells, and immersed to adnate apothecia. The thalline exciple is composed of subparaplectenchymatous cortex.

Peltula clavata and *P. lingulata* are genetically close to *P. anthracina*. *Peltula michroacanensis* (de Lesd.) Wetmore is not genetically close but has a squamulose thallus without ornaments as well as *P. anthracina*. Therefore, the upper cortex is not developed in the first species (Wetmore 1971), whereas it has 3–5 layers of isodiametric cells in the second species.

The thallus of *Peltula obscurans* var. *deserticola* (Zahlbr.) Wetmore is squamulose and has 105–240 µm thick, whereas *P. anthracina* has peltate and squamulose thallus with 110–225 µm thick. The cortex of *P. obscurans* var. *deserticola* is not developed and the *P. anthracina* cortex is subparaplectenchymatous.

Peltula leptophylla (Vain.) Büdel & M. Schultz, The Lichenologist: 324, 2018.
Basionym: *Heppia leptophylla* Vain., Acta Soc. Flora Fauna Fenn. 7(1): 216, 1890. Type: BRAZIL, RIO DE JANEIRO: "supra rupem graniticam litoralem, 1885, leg. *E. Vainio n. 135*", (holotype UPS (L-013539), isotype TUR-VAIN 12474 (Lich. Bras. Exs. 1891)).
Examined material: Corumbá municipality, Pantanal from Paraguai subregion, RPPN Rumo ao Oeste, Guaiba Bay, saxicolous, 17°44'18.10"S, 57°41'27.80"W, 91 m alt., 28.XI.2010, leg., *T.H. Stefanello et al. 379*, pr. p. (CGMS, GenBank accession MW267848).

Peltula leptophylla is characterized by squamulose thallus, 1.0–1.5 mm long., crenulate and laciniate (Vainio 1890).

The species was described originally by Vainio (1890) from a specimen collected in Rio de Janeiro, but the first generated sequence belongs to a Mexican specimen (Kauff *et al.* 2018).

Peltula leptophylla has been reported for the first time to Mato Grosso do Sul state.

Peltula lingulata (Vain.) Swinscow & Krog, Norweg. J. Agric. Sci. 26(3): 220, 1979.
Basionym: ≡*Heppia lingulata* Vain., Beih. Nova Hedwigia 37: 43, 1898. Type: UGANDA AND DEMOCRATIC REPUBLIC OF THE CONGO: the Ruwenzori mountains, located on the border, "In rupe gneissacea ad Mbuyuri", s/d (holotype TUR?).
Examined material: Ladário, Band'Alta farm, 19°10'59.00"S, 57°32'19.70"W, 208 m. alt., exposed, on lateritic bench, 4.IX. 2010. *L.S. Canêz et al. 3364* (CGMS 31717, GenBank accession MW267847).

Peltula lingulata is characterized by tongue-shape squamules, with 0.3–0.4 × 1.0–2.0 mm (Vainio 1898).

The *Peltula lingulata* type was collected originally in Ruwenzoni mountains, without more information, and described for the African continent. The first generated sequence belongs to a South African specimen.

The *P. lingulata* already was reported for Brazil in the state of Rio Grande do Sul (Fleig 1995), but this is the first record for the state of Mato Grosso do Sul.

Discussion

Phylogenetic studies have altered the circumscriptions, taxonomy, known distribution, and species numbers of inconspicuous cyanolichens (Ertz *et al.* 2017; Fryday *et al.* 2017; Park *et al.* 2018; Liu *et al.* 2018). Groups that have been classified according to morphological characters often do not correspond to phylogenetic patterns. An example is *Peltulaceae*, which was proposed with three genera, *Peltula*, *Phyllopeltula* and *Neoheppia*, separated mainly by the thalli type, but which proved to be monogeneric after a six-loci analysis. In this sense, Kauff *et al.* (2018) underlined that new species of *Peltula* should be carefully proposed with a clear connection between the defined operational taxonomic units (OTU) and the morphological analyses. Therefore, *Peltula anthracina* is proposed here as new to science based on an integrative approach through morphological, anatomical and molecular analyses.

The *P. anthracina* specimens were preliminarily determined as *P. auriculata* (Canêz *et al.*, personal communication), but they do not have the characteristic of ear-shape squamules. Furthermore, *P. auriculata* have thallus with olive-brown color 200–300(–500) µm thick, and the upper cortex is composed of globose hyphal cells 0–5 µm (one layer of cells) thick (Schultz *et al.* 2000), whereas *P. anthracina* has a thallus colored black to greenish black, 110–225 µm thick, and the upper cortex composed by subparaplectenchymatous cells that are 15–25 µm (3–5 layers of cells) thick. *Peltula clavata* has a thallus minutely subfruticose and the surface covered by scattered minute isidia (Wetmore 1971), whereas *P. anthracina* has a thallus peltate to squamulose and the upper surface without

ornaments; and *Peltula lingulata* is characterized by a subfruticose squamulose thallus tongue-shaped, which differs from the squamulose thallus of *P. anthracina*. The generated sequences of *P. anthracina* also resulted in a distinct lineage that separated from the *P. clavata* (MF766348), *P. lingulata* (MF766360 and LSC3364) and *P. auriculata* lineages, corroborating the morphological and anatomical analysis.

The species were found in a wet region but with arid microclimatic features in which *Peltula* species are typically found. The Pantanal is a dynamic ecosystem with different phytophysiognomies that are shaped and influenced by a flood regime, where seasons are well-defined by wet (October-April) and dry (May-September) seasons. This region is influenced by the Amazon, Atlantic Forest, Cerrado and Chaco, where high biodiversity is found for different groups of animals, plants (Nunes da Cunha *et al.* 2007; Junk *et al.* 2014), and possibly lichenized fungi. However, the dry season and mostly the anthropogenic activities associated with extensive agriculture has suggested that the Pantanal biome, though an elevated occurrence of fires, (Oliveira-Junior *et al.* 2020) is associated with changes in the vegetation shape, structure, and composition (Araújo *et al.* 2017).

With this study, the number of *Peltula* species has doubled for the Mato Grosso do Sul, and new field studies should reveal even greater lichen biodiversity for the region. Therefore, the conservation of natural vegetation is key to preserving the diversity of lichens, and ecological studies are key to understanding the response of these organisms to climate change in which the Pantanal of Mato Grosso do Sul is undergoing.

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