

An integrative study of *Parmotrema* (*Parmeliaceae*) from the Brazilian Cerrado

Introduction

Lichenized fungi, popularly called lichens, are symbiotic associations between one or more fungi and green algae or cyanobacteria, or both (Spribille et al., 2016; Lendemer et al., 2019). Among the more than 19.000 species known of lichenized fungi, *Parmeliaceae* is the most diverse family, containing about 2.700 species, and having the southern hemisphere as the main center of its diversification (Thell et al., 2012; Lücking et al., 2017). *Parmotrema* A. Massal. is the third-largest genus of *Parmeliaceae* (ca. 350 spp.), highly diverse in regions with tropical and subtropical climate (Blanco et al., 2005; Lücking et al., 2017). In Brazil, a country with a great diversity of *Parmeliaceae*, about 90 species of *Parmotrema* are known (Spielmann, pers. comm.).

The genus *Parmotrema* was morphologically segregated from *Parmelia* s.l in 1860 by Massalongo due to the presence of perforated apothecia (Massalongo, 1860). However, later molecular studies showed the great morphological and chemical variation of the genus, grouping species previously classified as different genera under the name *Parmotrema* (Blanco et al., 2005; Divakar et al., 2017). Molecular biology approaches, as the DNA barcoding, increased the understanding of the lichenized fungi diversity, mainly within groups with cryptic species, species complexes, or those with morphological and chemical plasticity (Hebert et al., 2003; Leavitt et al. 2016). However, one of the limiting factors of the success rates of DNA barcoding is the correct taxonomic identification of the specimens sequenced and the availability of region-specific sequences in public databases (Begerow et al., 2010; Leavitt et al., 2014).

Such difficulty in using the DNA barcode approach in places with little knowledge about the diversity of lichens, associated with the lack of a database of molecular sequences about these species is the reality of some megadiverse countries, like Brazil. Among the diverse vegetation formations found in Brazil, the Cerrado, the second largest biome in the Brazilian territory, has a great diversity of flora and fauna, as well as being considered one of the world's biodiversity hotspot (Myers et al., 2000). Also, the west portion of the Cerrado is a transitional area, mixing the Amazon biomes to the north with Pantanal wetlands and Atlantic Forest to the southwest (Werneck, 2011), reflecting a strategic location for a high diversity of lichens. Studies on *Parmeliaceae* in the Cerrado are rare, being restricted to its northeast and southeast regions (Marcelli 1993; Jungbluth 2006; Cunha, 2012).

Thus, this study aims to use an integrative approach based on morphological and chemical characteristics associated with the DNA barcoding (using the nuITS region) to identify the *Parmotrema* species found in Cerrado, but focusing in the west part of it, especially the states of Mato Grosso and Mato Grosso do Sul. In addition, to reinforce the support values of the branches and avoid inconsistencies in the phylogenetic positions of the species, we will also generate sequences from other DNA regions, as the small mitochondrial subunit (mtSSU) and the large subunit (nuLSU).

Material and Methods

The *Parmotrema* samples will be obtained from collections in the states of Mato Grosso and Mato Grosso do Sul, as well as from specimens deposited at the CGMS Herbarium, which has the largest collection of lichens from Cerrado. However, molecular studies will be performed only on fresh specimens. Morphological identifications will be performed using a stereomicroscope and optical microscope; and the recognition of the chemical components of the samples will be carried out by thin-layer chromatography (TLC) (Orange et al., 2010).

For the molecular studies, the extraction of genomic DNA will be performed using the Wizard® Genomic DNA Purification Kit (Promega), following the protocol of the manufacturer. The nuITS, mtSSU, and nuLSU DNA regions will be amplified using specific primers, and the PCR products will be sequenced. The sequences generated will be compared to those available at GenBank, using for phylogenetic analysis those that are closest.

For multiple sequence alignments, we will use the MAFFT v7 program (Katho and Standley, 2013). We will verify the phylogenetic position and monophyly of the species through the construction of phylogenetic trees using the Markov Bayesian Monte Carlo (B / MCMC), and

Maximum Likelihood (ML) approaches. Phylogenetic trees based on ML will be built using the RAxML v8 program (Stamatakis, 2014), using the GTRGAMMA model with 1000 bootstrap replications. For the Bayesian inference, we will use the program MrBayes 3.2.7 (Ronquist et al. 2012) with four chains of 10 million generations.

Three approaches of sequence analyses of the nuITS region will be used to assist the delimitation of *Parmotrema* species: (1) phylogenetic position; (2) intra- and interspecific genetic distances (Del-Prado et al., 2010); and (3) automatic delimitation models as ABGD (Puillandre et al., 2012) and PTP (Zhang et al., 2013).

The study will be developed with the support of specialists in different areas of the lichenology, such as Ph.D. Adriano Spielmann (*Parmotrema* taxonomy), Ph.D. Andre Aptroot (lichen taxonomy), Ph.D. Neli Honda (lichen chemistry), Ph.D. Luciana Canêz (*Parmeliaceae* taxonomy) and Ph.D. Aline Lorenz (molecular biology).

Objectives

We expect to help the understanding of the diversity of *Parmotrema* from the Brazilian Cerrado, as well as to find and describe new species belonging to the genus. Also, we hope to contribute to the scientific community, developing an integrative study that provides taxonomic (morphological and chemical) and molecular data of the species found. Since Brazil, even though it is a megadiverse country in species of *Parmeliaceae* according to taxonomic studies punctual out over the years, little is still known about its real diversity, and there is still a lack of a reliable public database of DNA sequences from Brazilian specimens.

Other relevant information

I have a Ph.D. fellowship from the Brazilian government (agency: CAPES - Brazilian Federal Agency for the Support and Evaluation of Graduate Education). However, this financial aid only subsidizes my personal expenses. Therefore, for the execution of my thesis, there is no financial source to help with project expenses. The study of *Parmotrema* in the Brazilian Cerrado is part of one of the chapters of my thesis, and the financial assistance provided by the International Association for Plant Taxonomy (IATP) will be extremely important for its realization because with this money we will conduct the molecular analyses of *Parmotrema*, thus contributing to the development of an accurate work for its later publication.

Literature citations

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