Online Perspectives: Biology & Health
Dec/2022, v.12, n.43, p. 1 -17
ISSN: 2236-8868 (Online)
DOI: 10.25242/8868124320222618

# RICHNESS INDEX OF ENDOPHYTIC FUNGI FROM RIO DE JANEIRO RESTINGA

*Vicente Mussi-Dias e Maria das Graças Machado Freire*

## ABSTRACT

MUSSI-DIAS, V.; FREIRE, M.G.M. Richness index of endophytic fungi from Rio de Janeiro restinga. **Online Perspectives: Biology & Health**, v.12, n.43, p. 1 - 17, 2022.

The preservation of the Restinga biome is of great importance, both ecologically and socially, and this ecosystem is threatened mainly by anthropic actions, with little known about its fungal diversity. Endophytic fungi, besides contributing to the prospecting of new efficient compounds in industry and agriculture, can indicate the richness and abundance of species in the areas where they are found. Thus, the objective of this work was to carry out an inventory of endophytic fungi prospecting from plant species in the Açu, Gargaú, Grussaí, and Jurubatiba restingas, located in the north of Rio de Janeiro State, Brazil. Leaves of 78 plant species were collected, from which 1787 isolates were obtained in culture medium, identified in 36 different genera. After analyzing the data of richness and abundance of the fungi found, the biodiversity of the four restingas was estimated, indicating a high diversity in the ecosystem, being the restinga of Açu the most preserved and the restinga of Gargaú the most degraded when evaluated by Margalef Richness Index.

**Keywords:** Biodiversity; Local-regional comparisons; Diversity indices.

[1]Researcher Clínica Fitossanitária - Laboratório de Entomologia e Fitopatologia (LEF) – Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF; Av. Alberto Lamego, 2000 – Parque Califórnia, 28013-602 – Campos dos Goytacazes, RJ, Brasil.
[2]Researcher Laboratório de Química e Biomoléculas (LAQUIBIO)/Centro de Pesquisa e Pós-graduação (CPPG)/ Institutos Superiores de Ensino do CENSA – ISECENSA, Rua Salvador Correa, 139, Centro, Campos dos Goytacazes, RJ, Brasil
(*) e-mail: vimdias@yahoo.com.br

Received: 23/11/2022 Accepted: 17/01/2023 Published online: 04/03/2023

Revista Perspectivas Online: Biológicas & Saúde
Dez/2022, v.12, n.43, p. 1 -17
ISSN: 2236-8868 (Online)
DOI: 10.25242/8868124320222618

# ÍNDICE DA RIQUEZA DE FUNGOS ENDOFÍTICOS DE RESTINGA DO RIO DE JANEIRO

*Vicente Mussi-Dias e Maria das Graças Machado Freire*

## RESUMO

MUSSI-DIAS, V.; FREIRE, M.G.M. Índice da riqueza de fungos endofíticos de restinga do Rio de Janeiro. **Perspectivas Online: Biológicas & Saúde**, v.12, n.43, p. 1 - 17, 2022.

A preservação do bioma Restinga é de grande importância, tanto ecológica quanto social, estando este ecossistema ameaçado principalmente por ações antrópicas, com o pouco que se sabe sobre sua diversidade de fungos. Os fungos endofíticos, além de contribuir com a prospecção de novos compostos eficientes na indústria e na agricultura, podem indicar riqueza e abundância de espécies nas áreas onde se encontram. Assim, o objetivo deste trabalho for realizar um inventário de prospecção de fungos endofíticos de espécies vegetais nas restingas do Açu, Gargaú, Grussaí e de Jurubatiba, localizadas no norte do estado do Rio de Janeiro, Brasil. Coletaram-se folhas de 78 espécies vegetais, das quais foram obtidos em meio de cultura 1787 isolados, identificados em 36 diferentes gêneros. Após análise dos dados de riqueza e abundância dos fungos encontrados, estimou-se a biodiversidade das quatro restingas, indicando alta diversidade no ecossistema, sendo a restinga do Açu a mais preservada e a restinga de Gargaú a mais degradada quando avaliadas pelo Índice de Riqueza de Margalef.

**Palavras-chave:** Biodiversidade; Comparações locais-regionais; Índices de diversidade.

[1]Clínica Fitossanitária - Laboratório de Entomologia e Fitopatologia (LEF) – Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF; Av. Alberto Lamego, 2000 – Parque Califórnia, 28013-602 – Campos dos Goytacazes, RJ, Brasil.
[2]Laboratório de Química e Biomoléculas (LAQUIBIO)/Centro de Pesquisa e Pós-graduação (CPPG)/ Institutos Superiores de Ensino do CENSA – ISECENSA, Rua Salvador Correa, 139, Centro, Campos dos Goytacazes, RJ, Brasil
(*) e-mail: vimdias@yahoo.com.br

Data de recebimento: 23/11/2022 Aceito para publicação: 17/01/2023 Data de publicação: 04/03/2023

## 1. INTRODUCTION

Brazil has enormous biodiversity, which allows it to be characterized as a great potential of natural resources. However, its unsustainable growth has led to the loss of most of its biomes (Amazon, Atlantic Forest, Cerrado, Caatinga, Pantanal, and Pampa) and the biological groups that make up the Brazilian biodiversity, still little explored, including endophytic fungi (ALEIXO et al., 2010; PILATTI et al., 2011).

Endophytic fungi are defined as the fungi which live the whole or part of their lifecycle inhabiting intra-and/or inter-cellularly inside the healthy plants, naturally causing no apparent symptoms of disease (KAMEL et al., 2013).

Some studies on endophytes in different Brazilian biomes have already been published. In the Amazon, more specifically in the state of Tocantins, endophytic microorganisms associated with *Bellucia grossularioides* (L.) Triana was isolated and tested against the growth of bacteria of the genus *Staphylococcus* Rosenbach 1884 and fungi of the genera *Candida* Berkhout and *Aspergillus* P. Micheli ex Haller (MARTINS et al., 2016). Vaz et al. (2018) also investigated the fungal endophytic communities in two preservation areas (Anavilhanas National Park - ANP and Caxiuanã National Forest - CNF), in the same biome.

In Pantanal biome, Galvão et al. (2014) verified the presence of dark septate endophytic fungi collected in the Pantanal of Poconé. In the Cerrado, Detmann et al. (2008) studied the mycorrhizal association of different native species collected in the National Forest of Paraopeba-MG. Cafêu, et al. (2005) isolated endophytes from *Palicourea marcgravii* St. Hil., with emphasis on studies with the fungus *Xylaria* Hill ex Schrank, due to the activity of its metabolites against the phytopathogenic fungi *Cladosporium cladosporioides* (Fresen.) G.A. de Vries and *C. sphaerospermum* Penz.

In Caatinga, in Ceará state, Gonçalves et al. (2016) studied the pathogenicity of more than 70 fungi isolated from plants and Pires et al. (2015) studied fungi from the cacti *Cereus jamacaru* DC, *Opuntia ficus-indica* (L.) Mill. and *Pilosocereus gounellei* (F.A.C. Weber) Byles & G.D. Rowley, from the Brazilian Dry Tropical Forest against different bacteria. Meanwhile, in the Pampa biome, Varreira (2018) evaluated the enzymatic production of four genera of the fungi *Acremoniun* Link., *Aspergillus*, *Fusarium* Link. and *Penicillium* Link. from that vegetation.

And finally, in the Atlantic Forest biome, Vaz et al. (2009) examine the antimicrobial activity of endophytic fungi isolated from 54 species of Orchidaceae collected on The Ecological Reserve of Caraça's Sanctuary (Minas Gerais). Gomes and Fortuna (2020) identified endophytic microfungi associated with two species of bromeliads, *Aechmea alba* Mez and *Vriesea procera* Mez, in the state of Bahia.

In this same Atlantic Forest biome is found, among others, the Restinga ecosystem (MARTINS, 2012), "defined as sandy coastal plain of marine origin, covering beaches, sandy ridges, dunes, depressions between ridges and depressions between dunes with their respective marshes, ponds, swamps, and lagoons, whose vegetation and fauna are adapted to local environmental conditions starting at the seashore and may reach the first elevations of the Serra do Mar" (CONAMA 2009; CONAMA, 2012).

In the North Fluminense region, these restingas compose a unique environment with native plants adapted to the harsh conditions of this environment, such as strong winds, high temperatures luminosity, and higher salinity, where microbial communities are little explored

(FREIRE et al., 2016; MUSSI-DIAS et al., 2018; BEZERRA et al., 2020). Such conditions, may induce plant-fungus relationship adaptations and provide the discovery of new fungal species, as well as metabolites produced by them, because the numerous applications of secondary metabolites produced by endophytic fungi, have raised interest in natural products for applications in industry (RANA et al., 2019) constituting, therefore, another need to know and disclose the presence and relationship between species in areas little explored.

Combining the plant diversity of the restingas with the interests of mycological prospection for scientific purposes, the objective of this work was to explore the endophytic fungi communities of different native plants from the restingas of the North Fluminense region in order to start an inventory of this unknown and still little explored diversity, as well as to apply the richness and abundance of these fungi to distinguish between these areas of the ecosystem.

## 2. MATERIAL AND METHODS

### 2.1. Sample collection and endophyte isolation

The plants were collected between the months of June 2013 and June 2015 in four distinct areas of restinga in the northern Fluminense, between the municipalities of Macaé (22° 22' 18" South and 41° 47' 9" West) and São João da Barra (21° 38' 24" South and 41° 03' 03" West).

At collection, the selected leaf samples were alive, healthy, or without apparent lesions and adhered to the plant. All plant species were compared and identified based on species already deposited in the Herbarium of the Botanical Garden of Rio de Janeiro.

### 2.2. Isolation and identification of endophytic fungi

The isolation of endophytic fungi followed the methodology described by Freire et al., 2017. Fresh leaves were collected from healthy plants, washed with running tap water and cut into 0.6 cm diameter pieces, which were surfacesterilized with sequential washes of 70% EtOH (2 min.), 10% bleach (1% NaOCl; 2 min.), 70% EtOH (30 s) and sterile distilled water (MUSSI-DIAS, et al., 2012). The segments were plated onto 2% potato dextrose agar (PDA) and incubated at room temperature (aproximadamente 28º C) with approximately 12 h light/dark cycles. The morphological identification of the isolates was carried out by observations of the structures of the axenic colonies. Samples of all fungi isolates were deposited in the culture collection in the Laboratory of Chemistry and Biomolecules (LAQUIBIO/ISECENSA), Brazil, which are kept under sterile distilled water (CASTELLANI, 1964).

The identification of the fungal specimens, at the genus level, was performed based on the joint morphology of the reproductive and colonial structures according to specialized literature, made with the aid of optical light and stereoscopic microscopes (SUTTON, 1980; HANLIN & MENEZES, 1996; BARNETT & HUNTER, 1998).

### 2.3. Statistics

The data regarding the sandbanks collected plant species, isolated and identified endophytic fungi were prepared in a Microsoft Excel® spreadsheet for frequency analysis and graphs preparation. To evaluate the diversity of fungal isolates obtained from plant samples in the four collection areas, the PAST software (HAMMER et al., 2001) was used, calculating the Margalef Richness Index (d), by the equation: $d = (S-1)/\ln(N)$, where S = number of species (genus) and N = number of individuals (fungal isolates).

## 3. RESULTS AND DISCUSSION

The number of sampled plant species varied in each sandbank: Grussaí (33), Açu (32), Gargaú (37), and Jurubatiba (31). In all plants, endophytic fungi cultivable in culture medium were obtained, whose relative frequency ranged from 9 to 39%, depending on the sandbank explored. Higher numbers of isolates were obtained from plants collected in Restinga do Açu, Jurubatiba, Grussaí, and Gargaú, respectively (Figure 1). Brazilian biomes such as Caatinga (SANTOS, 2012), Amazon Rainforest (BANHOS et al., 2014), and Atlantic Forest (OLIVEIRA et al., 2014), have been little studied, but in all the surveyed plants endophytic fungi have been found, varying in frequency and richness, usually related to studies of bioprospecting of biotic compounds produced by these microorganisms, not having been found in the literature studies considering the relationship of a large number of plant species and their endophytic fungi in the same ecosystem.

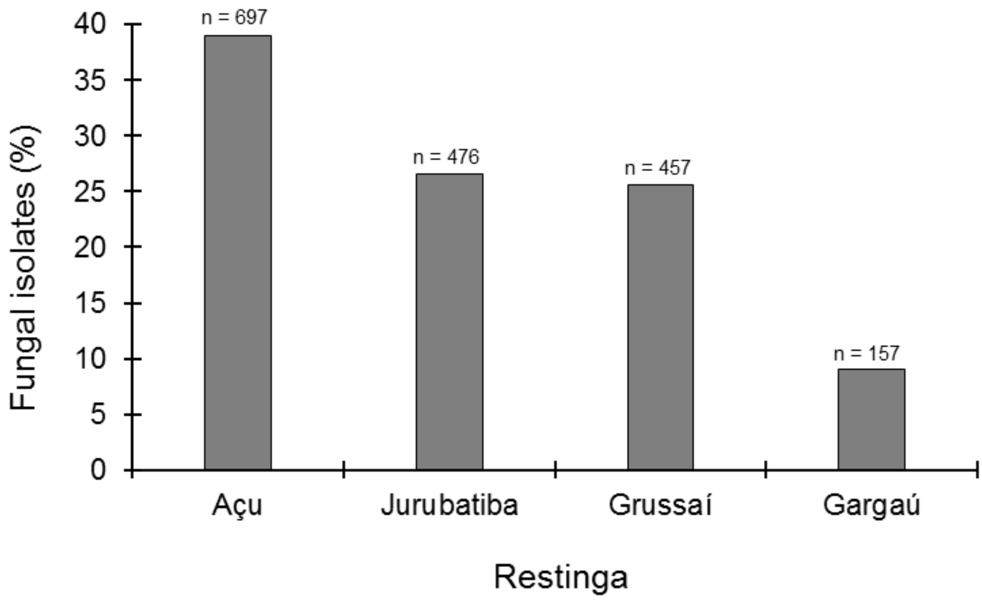


Figure 1: Quantitation of endophytic fungal isolates obtained from four restinga ecosystems in northern Rio de Janeiro State, Brazil.

In our survey, we obtained and recorded a total of 1787 endophytic fungal isolates from 78 plant species belonging to 39 botanical families (Figure 2A), found in the explored restingas (Table 1).

By morphological analysis of most isolates and by the similarity between colonies of these fungi it was possible to group them into 36 different genera. Of this total, half were identified as *Nigrospora* Zimm or as non-sporulated species. The other half, comprised other genera (Figure 2B; Figure 3).

The fungus *Nigrospora* is commonly isolated from the environment and is considered a fungus with cosmopolitan distribution and a wide range of ecological niches, from endophytic and saprophytic (THALAVAIPANDIAN et al., 2011; UZOR et al., 2015) to plant and human pathogen (KINDO et al., 2014; WANG et al., 2017). It has also been studied as extremely interesting as a source of natural products and because of its potential industrial applications (CHEN et al., 2016). Such importance can be attributed to the other genera of fungi found in smaller quantities in this work, being, most of them already reported as endophytes.

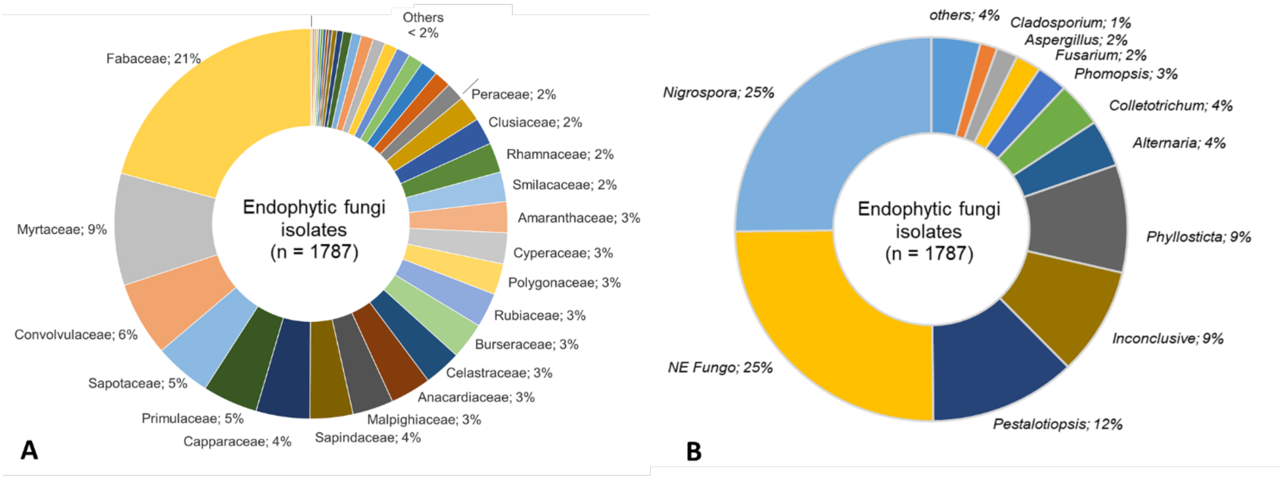


Figure 2: Relative frequency of endophytic fungi distributed among botanical families (A) and identified by genera (B), obtained from plant species of four different restinga ecosystems of northern Rio de Janeiro State, Brazil.

The number of fungi not identified due to lack of sporulation (morphological identification) was high. Similar results were obtained by other authors (PEREIRA et al., 1993; SURYANARAYANAN and KUMARESAN, 2000; PHOTITA et al., 2001). Hanada et al. (2010) studied endophytic fungi of *Theobroma cacao* L. and *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. from Amazonas and Bahia, cited 35% of isolates that showed no sporulation. These values are higher than those found in our survey (25%). This absence of spore production may indicate the lack of adaptation of these fungi to the artificial culture medium and the conditions used in the isolation, since the environment inside the plant tends to be different (COSTA et al., 2012). On the other hand, they may be related to those fungi that do not sporulate, classified within the group "*Mycelia sterilia*".

The highest percentage of fungi occurred on plants in the Fabaceae and Myrtaceae families, with absolute frequencies ranging from 2 to 372 isolates per family. The plant species *Cynophalla flexuosa* (L.) J. Presl and *Andira fraxinifolia* Benth provided, individually, higher numbers of isolates (Figure 4). The restinga plant community exhibits high endophytic fungal diversity, as more than 30% of the species were colonized by more than 20 fungal isolates. The endophytic niche is a rich source of microorganisms that can promote benefits to plants (BARROW et al. 2008), as well as being more diverse in tropical plants than in temperate plants (OLIVEIRA et al., 2014).

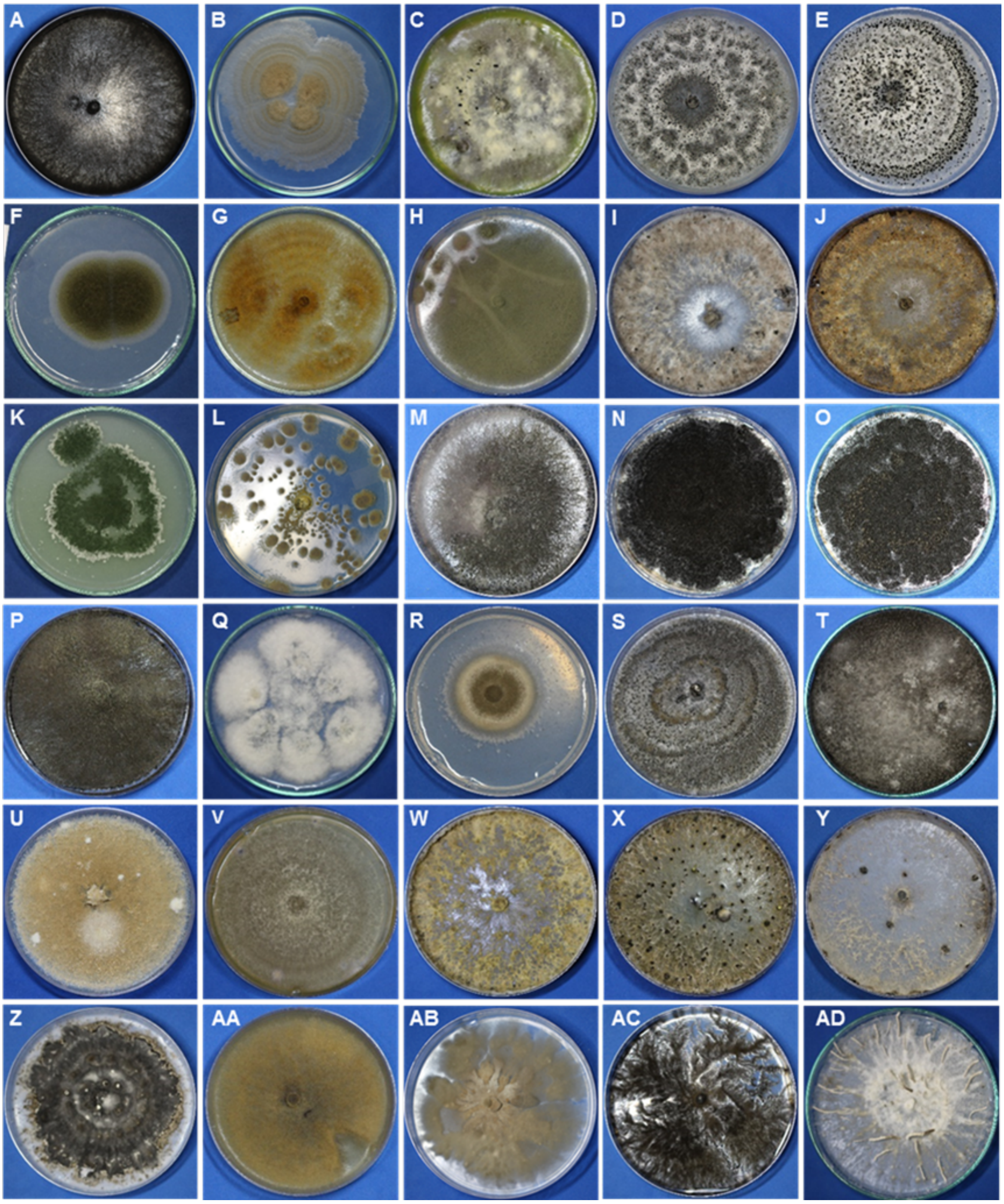


Figure 3: Endophytic fungal colonies were obtained from several plant species from restinga ecosystems in northern Rio de Janeiro state, Brazil. Genera: A) *Exserohilum*; B) *Gliocladium*; C, D and E) *Pestalotiopsis*; F) *Cladosporium*; G) *Curvularia*; H) *Penicillium*; I and J) *Phoma*; K) *Trichoderma*; L) *Aspergillus*; M) *Bipolaris*; N, O and P) *Guignardia*; Q) *Beauveria*; R) *Alternaria*; S) *Colletotrichum*; T) *Nigrospora*; U and V) *Fusarium*; W, X and Y) *Phomopsis*; Z, AA, AB, AC and AD) non sporulated isolates. Reprinted with permission of Freire et al., (2016).

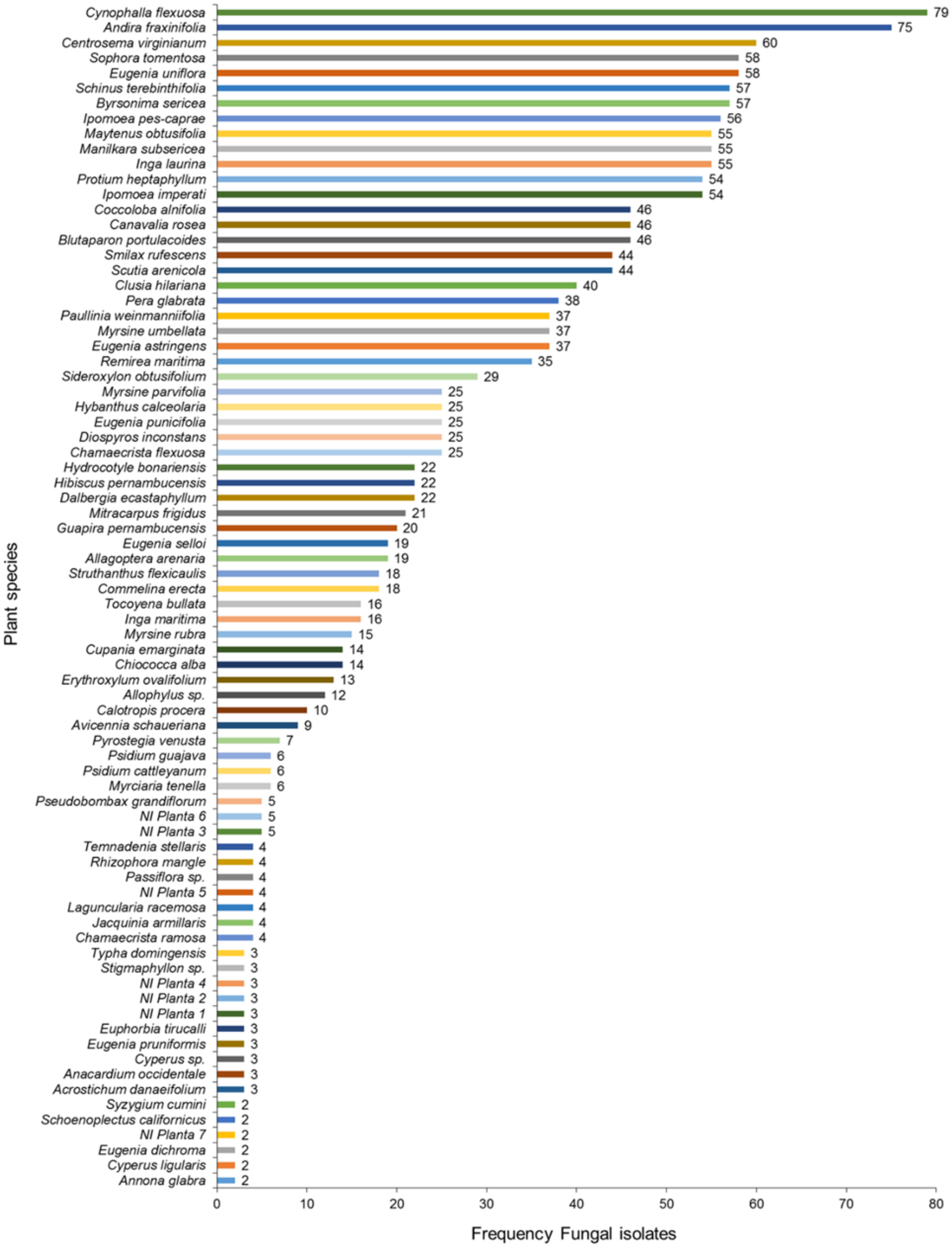


Figure 4: Absolute frequency of endophytic fungal isolates obtained from host plants in restinga ecosystems in northern Rio de Janeiro State, Brazil. (NI= plant in process of identification).

From all plants collected, 36 genera of fungi were identified: *Acremonium* Link, *Alternaria* Nees, *Aspergillus*, *Chaetomium* Kunze, *Cladosporium* Link, *Cochliobolus* Drechsler, *Colletotrichum* Corda, *Curvularia* Boedijn, *Diaporthe* Fuckel, *Eurotium* Link, *Exserohilum* K.J. Leonard & Suggs, *Fusarium*, *Glomerella* Spauld. & H. Schrenk, *Guignardia* Viala & Ravaz, *Helminthosporium* Link, *Lasiodiplodia* Ellis & Everh., *Lentinus* Fr., *Macrophoma* (Sacc.) Berl. & Voglino, *Mortierella* Coem., *Neofusicoccum* Crous, Slippers & A.J.L. Phillips, *Nigrospora*, *Penicillium*, *Periconia* Tode, *Pestalotiopsis* Steyaert, *Phaeobotryosphaeria* Speg., *Phanerochaete* P. Karst., *Phoma* Sacc., *Phomopsis* Sacc. &

Roum., *Phyllosticta* Pers., *Rhinocladiella* Nannf., *Setosphaeria* K.J. Leonard & Suggs, *Stemphylium* Wallr., *Stenella* Syd., *Talaromyces* C.R.Benj., *Torula* Pers. and *Trichoderma* Pers.

Nine genera were common to all four restingas, regardless of the plant from which they were isolated. In addition, eight other genera occurred exclusively in the Gargaú and Grussaí restingas. In the Açu sandbank, five exclusive genera were found and in Jurubatiba, no exclusive genera were recorded (Figure 5).

All fungal genera found in this study have been reported as endophytes of plants in several natural environments or under anthropogenic action (HANADA et al., 2010; HUANG et al., 2008; BEZERRA et al., 2012; PERŠOH, 2013; VIEIRA et al., 2012; GAUTAM, 2013; VIEIRA et al, 2014; LUO et al., 2015; RAJAMANIKYAM et al., 2017; MAADON et al., 2018).

Environmental factors linked to the host may be involved in the endophytic composition, however, the plant contributes to the establishment of the fungi, since its endogenous community differs among plants growing in the same area, as found in the work performed by Costa et al., (2012), whose fungal similarity index among mangrove plant species was relatively low. In our survey, the pattern of host preference was evaluated only at the level of the number of fungal isolates, regardless of their identification (Figure 4). Further analyses should be carried out to obtain diversity indices, considering each plant as an isolated environment (alpha diversity). So far, there was no correlation between host species preference as a source of specific genera of endophytic fungi from restinga, suggesting a more detailed view of the diversity that was not known until now in this environment.

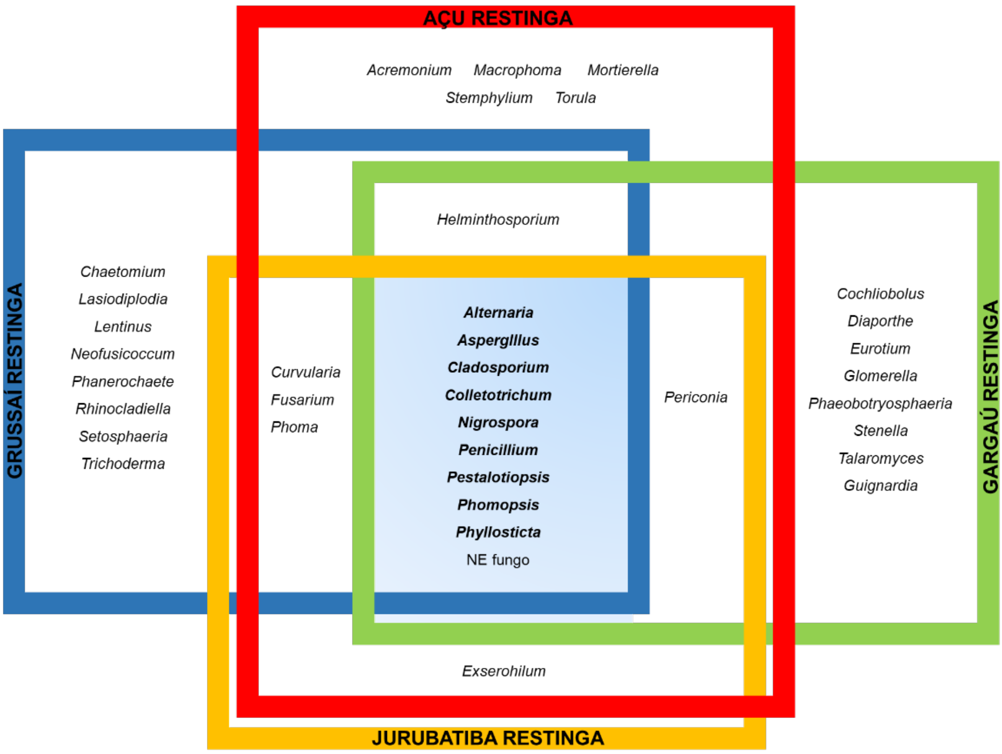


Figure 5: Distribution diagram of the 36 endophytic fungal genera isolated from plants of four restingas in the north of Rio de Janeiro State, Brazil. Each rectangle with a colored border represents a restinga and the overlap between them gives rise to intersections defined by the common or exclusive occurrence of the fungi among them. (NE = unsporulated fungus).

Table 1: Plant species collected from four restinga ecosystems in the north of Rio de Janeiro State, Brazil, which were used to study the diversity of endophytic fungi

| PLANT SPECIES | BOTANICAL FAMILY | RESTINGA* | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| *Acrostichum danaeifolium* | Pteridaceae | | | x | |
| *Allagoptera arenaria* | Arecaceae | x | | | x |
| *Allophylus* sp. | Sapindaceae | | | | x |
| *Anacardium occidentale* | Anacardiaceae | | | x | |
| *Andira fraxinifolia* | Fabaceae | x | x | | |
| *Annona glabra* | Annonaceae | | | x | |
| *Avicennia schaueriana* | Acanthaceae | | x | x | |
| *Blutaparon portulacoides* | Amaranthaceae | x | x | | x |
| *Byrsonima sericea* | Malpighiaceae | x | x | x | x |
| *Calotropis procera* | Apocynaceae | | x | | |
| *Canavalia rosea* | Fabaceae | x | x | | x |
| *Centrosema virginianum* | Fabaceae | x | x | x | x |
| *Chamaecrista flexuosa* | Fabaceae | x | | | |
| *Chamaecrista ramosa* | Fabaceae | | | | x |
| *Chiococca alba* | Rubiaceae | | x | | |
| *Clusia hilariana* | Clusiaceae | x | x | x | x |
| *Coccoloba alnifolia* | Polygonaceae | x | x | x | |
| *Commelina erecta* | Commelinaceae | x | x | | |
| *Cupania emarginata* | Sapindaceae | | | | x |
| *Cynophalla flexuosa* | Capparaceae | x | x | | x |
| *Cyperus ligularis* | Cyperaceae | | | x | |
| *Cyperus* sp. | Cyperaceae | | | x | |
| *Dalbergia ecastaphyllum* | Fabaceae | | x | x | |
| *Diospyros inconstans* | Ebenaceae | x | | | |
| *Erythroxylum ovalifolium* | Erythroxylaceae | | | | x |
| *Eugenia astringens* | Myrtaceae | x | | x | x |
| *Eugenia dichroma* | Myrtaceae | x | | | |
| *Eugenia pruniformis* | Myrtaceae | | | x | |
| *Eugenia punicifolia* | Myrtaceae | x | x | | |
| *Eugenia selloi* | Myrtaceae | | | | x |
| *Eugenia uniflora* | Myrtaceae | x | x | x | x |
| *Euphorbia tirucalli* | Euphorbiaceae | | x | | |
| *Guapira pernambucensis* | Nyctaginaceae | | | | x |
| *Hibiscus pernambucensis* | Malvaceae | | x | x | |
| *Hybanthus calceolaria* | Violaceae | | x | | |
| *Hydrocotyle bonariensis* | Araliaceae | x | x | | |
| *Inga laurina* | Fabaceae | x | x | | x |
| *Inga maritima* | Fabaceae | | | | x |
| *Ipomoea imperati* | Convolvulaceae | x | x | | x |
| *Ipomoea pes-caprae* | Convolvulaceae | x | x | x | x |
| *Jacquinia armillaris* | Primulaceae | | | | x |
| *Laguncularia racemosa* | Combretaceae | | x | | |
| *Manilkara subsericea* | Sapotaceae | x | x | | x |
| *Maytenus obtusifolia* | Celastraceae | x | x | | |
| *Mitracarpus frigidus* | Rubiaceae | x | | | |
| *Myrciaria tenella* | Myrtaceae | | | x | |
| *Myrsine parvifolia* | Primulaceae | x | | | x |
| *Myrsine rubra* | Primulaceae | | | | x |
| *Myrsine umbellata* | Primulaceae | | | | x |
| **NI 1 | Fabaceae | | | x | |
| **NI 2 | Fabaceae | | | x | |
| **NI 3 | Asteraceae | | | x | |
| **NI 4 | **NI | | | x | |
| **NI 5 | Cyperaceae | | | x | |
| **NI 6 | Fabaceae | | | x | |
| **NI 7 | Myrtaceae | | x | | |
| *Passiflora* sp. | Passifloraceae | | | x | |
| *Paullinia weinmanniifolia* | Sapindaceae | x | x | | |
| *Pera glabrata* | Peraceae | x | x | x | |

| | | | | | |
|---|---|---|---|---|---|
| *Protium heptaphyllum* | Burseraceae | x | | | x |
| *Pseudobombax grandiflorum* | Malvaceae | | | x | |
| *Psidium cattleyanum* | Myrtaceae | | | x | |
| *Psidium guajava* | Myrtaceae | | | x | |
| *Pyrostegia venusta* | Bignoniaceae | | | x | |
| *Remirea maritima* | Cyperaceae | x | x | | x |
| *Rhizophora mangle* | Rhizophoraceae | | | x | |
| *Schinus terebinthifolia* | Anacardiaceae | x | x | x | x |
| *Schoenoplectus californicus* | Cyperaceae | | | x | |
| *Scutia arenicola* | Rhamnaceae | x | x | | |
| *Sideroxylon obtusifolium* | Sapotaceae | x | | | x |
| *Smilax rufescens* | Smilacaceae | x | x | x | x |
| *Sophora tomentosa* | Fabaceae | | | | x |
| *Stigmaphyllon* sp. | Malpighiaceae | | | x | |
| *Struthanthus flexicaulis* | Loranthaceae | | x | | |
| *Syzygium cumini* | Myrtaceae | | | x | |
| *Temnadenia stellaris* | Apocynaceae | | | x | |
| *Tocoyena bullata* | Rubiaceae | | | | x |
| *Typha domingensis* | Typhaceae | | | x | |

*RESTINGA: 1 = Açu Restinga; 2 = Grussaí Restinga; 3 = Gargaú Restinga; 4 = Jurubatiba restinga; **NI: unidentified.

The biodiversity of endophytic fungi from the four restingas, based on the identification of isolates, as a function of the total number of endophytes obtained from all sampled plants, was high.

When the Margalef Richness Index was applied to all fungi obtained from the four studied areas, regardless of the plant species, higher diversity was observed when compared to the indices obtained for each restinga separately (Figure 6). The highest diversity index was detected for the restinga of Gargaú, followed by the restingas of Grussaí, Açu, and Jurubatiba. There was some variability in the diversity related to each restinga, and, under this generalized view of the collected volume of isolate, the restinga of Gargaú presented the highest diversity value, while the restingas of Grussaí, Açu, and Jurubatiba presented decreasing indices, respectively. This diversity is represented by the four circular crowns (Figure 6), whose colors, in each one, represent the abundance of the most frequent fungal species (genera).

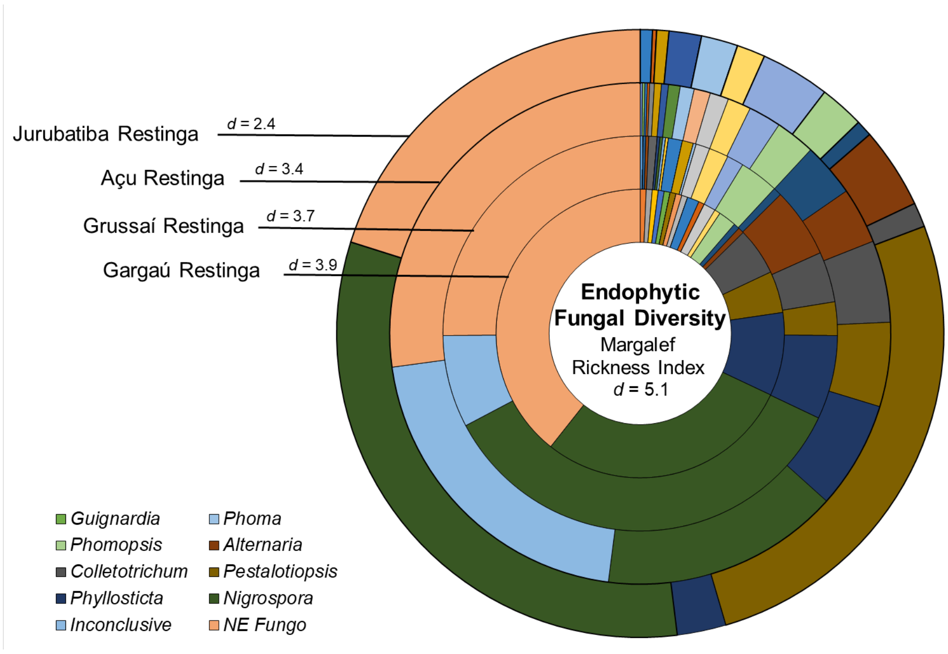

Figure 6: Endophytic fungal diversity of restinga ecosystems from northern Rio de Janeiro State, Brazil. Each circular crown represents a sandbank and its sections the intensity of occurrence of the identified genera.

The restingas of Açu and Jurubatiba are more preserved when compared to the restingas of Grussaí and Gargaú, the latter two with accentuated human degradation. If we consider the richness indices presented in Figure 6, the diversity of fungi seems to be inversely proportional to the stage or degree of degradation of the restingas, because, according to Odum (1988), diversity is usually higher in natural ecosystems that show great stability, while in ecosystems with anthropic interference the uniformity is low, resulting in the high dominance of a few species.

Analyzing this way and considering the variations between the plant species collected in different areas, to obtain the fungi, most of the time, what we have is only an estimate of the diversity in these areas, which is necessary for the analysis of patterns that may arise (MELO, 2008).

From this view, it was possible to select, for a more standardized comparison, only the fungi obtained from the same plant species occurring in the four restingas. Thus, the patterns of fungal richness among the restingas (Figure 7), under this restriction, the restinga of Açu can be considered with higher diversity, and the most preserved, because the general index for diversity was almost the same. The least diversity was presented by the Gargaú restinga, which is considered the most degraded.

The Margalef Index (1956) shows the specific richness and refers to the total number of individuals. The higher its value, the greater the diversity of the sampled community. Margalef's Richness Index was useful for comparing the sandbanks because it distinguished the communities by the number of fungal genera per number of individuals found, considering the size of each sample. Thus, there is a tendency to assume a constant value about to increases in sampling effort (MELO, 2008). Besides this, other diversity indexes (Fisher, Menhinick, and Shannon) were tested and, in all of them, the same trends were followed for fungal diversity in the four restingas (data not shown).

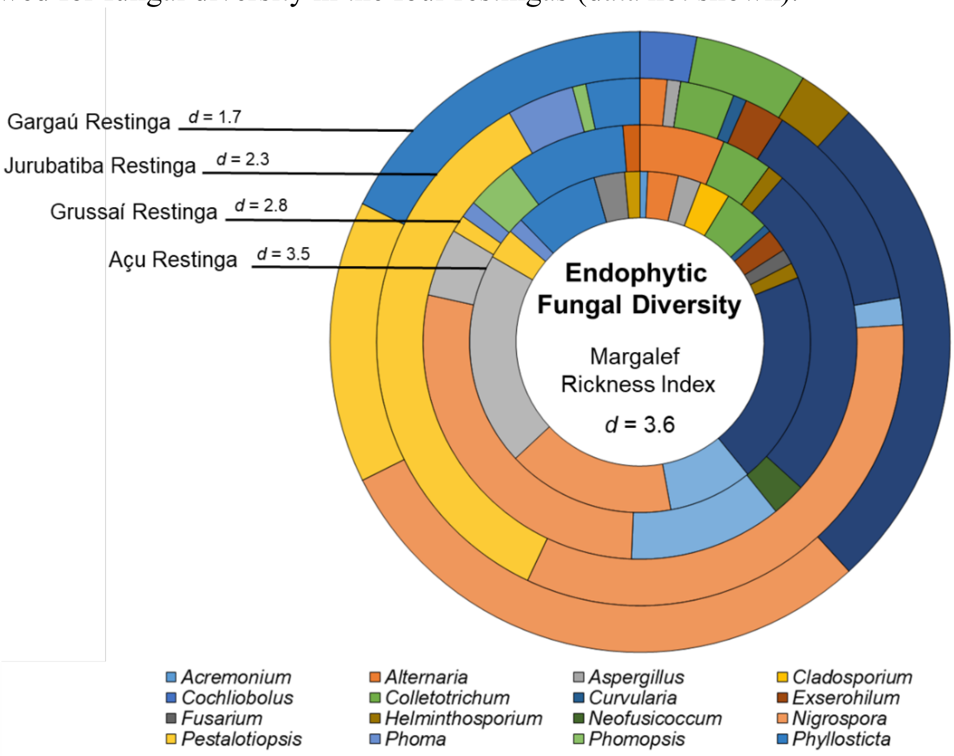

Figure 7: Diversity of endophytic fungi, obtained from the same plant species in four sandbanks in the north of Rio de Janeiro State, Brazil. Each circular crown represents a sandbank and its sections the intensity of occurrence of the identified genera.

## 4. CONSIDERATIONS

Although there has been a small increase in the number of studies on endophytic fungi in restinga areas, practically nothing is known about the presence and identification of these organisms associated with plant species of this ecosystem. This represents a potential source of species diversity and raises the need to know their presence, relationships, and applicability, since the restingas are considered unique spaces because they converge with other biomes, making it a different environment for the prospection of new fungal species. Thus, the limits of diversity (species richness) observed in the composition of these environments can serve as a preliminary parameter in the evaluation and comparison of biodiversity and preservation of these areas.

## 6. REFERENCES

ALEIXO, A., ALBERNAZ, A.L., GRELLE, C.E.V., VALE, M., RANGEL, T.F. Mudanças Climáticas e a Biodiversidade dos Biomas Brasileiros: Passado, Presente e Futuro. **Natureza & Conservação**, v. 8, n.2. p.194-196, 2010.

BANHOS, E.F., SOUZA, A.Q.L., ANDRADE, J.C., SOUZA, A.D.L., KOOLEN, H.H.F., ALBUQUERQUE, P.M. Endophytic fungi from *Myrcia guianensis* at the Brazilian Amazon: Distribution and bioactivity. **Brazilian Journal of Microbiology,** v. 45, n.1, p. 153-161. 2014.

BARNETT, H.L., HUNTER, B.B. **Illustrated genera of imperfect fungi**. 4.ed. Minnesota: APS Press, 1998. 218p.

BROWN, K.B., HYDE, K.D., GUEST, D.I. Preliminary studies on endophytic fungal communities of Musa acuminata species complex in Hong Kong and Australia. **Fungal Diversity, v.**1, p. 27-51. 1998.

BEZERRA, G.A.; MUSSI-DIAS, V.; SANTOS, P.H.D.; CARVALHO, B.M.; POLTRONIERI, T.P.S.; SILVEIRA, S.F. Endophytic fungi associated with the restinga bromeliads the National Park of Jurubatiba Restinga, Rio de Janeiro - Brazil. **Research, Society and Development**, [S. l.], v. 9, n. 7, p. e971974298, 2020. DOI: 10.33448/rsd-v9i7.4298. Disponível em: https://www.rsdjournal.org/index.php/rsd/article/view/4298. Acesso em: 27 oct. 2021.

BEZERRA, J.D., SANTOS, M.G., SVEDESE, V.M., LIMA, D.M., FERNANDES, M.J., PAIVA, L.M., SOUZA-MOTTA, C.M. Richness of endophytic fungi isolated from Opuntia ficus-indica Mill. (Cactaceae) and preliminary screening for enzyme production. **World J Microbiol Biotechnol**, v. 28, n.5, p.1989-95. 2012. doi: 10.1007/s11274-011-1001-2.

CAFÊU, M.C., SILVA, G.H., TELES, H.L., BOLZANI, V.S., ARAÚJO, A.R., YOUNG, M.C.M., PFENNING, L.H. Substâncias antifúngicas de xylaria sp., um fungo endofítico isolado de palicourea marcgravii (RUBIACEAE). **Química Nova**, v. 28, n. 6, p. 991-995, 2005.

CASTELLANI, A. The “water cultivation” of pathogenic fungi, **Annales des Societes Belges de Medecine Tropicale de Parasitologie, et de Mycologie**, v.44, n.2, p. 217-220. 1964.

CHEN, Z., DONG, Z., WEN, J. et al. A new sesquiterpene from the endophytic fungus Nigrospora sphaerica. **Records of Natural Products,** v.10, p.307–310. 2016.

CONAMA - **Conselho Nacional do Meio Ambiente, Resolution No 417, DOU nº 224**, Available at http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=617, 2009.

CONAMA - **Conselho Nacional do Meio Ambiente, Resolution No 453, DOU nº 192**, Available at http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=685, 2012.

COSTA, I.P.M.W, MAIA, L.C., CAVALCANTI, M.A. Diversity of leaf endophytic fungi in mangrove plants of northeast Brazil. **Brazilian Journal of Microbiology**, 1165-1173. 2012.

DETMANN, K.S.C., DELGADO, M.N., REBELLO, V. P. A., LEITE, T.S., AZEVEDO, A.A., KASUYA, M.C.M., ALMEIDA, A.M. Comparação de métodos para a observação de fungos micorrízicos arbusculares e endofíticos do tipo dark septate em espécies nativas de cerrado. **R. Bras. Ci. Solo**, v. 32, p.1883-1890, 2008.

FREIRE, M.G.M., MUSSI-DIAS, V., COUTINHO, H.S., HERNANDEZ, G.A.F., TAVARES, L.P.S. Bioprospecção da flora fúngica endofítica da reserva de Jurubatiba. **Persp Online: Biol & Saúde**, v.21, n.6, p. 6-13, 2016.

FREIRE, M.G.M., MUSSI-DIAS, V., MATTOSO, T. C., HENK, D.A., MENDES, A., MACEDO, M.L.R., TURATTI, C., MACHADO, S.W., SAMUELS, R.I. Survey of endophytic Alternaria species isolated from plants in the Brazilian restinga biome. **IOSR Journal of Pharmacy and Biological Sciences**, v. 12, n. 2, p. 84-94, 2017.

GAUTAM, A.K. Diversity of fungal endophytes in some medicinal plants of Himachal Pradesh, India, **Archives of Phytopathology and Plant Protection**, 2013. DOI: 10.1080/03235408.2013.813678

GALVÃO, M.L., SIQUEIRA, K.A., CUNHA, C.N., SOARES, M.A. Associação entre fungos e sistema radicular de plantas do pantanal mato-grossense. **Gl. Sci Technol**, v. 07, n. 02, p.56 – 62, 2014.

GOMES, C.V., FORTUNA, J.L. Microfungos endofíticos e epifíticos em folhas de bromélias em um fragmento de Mata Atlântica no Extremo Sul da Bahia. **UNISANTA Bioscience**, v. 9, n. 4, p. 356 – 370, 2020.

GONÇALVES, F.J.T., FREIRE, F.C.O., LIMA, J.S., J.G.M., CÂMARA, M.P.S. Patogenicidade de espécies de Botryosphaeriaceae endofíticas de plantas da Caatinga do estado do Ceará em manga e umbu-cajá. **Summa Phytopathol**., v. 42, n. 1, p. 43-52, 2016.

HAMMER, O. HARPER, D.A.T., RYAN, P.D. **PAST: paleontological statistic software package for education and data analysis**. Oxford: Blackwell, 2001. 351p.

HANADA, R.E., POMELLA, A.W., COSTA, H.S., BEZERRA, J.L., LOGUERCIO, L.L., PEREIRA, J.O. Endophytic fungal diversity in Theobroma cacao (cacao) and T. grandiflorum (cupuaçu) trees and their potential for growth promotion and biocontrol of black-pod disease. **Fungal Biol**., 114(11-12):901-10. 2010. doi: 10.1016/j.funbio.2010.08.006.

HANLIN, R.T., MENEZES, M. **Gêneros ilustrados de ascomicetos**. Recife: Imprensa da Universidade Federal Rural de Pernambuco, 1996. 274p.

HUANG, W.Y., CAI, Y.Z., HYDE, K.D., CROKE, H, SUN, M. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. **Fungal Diversity**, v.33, p. 61-75. 2008.

KAMEL, A., ABD-ELSALAM, K.A., HASHIM, A.F. Hidden fungi as microbial and nano-factories for anticancer agents. **Fungal Genom Biol**, 3: e115., 2013. DOI:10.4172/2165-8056.1000e115.

KINDO, A.J., SUBRAMANIAN, A., SURESH, K. Nigrospora sphaerica causing corneal ulcer in an immunocompetent woman: a case report**. International Journal of Case Reports and Images**, v.5. p. 675–679. 2014.

LUO, Z.P., LIN, H.Y., DING, W.B., HE, H.L., LI, Y.Z. Phylogenetic Diversity and Antifungal Activity of Endophytic Fungi Associated with *Tephrosia purpurea*. **Mycobiology**, v.43, n.4, p.435-43. 2015. doi: 10.5941/MYCO.2015.43.4.435.

MAADON, S.N., WAKID, S.A., ZAINUDIN, RUSLI, L.S., ZAN, M.S.M., HASAN, N., SHAH, N.A., ROHANI, E.R. Isolation and identification of endophytic fungi from UiTM Reserve Forest, Negeri Sembilan. **Sains Malaysiana**, v.47, n.12, p. 3025–3030. 2018. http://dx.doi.org/10.17576/jsm-2018-4712-12.

MARGALEF, R. Información y diversidad específica en las comunidades de organismos. **Investigación Pesquera, Barcelona**, v.3, p. 99-106, 1956.

MARTINS, M.L.L. Fitofisionomia das formações vegetais da Restinga da Área de Proteção Ambiental (APA) de Guaibim, Valença, Bahia, Brasil. **R. Bras. Bioci**., v. 10, n. 1, p. 66-73, 2012.

MARTINS, R.T M.C., BORGES, A.K P., ARMIATO, A.M., PIMENTA, R.S. Comunidade de fungos endofíticos associados a planta etnomedicinal amazônica bellucia grossularioides (l) trianna e avaliação de suas propriedades antimicrobianas. **Revista Desafios** v. 3, n. 2, 2016. DOI: http://dx.doi.org/10.20873/uft.2359-3652.2016v3n2p112.

MELO, A.S. What do we win 'confounding' species richness and evenness in a diversity index? **Biota Neotrop**., v.8, n.3, p. 21-27. 2008 http://www.biotaneotropica.org.br/v8n3/en/abstract?point-of-view+bn00108032008.

MUSSI-DIAS, V., ARAÚJO, A.C.O., SILVEIRA, S.F.S., ROCABADO, J.M.A., ARAÚJO, K.L. Fungos endofíticos associados a plantas medicinais, **Rev Bras Pl Med**, v.14, n.2, p. 261-266, 2012.

MUSSI-DIAS, V., FERNANDES, D.C., FREIRE, M.G.M. Criação do banco de dados da micoteca do Laboratório de Química e Biomoléculas (LAQUIBIO) do ISECENSA. **Perspectivas Online: Biológicas & Saúde**, v.8, n.28, p. 92-102, 2018.

ODUM, E. **Ecologia**. Tradução de Christopher J. Tribe. Rio de Janeiro: Guanabara, 1988.

OLIVEIRA, S.F., BOCAYUVA, M.F., VELOSO, T.G., BAZZOLLI, D.M., DA SILVA, C.C., PEREIRA, O.L., KASUYA, M.C. Endophytic and mycorrhizal fungi associated with roots of endangered native orchids from the Atlantic Forest, Brazil. **Mycorrhiza**. V.24, n.1, p.55-64. 2014. doi: 10.1007/s00572-013-0512-0.

PERŠOH, D. Factors shaping community structure of endophytic fungi–evidence from the Pinus-Viscum-system. **Fungal Diversity**, v.60, p. 55–69. 2013. DOI 10.1007/s13225-013-0225-x.

PEREIRA, J.O., AZEVEDO, J.L., PETRINI, O. Endophytic fungi of *Stylosanthes*: A first report. **Mycologia,** v.85, p. 362-364. 1993.

PHOTITA, W., LUMYONG, S., LUMYONG, P., HYDE, K.D. Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. **Mycol. Res**., v.105, p. 1508-1513. 2001.

PILATTI, F.K., AGUIAR, T., SIMÕES, T. BENSON, E.E., VIAN, A.M. In vitro and cryogenic preservation of plant biodiversity in Brazil. **In Vitro Cell. Dev. Biol. -Plant**, v.47, p.82–98 2011. https://doi.org/10.1007/s11627-010-9302-y.

PIRES, I.M.O., SILVA, A.V., SANTOS, M.G.S., BEZERRA, J.D.P., BARBOSA, R.N., SILVA, D.C.V., SVEDESE, V.M., SOUZA-MOTTA, C.M., PAIVA, L.M. Potencial antibacteriano de fungos endofíticos de cactos da caatinga, uma floresta tropical seca no nordeste do Brasil. **Gaia Scientia**. Edição Especial Cactaceae, v. 9, n.2, p. 155-161, 2015.

RAJAMANIKYAM, M., VADLAPUDI, V., AMANCHY, R., UPADHYAYULA, S.M. Endophytic fungi as novel resources of natural therapeutics. **Brazilian Archives of Biology and Technology**, v.60: e17160542 Jan/Dec 2017.

RANA K.L., KOUR, D., SHEIKH, I, et al. Endophytic Fungi: Biodiversity, Ecological Significance, and Potential Industrial Applications. In: YADAV A., MISHRA S., SINGH S., GUPTA A. (eds) Recent Advancement in White Biotechnology Through Fungi. **Fungal Biology**. https://doi.org/10.1007/978-3-030-10480-1_1.

SANTOS, S.N. **Bioprospecção de biomoléculas isoladas de fungos endofíticos de Combretum leprosum do bioma caatinga**. Tese (doutorado em ciencias: microbiologia agrícola). Universidade de São Paulo. ESALQ – Piracicaba, 2012. 182p.

SURYANARAYANAN, T.S., KUMARESAN, V. Endophytic fungi of some halophytes from an estuarine mangrove forest. ***Mycol. Res***., v.104, p. 1465-1467. 2000.

SUTTON, B.C. **The coelomycetes:** fungi imperfecti with pycnidia acervuli and stromata. Kew, Surrey, England: Commonwealth Mycological Institute, 1980. 696p.

THALAVAIPANDIAN, A., RAMESH, V., BAGYALAKSHMI, et al. Diversity of fungal endophytes in medicinal plants of Courtallam hills, Western Ghats, India. **Mycosphere**, v.2, p. 575–582. 2011.

UZOR, P.F., EBRAHIM, W., OSADEBE, P.O., et al. Metabolites from Combretum dolichopetalum and its associated endophytic fungus Nigrospora oryzae – evidence for a metabolic partnership. **Fitoterapia,** v.105, p. 147–150. 2015.

VARREIRA, J.S. **Avaliação de atividades enzimáticas, lipase, protease e quitinase de fungos provenientes de solo do Bioma Pampa**. Monografia (Graduação em Ciências Biológicas - Bacharelado) – Universidade do Vale do Taquari - Univates, Lajeado, 03 dez. 2018. Disponível em: http://hdl.handle.net/10737/2382.

VAZ, A.B.M., FONSECA, P.L.C., BADOTTI, F. et al. A multiscale study of fungal endophyte communities of the foliar endosphere of native rubber trees in Eastern Amazon. **Sci Rep**, v.8, n.16151, 2018. https://doi.org/10.1038/s41598-018-34619-w.

VAZ, A.B.M., MOTA, R.C., BOMFIM, M.R.Q., VIEIRA, M.L.A., ZANI, C.L., ROSA, C.A., ROSA, L.H. Antimicrobial activity of endophytic fungi associated with Orchidaceae in Brazil. **Canadian Journal of Microbiology**, v. 55, n. 12. 1381–1391, 2009.

VIEIRA, M.L., HUGHES, A.F., GIL, V.B., VAZ, A.B. et al. Diversity and antimicrobial activities of the fungal endophyte community associated with the traditional Brazilian medicinal plant *Solanum cernuum* Vell. (Solanaceae). **Can. J. Microbiol**. v. 58, p. 54-66. 2012.

VIEIRA, M.L., JOHANN, S., HUGHES, F.M., ROSA, C.A., ROSA, L.H. The diversity and antimicrobial activity of endophytic fungi associated with medicinal plant Baccharis trimera (Asteraceae) from the Brazilian savannah. **Can J Microbiol**. v. 60, n.12, p. 847-56. 2014. doi: 10.1139/cjm-2014-0449. PMID: 25403761.

WANG, M., LIU, F., CROUS, P.W., CAI, L. Phylogenetic reassessment of Nigrospora: Ubiquitous endophytes, plant and human pathogens. **Persoonia**, 39, 118–142. 2017. https://doi.org/10.3767/persoonia.2017.39.06.