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Reference collection of plant phytoliths from the *Caatinga* biome, Northeast Brazil

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ABSTRACT

Located in northeastern Brazil, the *Caatinga* biome is characterized by a semi-arid climate with vegetation adapted to high temperatures, low precipitation and uneven rainfall distribution throughout the year. The unjustified belief that the *Caatinga* ecosystem is poor in biodiversity and endemism, makes it the most undervalued and botanically unknown Brazilian biome, despite there being several phytogeographical areas, and a significant number of rare endemic taxa. Phytoliths are biomineralizations of silica (Si) that precipitate inside or between plant cells during the process of photosynthesis or evapotranspiration, giving the plant organism a series of benefits and evolutionary advantages. In this study, 33 species from 16 different families typical to the region were analyzed, in order to establish modern reference collections enabling further environmental reconstitution studies using phytoliths as proxy, contributing, therefore, to improved knowledge on the *Caatinga* biome. The results showed that *Caatinga* plants produce a large amount of biomineralizations, the most prevalent being tracheids, followed by polyhedral, globular granulate, elongate and trichomes (lanceolate hair cells), among others. The types of silicification found may be related to the environmental characteristics of the *Caatinga*, whereby plants develop mechanisms against desiccation. The deposition of Si in plant cells may be a survival strategy in this environment. Despite some limitations, phytoliths have been shown to be promising tools for improving knowledge on the vegetation of this region, as well as for use in paleoenvironmental studies in the biome.

1. Introduction

In Brazil there are no deserts, but there is a semi-arid region, with unique species and characteristics, called *Caatinga*. The name *Caatinga* has a Tupi-Guarani origin and means white forest. This name appropriately characterizes the physical aspect of the vegetation during the dry season, when the leaves fall and only the bright white trunks of trees and shrubs remain on the dry landscape (Albuquerque and Bandeira, 1995).

It is the only exclusively Brazilian ecosystem, composed of a mosaic of dry forests and shrub vegetation (steppe savanna), with enclaves of tropical mountainous rainforest (Tabarelli and Cardoso da Silva, 2003). As it does not show the green exuberance of the tropical forests, which are so important in Brazil, and due to the phytophysiognomy dominated by cacti and shrubs, *Caatinga* vegetation unduly suggests low diversification of fauna and flora. Among the Brazilian biomes, the *Caatinga* is probably the most undervalued and poorly known scientifically (Coe et al., 2017), which is due to an unjustified belief that the *Caatinga* is the result of the modification of another plant formation, associated with a very low diversity of plants, without endemic species and highly modified by human actions (Giulietti et al., 2002). Although quite altered, particularly on the plains, the *Caatinga* is a biome of wide biodiversity, with biological relevance and peculiar beauty, especially with respect to the multiplicity of plant communities, formed by a range of combinations of soil types and microclimatic variations. In addition, there is a significant proportion of rare endemic taxa, many of which

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are commonly used by the population for their therapeutic properties.

The strategies used by species to survive during periods of rainfall are numerous and of great interest. Many plants lose their leaves to reduce water loss during periods of drought stress; several herbs have annual life cycles, growing and blooming in the rainy season; cacti and bromeliads accumulate water in their tissues and there is a predominance of shrubs and small trees on the landscape (Leal et al., 2003). The strongly xerophytic character of native *Caatinga* plants demonstrates beyond any doubt that the semi-arid nature of the region does not come from centuries ago, but probably developed millions of years ago.

Phytoliths are microscopic particles formed as a result of root absorption of silicic acid [$Si(OH_4)$] from the soil solution, followed by deposition and biomineralization, which are then incorporated into the soil through decomposition of plant remains (Piperno, 1988). These siliceous structures can be found inside or between cells of many living plants. In addition, they are generally found in the finer fractions of the soil, where the entire plant or tissue parts have decomposed (Piperno, 1988). After being recovered from the soil, these microfossils have configurations that can be associated with their original vegetation. Phytoliths can be classified by taxonomy or by their physical characteristics and are usually studied through analysis of their assemblages. After identifying phytolith morphotypes, the calculation of phytolyth indices helps to infer some important environmental parameters, such as tree density and adaptation to drought or water stress (Bremond et al., 2005a, b; Messager et al., 2010; Coe et al., 2012; Coe and Osterrieth, 2014; Deya et al., 2015; Issaharou-Matchi et al., 2016; Li et al., 2018).

The objective of this study is to analyze the presence of phytoliths in plant samples from different phytophysiognomies of the *Caatinga* biome located in the states of Rio Grande do Norte and Ceará, with the aim of improving understanding of this vegetation and its biomineralization process of silica, in order to establish modern reference collections enabling further environmental reconstitution studies in this biome using phytoliths as proxy, thus contributing to better knowledge of the *Caatinga* biome.

2. Materials and methods

2.1. Study area

Caatinga is the only large Brazilian natural region whose limits are entirely restricted to the national territory, occupying mainly the Northeast Region. It extends from 2° 54′ to 17° 21′ S and from 38° to 43 °W, comprising an area of approximately 800,000 km^2, representing 70% of the Northeast Region and 11% of Brazilian territory (Fig. 1).

In Northeast Brazil, most *Caatinga* areas are located in inter-plateau depressions (Ab'Sáber, 1974). In general, this province extends over undulating pediplanes (Andrade-Lima, 1981), exposed from Cretaceous or Tertiary sediments covering the Brazilian pre-Cambrian basaltic shield. The vegetation varies with the topography, with savanna vegetation on top of the plateaus, coastal plateaus, tropical or semi-deciduous forests at the top of the mountains and dry forests or arboreal *caatinga* on the slopes and inselbergs (Prado, 2003).

As a result of the origin of the *Caatinga* substrate, the soils are stony and shallow, with the depths of the background rock sparsely decomposed and many outcrops of massive rocks (Tricart, 1961; Ab'Sáber, 1974).

The climate of the Northeast Region, classified as semi-arid tropical, or as Bsh type, according to the Köppen classification, is characterized by high temperatures and sparse irregular rainfall, limited in most of the area to a very short period of the year (2–3 months). These rains, often of great intensity, lead to flooding of reservoirs and watercourses, as well as intense surface runoff, with little or no infiltration, accentuating and aggravating soil erosion every year. Catastrophic phenomena like droughts and floods are very common. However, what most characterizes the region is the complete absence of rain in some years (Nimer, 1972).

In the *Caatinga*, the climate exerts a great influence on the establishment of vegetal formations. *Caatinga* vegetation always seems to be associated with high water stress, indicating a complex network of vegetation types determined by climatic factors (Reis, 1976).

The vegetation of the *Caatinga* has about 5000 species of angiosperms with approximately 300 endemic species (Giulietti et al., 2002). The interaction of factors such as soil type, altitude and precipitation index permits the recognition of different landscape units, forming a mosaic of diverse vegetation. In general, the *Caatinga* is characterized by a type of xeromorphic vegetation composed of low growth forests, often with a discontinuous canopy and deciduous foliage in the dry season with trees and shrubs commonly endowed with thorns or spines. The most common plant families are Fabaceae, Euphorbiaceae, Malvaceae, Asteraceae and Cactaceae, among others. Trees and shrubs are the predominant lifeforms and in most of the biome the canopy does not exceed 8 m in height. The herbaceous stratum is composed mainly of annual plants and is absent for about seven months of the year, appearing more frequently in the rainy season through seed germination in annual species or regrowth from storage structures (geophysical species) (Coe and Sousa, 2014).

2.2. Materials

The samples were collected in the states of Ceará and Rio Grande do Norte, in the area occupied by the *Caatinga* biome and belonging to the Northern Sertaneja Depression Ecoregion (Fig. 1). The species were identified and deposited in the herbarium of the Federal Rural University of the Semi-Arid Region. Samples were collected from 33 plants of 16 families (Table 1 and Fig. 2) using the most common *Caatinga* species in the collection area, according to Leal et al. (2003), as criterion for selection.

2.3. Methods

2.3.1. Extraction of phytoliths

For the extraction of phytoliths, 3 g of leaves were used. The extraction was carried out by removing the organic matter with a solution of 1 part nitric acid (HNO_3) to 4 parts sulfuric acid (H_2SO_4) and heating the material for 3 h. Subsequently 10–20 ml of Hydrogen Peroxide (H_2O_2) was added.

2.3.2. Counting

Phytoliths were mounted on microscope slides in pure clove oil for 3D observation and Entellan® for counting with 500x and 630x magnification. The same amount of material (25 µl) was used on all slides, which were observed in their entirety. Phytolith production was classified, based on the counting of 3 horizontal transects, as very high (> 300 phytoliths), high (100–300 phytoliths), medium (50–100 phytoliths), low (10–50 phytoliths) or rare (< 10 phytoliths).

2.3.3. Types of phytoliths

Phytoliths were classified according to the classification of Twiss (1969, 1992); expanded and supplemented by descriptions of phytolith forms from Mulholland (1989), Fredlund and Tieszen (1994), Kondo et al. (1994), Alexandre et al. (1997), Strömberg (2004) and Mercader et al. (2009) and named according to the International Code for Nomenclature of Phytoliths 1.0 (Madella et al., 2005). The types of biomineralizations that constituted > 50% of the sample were classified as predominant; 10 to 50% as some; and < 10% as rare.

2.3.4. Multivariate analyses

Principal component analysis (PCA) was performed using the Fitopac2 program (Shepherd, 2010), version 2.1, using the presence and absence of the main types of phytoliths found in the analyzed