Discovering the Diversity of Cultivable Endophytic Fungi in a Decumbent Subshrub Endemic of the Brazilian Tropical Savanna

Jefferson Brendon Almeida dos Reis
jeffersonalmeidareis@gmail.com
University of Brasília (UnB)
Jadson Diogo Pereira Bezerra
Federal University of Goiás (UFG)
Helson Mario Martins Vale
University of Brasília (UnB)

Research Article

Keywords: Ascomycota, Cerrado, Colletotrichum, Diaporthe, ITS, Peltaea polymorpha

Posted Date: September 28th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3372991/v1

Additional Declarations: No competing interests reported.

Abstract

The diversity of cultivable endophytic fungi in native subshrubs of the Brazilian Cerrado is largely unknown. Given the lack of knowledge, this study investigated the cultivable endophytic mycobiome of stems, leaves, and flowers of *Peltaea polymorpha* (Malvaceae). In total, 208 endophytic fungi were isolated, 95 from stems, 65 from leaves, and 48 from flowers. The isolates were classified as ascomycetes belonging to three classes, eight orders, ten families, 12 genera, and 31 species. Diaporthe, Nigrospora, and Colletrotrichum were the dominant genera in the three analyzed organs. The richness estimators suggested that the number of species might be slightly higher than observed. The highest values for the Shannon and Simpson diversity indices were observed in stems. Beta diversity showed overlapping of fungal communities in different organs, with a high rate of sharing of taxa. Furthermore, the dominant primary fungal lifestyles were plant pathogens and saprobes. Our findings show that the cultivable endophytic fungal community of *P. polymorpha* is species-rich and that communities in different organs share numerous genera and species. Finally, our findings reinforce the importance of the Brazilian Cerrado as a reservoir of fungal species to contribute to the national and global estimations of mycodiversity.

INTRODUCTION

Endophytic fungi are microorganisms that reside within plant tissues without causing symptoms of disease (Jia et al., 2016). These microorganisms are present in all plant species with varying richness and diversity, which are affected by plant species, seasonality, plant tissue and age, and geographic location, among other factors (Aly et al., 2011; Fang 2019; Chi et al., 2019). The association between endophytic fungi and their hosts is a mutually beneficial relationship: the host plant provides the fungi with habitat and nutrition, while the fungi play critical roles in the growth, development, and fitness of their hosts, particularly under stress conditions (Pietro-Souza et al., 2017; El-Shafey et al., 2021). Some endophytic fungi have co-evolved with their plant hosts, so that they can produce valuable secondary metabolites (Gong et al., 2019; Pang et al., 2022), which may have numerous biotechnological applications (Yao et al., 2017; Liu et al., 2021; Wen et al., 2022). Due to its evolutionary, ecological, and biotechnological importance, endophytic fungi have been considered a current and promising source of research.

The community of endophytic fungi associated with plant species from various ecosystems has been examined by culture-independent (Chi et al., 2019; Durán et al., 2021; Wang et al., 2022) and culture-dependent methods (Li et al., 2016; Yao et al., 2017; Du et al., 2020; Wang et al., 2022). Both types of methodology offer advantages and disadvantages. Culture-independent approaches, such as metabarcoding, allow closer knowledge of the real diversity of these microbial communities (Chi et al., 2019; Durán et al., 2021; Wang et al., 2022). Culture-dependent approaches, in turn, are less efficient to infer ecological analysis (Chi et al., 2019; Durán et al., 2021; Wang et al., 2022). However, they are cheaper, more accessible and provide data on easily cultivated fungi, which represent a potential genetic and biotechnological resource (Li et al., 2016; Yao et al., 2017; Du et al., 2020; Wang et al., 2022). In addition, the combination of different culture media for the isolation of endophytic fungi has enabled an increase in the number of isolated fungal species (Fang et al., 2019).

The considerable advances in the methods used to study endophytic fungi have enabled the characterization of the endophytic mycobiome of numerous plant species (Yadav et al., 2016; Noriler et al., 2018; Martins et al., 2021; dos Reis et al., 2022b; dos Reis et al., 2023). However, knowledge about the endophytic mycobiome of small plants, decumbent subshrub in natural areas, remains underexplored. For example, in the Brazilian Neotropical savannah (Cerrado), endophytic fungal communities are mainly studied in medium to large plant species (Noriler et al., 2018;

lantas et al., 2021; Morais et al., 2022; dos Reis et al., 2023), while small plants such as *Peltaea polymorpha* (A.St.-Hil.) Krapov. & Cristobal remains unexplored.

Peltaea polymorpha (Malvaceae), a decumbent subshrub species, has subsessile flowers grouped at the apex of the branches and occurs in the Cerrado and Atlantic Forest in Brazil (Fernandes-Júnior & Konno, 2017). Although this species occurs widely in the Cerrado (Fernandes-Júnior & Konno, 2017), there are no known reports on the diversity of endophytic fungi associated with this species. Given the gap in knowledge and reinforcing the importance of studying the endophytic mycobiome of neglected species, we investigated the occurrence, composition, species, and functional diversity of cultivable endophytic fungi associated with flowers, leaves, and stems of the decumbent subshrub *P. polymorpha*.

MATERIALS AND METHODS

1.1. Study area and plant collection

Collections of *Peltaea polymorpha* were carried out in an area of Cerrado *sensu stricto* located in the Parque Nacional de Brasília, Distrito Federal, Brazil (15°’8"’8"S 48’1"’5"W) during the rainy season of December 2022 (Fig. 1). The climate in this region is tropical (Aw), with two distinct seasons, a dry (April-September) and a rainy (October-March) (Cardoso et al., 2014). The vegetation in the studied area is characterized by the presence of grasses along with tree and shrub strata (Cavararo, 2004; Eiten, 1972; Eiten 1994). Five healthy individuals of *Peltaea polymorpha* were collected. During plant collection, each individual was sectioned close to the ground and the whole plant (stems, leaves, and flower) was placed in a sterile plastic bag, packed in a thermal box, and sent to the laboratory. All sampled individuals were in the phenological stage of flowering and had a size of 25 ± 2 cm length, 5 to 7 leaves per individual, and a single flower. Samples were processed within 2 hours after the collection.

1.2. Isolation and Preservation of Endophytic Fungi

For the isolation of endophytic fungi, three individuals of *P. polymorpha* were selected, and the other two were used to prepare the culture medium. Plant organs (leaves, stems, and flowers) were separated, washed with running water to remove dust on the surface, and subjected to surface disinfection methods. The stems and leaves were submerged in 70% ethanol for 2 min, followed by submersion in 4% NaClO for 5 min, and washed five times with distilled and sterilised water (Yu et al., 2018). The flowers were immersed in 70% ethanol for 2 min, washed three times with 10% NaClO, and then rinsed 5 times with distilled and sterilised water (Niu et al., 2022). The water from the last rinse of the surface disinfection of plant organs was collected in falcon tubes, centrifuged at 3,000 rpm, and seeded in triplicate on Potato Dextrose Agar (PDA) as a negative control. Plant samples intended for isolation were cut with a sterile blade into segments of approximately 5 mm^2, totalling 81 segments per organ. Subsequently, the segments were placed on potato-dextrose-agar (PDA), malt extract agar (MEA), and PDA containing 10 g/L of macerated fragments of *P. polymorpha* (flowers, leaves, and stems) (PDAP). All culture media were supplemented with 150mg/L of chloramphenicol to prevent bacterial growth. The Petri plates were incubated at 25°C with a 12-hours photoperiod and examined daily for seven days. During the incubation period, any newly emerged mycelial growth from the plant fragments was collected immediately and transferred to a fresh PDA plate. The resulting fungal isolates were purified and stored in 2 mL microtubes containing 10% glycerol.

1.3. Identification of Endophytic Fungi

The purified fungal isolates were grouped based on their morphological characteristics, which included the colour of the colony (verse and obverse), growth rate, the appearance of the mycelium, shape, and structure of hyphae, colour of spores and exudates (Li et al., 2016). Fungal isolates were defined as being of the same morphotype if they had the same characteristics of the colony, mycelium, reproductive structures (when present), and exudates. Based on this, one representative isolate of each morphotype was selected to sequencing of the internal transcribed spacer region (ITS) of the nrDNA operon and intervening 5.8S nRNA gene.

TXXXdenophorentative isolates were grown on PDA and when colonies reached sufficient mycelial mass for DNA extraction, approximately 400 mg of mycelium was scraped from the colony. The scraped mycelium was added to a 2 mL microtube and DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI), following the manufactur'r's specifications. The DNA from the extraction was dissolved in 100 µl of nuclease-free water and stored at -20°C.

The ITS region was amplified with the primer pair V9G (Hoog & Gerrits van den Ende, 1998) and LR5 (Vilgalys & Hester, 1990). The PCR was performed in a 25 µL volume containing 20 ng of DNA, 0.4 µM of primer pair, 0.8 mM of dN'P's mix, 2.5 mM of MgCl, and 1.5 U of Taq Polymerase (Invitrogen, São Paulo Brazil). The PCR was cycled as follows: 5 min of initial denaturation at 95°C, followed by 35 cycles of the 30s of denaturation at 95°C, 45s of primer annealing at 53°C, 1min extension at 72°C, and a final extension of 5 min at 72ºC. PCR products were analysed by electrophoresis on a 1% agarose gel stained with GelRed ® (Biotium, Fremont, CA, USA) and visualized under UV light. Subsequently, the PCR products were purified with Exo-SAP (USB Corp, Germany) according to the manufactur'r's specifications and sent to sequencing using the same primers. The generated sequences were analysed for quality using the DNA Sequence Assembler v4 (2013) software (Heracle BioSoft, www.DnaBaser.com).

The ITS sequence data from endophytic fungi were submitted to GenBank and compared with those of the closest fungal species in the NCBI database using the BLASTn tool. Sequences with similarity above 94% were considered from the same genus, and those with similarity above 97% belonged to the same species (Li et al., 2016). Sequences were deposited in GenBank database (Table 2).

1.4. Data analysis

We used first-order Chao 1, Bootstrap, and Jackknife richness estimators to extrapolate the number of fungal genera and species observed in P. polymorpha using the "*estimateR*" and "*specpool*" functions of the Vegan package (Dixon, 2003). The relative abundance of isolated species among different plant tissue was calculated at different taxonomic levels using the R language packages "*dplyr"* and "*tidyverse*".

Alpha diversity among *P. polymorpha* organs was assessed using species richness 'S'), Shannon diversity 'H'), Simpson diversity (D), and Pielou evenness (J) indices using the Vegan package (Dixon, 2003). ANOVA was used to compare alpha diversity and fungal abundance in *P. polymorpha* organs. Beta diversity (similarities and dissimilarities) of communities across plant organs was calculated using the Bray-Curtis distance using the BiodiversityR (Kindt & Coe, 2005) and Vegan (Dixon, 2003) packages. Principal coordinate analysis (PCoA) was used to visualize special dissimilarities in the composition of endophytic fungal communities between organs. Bipartite networks were built to show patterns of species occurrence and sharing between organs using the Bipartite package (Dormann et al., 2008). Venn diagrams were constructed using the Bioinformatics & Evolutionary Genomics web tool (https://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl). We used FungalTraits (Põlme et al., 2020) to group isolates into primary and secondary functional guilds.

Statistical and diversity analyses were performed using R scripts version 4.3.0. The plots were generated using the ggplot2 package (Wickham, 2016).

RESULT

1.1. Culturable Endophytic Fungi

A total of 208 endophytic fungi were isolated from *P. polymorpha*, of which 95 were isolated from stems, 65 from leaves, and 48 from flowers (Table 1). Most isolates were recovered from MEA (89 isolates), followed by PDA (80) and PDAP (39). However, we did not observe significant differences in the number of isolates recovered from the different culture media ($p > 0.05$). According to morphological characteristics, isolates from stems, leaves, and flowers were previously characterized and grouped into 77 morphotypes (MT1-MT77), being 46 from stems, 24 from flowers, and 42 from leaves (Fig. 2).

Table 1
Number of endophytic fungal isolates per plant organs of *P. polymorpha* and culture media used to access the fungal community, Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil

Plant organs	PDA	MEA	PDAP	Total
Stems	41	43	11	95
Leaves	26	29	10	65
Flowers	13	17	18	48
Total	80	89	39	208

MEA: Malt Extract Agar; PDA: Potato Dextrose Agar; PDAP: PDA culture media containing 10 g/L of plant organs (flowers, leaves, and stem) of *P. polymorpha.*

Based on sequences of ITS region and using the BLASTn tool, the 77 morphotypes were classified as ascomycetes, belonging to three classes, eight orders, ten families, 12 genera, and 31 species (Table 2). Among the 77 morphotypes, 48 (62.33%) were classified at the species level (percent similarity > 97%) and 29 (37.66%) were classified only at the genus level (percent similarity < 97%).

Table 2
Endophytic fungi from flowers, leaves, and stems of *Peltaea polymorpha*.

Morphotypes	Nº Isolates	Closest GenBank match	Match	Identity (%)	GenBank access number of our sequences	Organ	Medium
MT1	1	*Nigrospora cooperae*	NR_185745.1	97.61	OR164510	Flowers	MEA
MT10	1	*Nigrospora cooperae*	NR_185745.1	98.26	OR164511	Flowers	PDA
MT11	1	*Colletotrichum aeschynomenes*	NR_120133.1	99.21	OR164512	Stem	PDA
MT12	2	*Colletotrichum* sp.	CP117792.1	91.91	OR164513	Stem	MEA
MT13	2	*Diaporthe* sp.	NR_172401.1	93.33	OR164514	Leaves and Stem	MEA and PDAP
MT14	1	*Nigrospora macarangae*	NR_174838.1	97.17	OR164515	Leaves	PDA
MT15	1	*Nigrospora* sp.	NR_185745.1	95.97	OR164516	Flowers	MEA
MT16	1	*Nigrospora* sp.	NR_174838.1	96.41	OR164517	Leaves	MEA
MT17	1	*Diaporthe miriciae*	KJ197282.1	97.44	OR164518	Leaves	PDAP
MT18	1	*Neopestalotiopsis terricola*	OP082294.1	98.21	OR164519	Stem	PDAP
MT19	1	*Epicoccum* sp.	LT592927.1	98.72	OR164520	Stem	PDAP
MT2	1	*Epicoccum andropogonearum*	OK442369.1	98.31	OR164521	Leaves	PDAP
MT20	1	*Nigrospora* sp.	NR_185745.1	96.20	OR164522	Leaves	PDAP
MT21	1	*Aspergillus eucalypticola*	OL711732.1	99.11	OR164523	Leaves	MEA
MT22	1	*Epicoccum phragmospora*	NR_165920.1	98.31	OR164524	Stem	PDA
MT23	1	*Didymella chlamydospora*	MK836111.1	97.02	OR164525	Flowers	MEA
MT24	2	*Pestalotiopsis telopeae*	ON180762.1	97.11	OR164526	Flowers and Leaves	MEA and PDAP
MT25	1	*Epicoccum andropogonearum*	OK442369.1	98.31	OR164527	Leaves	MEA
MT26	2	*Aspergillus foetidus*	NR_163668.1	98.72	OR230588	Stem	MEA and PDA

Morphotypes	Nº Isolates	Closest GenBank match	Match	Identity (%)	GenBank access number of our sequences	Organ	Medium
MT27	1	*Epicoccum endophyticum*	NR_172436.1	98.29	OR164528	Stem	PDA
MT28	5	*Diaporthe novem*	MH864504.1	98.02	OR164529	Leaves and Stem	PDA and PDAP
MT29	1	*Neurospora* sp.	MH862539.1	95.77	OR164530	Stem	MEA
MT3	3	*Cladosporium ipereniae*	NR_152290.1	99.12	OR164531	Flowers	MEA, PDA, and PDAP
MT30	4	*Auxarthron* sp.	MH872264.1	90.44	OR164532	Stem	MEA and PDA
MT31	1	*Neopestalotiopsis* sp.	NR_170739.1	95	OR164533	Flowers	MEA
MT32	1	*Diaporthe* sp.	EU552122.1	93.22	OR164534	Stem	MEA
MT33	2	*Colletotrichum* sp.	MK541034.1	96.30	OR164535	Flowers and Leaves	MEA and PDA
MT34	1	*Nigrospora vesicularifera*	NR_165927.1	99.31	OR164536	Flowers	PDA
MT35	1	*Neopestalotiopsis mesopotamica*	NR_145244.1	98	OR164537	Leaves	MEA
MT36	1	*Nigrospora pyriformis*	NR_153469.1	98.89	OR164538	Leaves	MEA
MT37	1	*Nigrospora cooperae*	NR_185745.1	98.87	OR164539	Leaves	PDA
MT38	14	*Nigrospora cooperae*	NR_185745.1	98.70	OR164540	Flowers, Leaves, and Stem	MEA, PDA, and PDAP
MT39	1	*Nigrospora sacchari-officinarum*	NR_165926.1	97.39	OR164541	Stem	PDAP
MT4	1	*Neopestalotiopsis terricola*	OP082294.1	99.09	OR164542	Stem	MEA
MT40	1	*Nigrospora macarangae*	NR_174838.1	97.17	OR164543	Flowers	PDA
MT41	3	*Epicoccum* sp.	LT592927.1	95.02	OR164544	Stem and Flowers	MEA

Morphotypes	Nº Isolates	Closest GenBank match	Match	Identity (%)	GenBank access number of our sequences	Organ	Medium
MT42	2	*Diaporthe* sp.	NR_152458.1	94.28	OR164545	Leaves and Stem	PDA
MT43	2	*Neurospora* sp.	MH862539.1	95.77	OR164546	Flowers and Stem	MEA
MT44	1	*Colletotrichum spicati*	OL842171.1	99.11	OR164547	Leaves	MEA
MT45	1	*Nigrospora pyriformis*	NR_153469.1	98.89	OR164548	Stem	MEA
MT46	2	*Epicoccum phragmospora*	NR_165920.1	98.31	OR164549	Stem	MEA and PDA
MT47	1	*Diaporthe terebinthifolii*	NR_111862.1	98.02	OR164550	Stem	PDA
MT48	15	*Diaporthe stewartii*	MH855768.1	97.31	OR164551	Flowers, Leaves, and Stem	MEA and PDA
MT49	11	*Colletotrichum lupini*	AJ301948.1	98.73	OR164552	Flowers, Leaves, and Stem	MEA, PDA, and PDAP
MT5	1	*Neurospora tetraspora*	NR_077163.1	98.55	OR164553	Leaves	PDA
MT50	3	*Colletotrichum* sp.	CP117792.1	93.66	OR164554	Leaves and Stem	PDA and PDAP
MT51	2	*Nigrospora* sp.	NR_185745.1	96.82	OR164555	Leaves and Stem	MEA
MT52	2	*Alternaria alstroemeriae*	NR_163686.1	100	OR164556	Flowers	PDAP
MT53	1	*Neurospora dictyophora*	MH862539.1	98.68	OR164557	Stem	MEA
MT54	1	*Neopestalotiopsis formicarum*	NR_145242.1	99.00	OR164558	Leaves	MEA
MT55	1	*Epicoccum endophyticum*	NR_172436.1	98.29	OR164559	Stem	PDAP
MT56	3	*Nigrospora* sp.	NR_153485.1	96.82	OR164560	Leaves	MEA and PDAP

Morphotypes	Nº Isolates	Closest GenBank match	Match	Identity (%)	GenBank access number of our sequences	Organ	Medium
MT57	4	*Diaporthe terebinthifolii*	NR_111862.1	98.21	OR164561	Flowers, Leaves, and Stem	MEA and PDA
MT58	4	*Neurospora tetraspora*	NR_077163.1	97.12	OR164562	Flowers, Leaves, and Stem	MEA and PDAP
MT59	5	*Neurospora dictyophora*	MH862539.1	98.68	OR164564	Leaves	MEA and PDA
MT6	1	*Diaporthe* sp.	EU552122.1	93.01	OR164563	Stem	PDAP
MT60	2	*Colletotrichum* sp.	CP117792.1	93.66	OR164565	Leaves and Stem	MEA and PDA
MT61	1	*Neopestalotiopsis cocois*	NR_156312.1	99.01	OR164566	Flowers	PDAP
MT62	1	*Nigrospora magnoliae*	NR_172443.1	98.72	OR164567	Stem	MEA
MT63	5	*Neopestalotiopsis terricola*	OP082294.1	99.66	OR164568	Leaves and Stem	MEA, PDA, and PDAP
MT64	2	*Nigrospora vesicularifera*	NR_165927.1	99.02	OR164569	Flowers and Leaves	MEA and PDA
MT65	12	*Diaporthe* sp.	NR_147539.1	95	OR164570	Flowers, Leaves, and Stem	MEA, PDA, and PDAP
MT66	1	*Diaporthe* sp.	NR_165874.1	83.51	OR230587	Stem	MEA
MT67	3	*Diaporthe* sp.	NR_147534.1	94.55	OR164571	Stem	MEA, PDA, and PDAP
MT68	10	*Diaporthe stewartii*	MH855768.1	97.62	OR164572	Flowers, Leaves, and Stem	MEA, PDA, and PDAP
MT69	6	*Diaporthe* sp.	NR_111847.1	96.01	OR164574	Leaves and Stem	MEA, PDA, and PDAP

Morphotypes	Nº Isolates	Closest GenBank match	Match	Identity (%)	GenBank access number of our sequences	Organ	Medium
MT7	2	*Epicoccum phragmospora*	NR_165920.1	98.31	OR164573	Stem	MEA and PDA
MT70	1	*Nigrospora* sp.	NR_185745.1	96.41	OR164575	Leaves	PDA
MT71	1	*Diaporthe* sp.	NR_147539.1	93.58	OR164576	Stem	MEA
MT72	4	*Neopestalotiopsis nebuloides*	OM417249.1	97.76	OR164577	Flowers and Stem	MEA, PDA, and PDAP
MT73	12	*Diaporthe* sp.	EU552122.1	91	OR164578	Flowers, Leaves, and Stem	MEA, PDA, and PDAP
MT74	9	*Diaporthe* sp.	EU552122.1	93.22	OR164579	Leaves and Stem	MEA, PDA, and PDAP
MT75	1	*Alternaria destruens*	NR_137143.1	99.76	OR164580	Stem	PDA
MT76	1	*Neopestalotiopsis terricola*	OP082294.1	98.21	OR164581	Leaves	PDAP
MT77	1	*Diaporthe* sp.	EU552122.1	93.22	OR164582	Leaves	PDA
MT8	4	*Diaporthe terebinthifolii*	NR_111862.1	98.23	OR164583	Leaves and Stem	PDA and PDAP
MT9	1	*Pestalotiopsis* sp.	NR_147560.1	96.16	OR164584	Leaves	PDAP

MEA: Malt Extract Agar; PDA: Potato Dextrose Agar; PDAP: PDA culture media containing 10 g/L of plant organs (flowers, leaves, and stems) of *P. polymorpha.*

1.2. Richness estimators

Genus-level richness estimators ranged from 12 to 13 genera in the three statistical methods employed (Fig. 3a-c). Extrapolated values for genera richness estimates were observed in Bootstrap and Jackknife 1, both estimating a richness of 13 genera. The Chao 1 richness estimator reached an asymptote, estimating a richness of 12 genera. Estimates of species richness ranged from 42 to 50 species using three statistical methods (Fig. 3d-f). The lowest levels of species richness were estimated by Bootstrap (42 species) and by Chao 1 (44 species), while the highest level of richness was estimated by Jackknife 1 (50 species). The three richness estimation methods suggested that the species level in *P. polymorpha* might be slightly higher than what we observed in our study.

1.3. Alpha and Beta diversity

We used the indices of species richness (S), Shannon diversity 'H'), Simpson diversity, and Pielou equity (J) to determine the alpha diversity among the different organs of *P. polymorpha*. Based on the total number of species, the highest number of species was observed in leaves (24 species), while stems and flowers had 22 species each. The highest value for the Shannon diversity index was observed in stems (1.70 ± 0.17), followed by leaves (1.51 ± 0.14) and flowers (1.18 ± 0.12). Likewise, the highest value for Simps'n's diversity was also observed in stems (0.76 ± 0.05), followed by leaves (0.72 ± 0.05), and flowers (0.66 ± 0.03). The highest values for species abundance uniformity, demonstrated by Pielou evenness (J), were observed in flowers (0.97 ± 0.01), followed by stems (0.92 ± 0.01), and leaves (0.89 ± 0.02).

Visualization of beta diversity using principal coordinate analysis (PCoA) based on the Bray-Curtis distance showed the homogeneity of cultivable endophytic fungal communities associated with different organs of *P. polymorpha* (Fig. 5). Axis 1 explained 16% of the variation, while axis 2 explained 12.5% of the variation, totalling 28.5% of explanation. Notably, there is an overlap of cultivable endophytic fungal community occurring in flowers, leaves, and stems of *P. polymorpha*. However, PCoA also demonstrates that the fungal community is more dispersed in flowers compared to communities in leaf and stems.

1.4. Relative abundance and co-occurrence

The class Sordariomycetes was dominant in all three organs (> 80%), followed by Dothideomycetes, with an abundance of 17.5% in flowers, 9.3% in stems, and 5.6% in leaves (Fig. 6a). The class Eurotiomycetes was exclusive to flowers and leaves, with respective abundances of 6.2% in stems and 1.4% in leaves (Fig. 6b). The order Diaporthales was dominant in stems (49.5%) and in leaves (32.5%), followed by Amphisphaeriales, with an abundance of 16.5% in stems and 34.8% in leaves (Fig. 6c). In flowers, the dominant orders were Amphisphaeriales (35.5%), Diaporthales (32.5%), and Glomerellales (12.5%). Diaporthaceae/*Diaporthe* were the dominant family and genus in all three organs (Fig. 6c-d).

The community of endophytic fungi in different organs of *P. polymorpha* was dominated by species of *Diaporthe* (Diaporthales, Diaporthaceae), *Nigrospora* (Xylariales, Apiosporaceae), and *Colletotrichum* (Glomerellales, Glomerellaceae) (Fig. 7). However, it should be considered that different profiles of occurrence and abundance of endophytic fungal species were observed among different plant organs. Unidentified species of *Diaporthe* constituted the dominant group in the three organs. Considering the isolates identified at species level, *Diaporthe stewartii* (17.52%) followed by *Colletotrichum lupini* (6.18%), and *Nigrospora cooperae* (6.18%) were the dominant groups in stems. In leaves and flowers, the most abundant species was *Nigrospora cooperae*, with an abundance of 13% and 14.5%, respectively.

In addition to differences in relative abundance among organs, we observed differences in the occurrence of genera and species (Fig. 8). In total, six genera were common to the three plant organs, namely *Colletotrichum*, *Diaporthe*, *Epicoccum*, *Neopestalotiopsis*, *Nigrospora*, and *Neurospora*. Regarding the exclusive genera of each organ, *Auxarthron* was found only in stems; while *Cladosporium* and *Didymella* were exclusive to flowers (Fig. 8a-b). In total, nine species were shared to flowers, leaves, and stems, namely *Colletotrichum* sp., *Colletotrichum lupini*, *Diaporthe* sp., *Diaporthe stewarti*, *Diaporthe terebinthifolii*, *Epicoccum* sp., *Nigrospora* sp., *Nigrospora cooperae*, and *Neurospora dictyophora* (Fig. 8c-d). Regarding the exclusive species of each plant organ, *Alternaria alstroemeriae*,

Cladosporium ipereniae, *Didymella chlamydospora*, *Neopestalotiopsis cocois*, and *Neopestalotiopsis* sp. were exclusive to flowers; *Aspergillus eucalypticola*, *Colletotrichum spiccati*, *Diaporthe miriciae*, *Neopestalotiopsis formicarum*, and *Pestalotiopsis* sp. were exclusive to leaves; and *Alternaria destruens*, *Aspergillus foetidus*, *Auxarthron* sp., *Colletotrichum aeschynomenes*, *Epicoccum endophyticum*,*Nigrospora sacchari-officinarum*, and *Nigrospora magnoliae* were exclusive to stems.

1.5. Functional guilds

The community of cultivable endophytic fungi in *P. polymorpha* was grouped into four primary and four secondary functional guilds according to FungalTraits (Põlme et al., 2020) (Fig. 9). The primary ecological functional groups were litter saprotroph, plant pathogen, soil saprotroph, and unspecified saprotroph; the secondary functional groups were animal decomposer, hypervariable, litter saprotroph and unclassified. The functional guilds observed in flowers and leaves were saprotroph, plant pathogen, and unspecified saprotroph, while those observed in stems were litter saprotroph, plant pathogen, soil saprotroph, and unspecified saprotroph. In the three organs, there was a predominance of plant pathogens (> 58.5%), followed by saprotroph litter (> 11.3%). Regarding the secondary functional guilds, saprotroph was the dominant guild in the three organs (> 52.5%).

DISCUSSION

The Brazilian Cerrado is an important reservoir of microbial diversity, which include endophytic fungi (Noriler et al., 2018; Iantas et al., 2021; Morais et al., 2022; dos Reis et al., 2023). The occurrence, composition, and diversity of endophytic fungal communities associated with native plants in this biome have been studied mainly in woody or medicinal plant species (Noriler et al., 2018; Iantas et al., 2021; Dos Santos et al., 2021; Morais et al., 2022; dos Reis et al., 2023). In contrast, the community of endophytic fungi associated with subshrub plant species has received minimal attention in ecological and other research areas. In this sense, we investigated the occurrence, composition, species, and functional diversity of endophytic fungi associated with flowers, leaves, and stems of the decumbent subshrub *P. polymorpha*. Our study, therefore, provides unprecedented data on the community of endophytic fungi associated with this plant species and reinforces the importance of plants native to the Cerrado as a reservoir of endophytic fungal species, since this biome is a hotspot of biodiversity (Myers et al., 2000).

There are numerous limitations to studying microbial communities using culture-dependent approaches. However, we chose culture-dependent methods to study and better understand the ecological functions and chemical biodiversity produced by these fungi in further surveys. In our study, 248 isolates were recovered from flowers, leaves, and stems of *P. polymorpha*, with the highest number of isolates obtained from stems (95 isolates), followed by leaves (65 isolates) and flowers (48 isolates). Our findings are similar to other studies that investigated the occurrence and diversity of endophytic fungi associated with different tissues or organs of plant species, which also observed variations in the number of isolates depending on the organ or tissue (Wang et al., 2015; Fang et al., 2019; Du et al., 2020). However, although we observed variations in the number of recovered isolates among different organs, these differences were not significant ($p > 0.05$).

All endophytic isolates in our study are Ascomycota. The prevalence of Ascomycota as members of the cultivable endophytic mycobiota from the Cerrado and other biomes is commonly reported (Wang et al., 2015; Fang et al., 2019; Du et al., 2020; Noriler et al., 2018; Morais et al., 2022; dos Reis et al., 2023), and this can be explained by methodological limitations intrinsic to culture-dependent approaches (dos Reis et al., 2022b; Bhunjun et al., 2023)

and the biology, evolution and functional plasticity of ascomycetes (Egidi et al., 2019; James et al., 2020). Ascomycota is the most diverse phylum in the kingdom Fungi, with at least 83,000 described fungal species (James et al., 2020). In addition, ascomycetes have important ecological characteristics, including a greater number of genes associated with nutrition, carbohydrate metabolism, competitive capabilities, and tolerance to abiotic and biotic stresses when compared to other fungal phyla (Egidi et al., 2019). Thus, ascomycetes' high species diversity and functional plasticity allow these organisms to become the dominant group in the niche in which they occur and be more easily isolated in culture-dependent approaches.

Based on sequences of ITS rDNA, representative morphotypes were classified into three classes, eight orders, ten families, and 12 genera. The ITS region is considered a primary genetic marker for classifying fungal species (Badotti et al., 2017; Lücking et al., 2020; dos Reis et al., 2022a), and is widely used in environmental studies aimed at understanding and characterizing the diversity of endophytic fungi (Noriler et al., 2018; Iantas et al., 2021; Dos Santos et al., 2021; Morais et al., 2022; dos Reis et al., 2023a). Notably, there are limitations to delimiting species using only ITS, since this region does not provide sufficient resolution to delimit closely related species and cryptic species in some genera (Badotti et al., 2017; Lücking et al., 2020). In our study, the ITS region provided a resolution to classify 62.33% of the morphotypes at the species level (similarity > 97%) and 37.66% of the morphotypes at the genus level (similarity < 97%). This classification allowed us to analyse ecological inferences on the occurrence and diversity of genera and species of the endophytic fungal community of *P. polymorpha.* Thus, although the ITS region has not provided a resolution to classify some morphotypes or may not resolve cryptic species, this marker will allow us to choose the next molecular markers in future phylogenetic studies to accurately delimit the taxa found here.

Among the fungal classes, Sordariomycetes was the dominant class in the three organs (> 80%), followed by Dothideomycetes, while Eurotiomycetes were exclusive to flowers and leaves. Diaporthales and Amphisphaeriales were the dominant and most species-rich orders in the three organs. The dominant genera were *Diaporthe*, *Nigrospora*, and *Colletotrichum*, with *Diaporthe* being the genus with the highest number of isolates/species. In addition, we observed the occurrence of species belonging to *Neurospora*, *Neopestalotiopsis*, and *Pestalotiopsis*. Species of *Diaporthe* and *Colletotrichum* are commonly reported as members of the cultivable endophytic mycobiota of plant species from tropical dry forests (Bezerra et al., 2012a, 2012b, 2013, 2015; Noriler et al., 2018; dos Reis et al., 2023). The prevalence of species belonging to these genera may be linked to the evolutionary, adaptive success, and phenotypic plasticity of these species (Gomes et al., 2013; Ma et al., 2018; Hilário et al., 2023). We should also consider that the dominant presence of these genera might be a result of the limitations of cultivation-dependent methods, which result in a low diversity of endophytic fungal species, with a tendency to isolate ubiquitous and fast-growing species, such as representatives of *Colletotrichum*, *Diaporthe*, *Neopestalotiopsis*, and *Pestalotiopsis* (Bhunjun et al., 2023). Richness estimator indices suggested that the genera and species richness of the cultivated endophytic fungal community of *P. polymorpha* is greater than that observed in this study. Furthermore, most of the isolates recovered in our study are fast-growing and ubiquitous fungal species. These results may be due to limitations in our methodology, from the choice of surface disinfection method, size of plant tissue fragments, isolation method, and incubation time (dos Reis et al., 2022a, b, dos Reis et al., 2023; Bhunjun et al., 2023). Furthermore, we can also suggest that, although we used different culture media to recover a greater number of fungal species, a greater number of samples could perhaps result in a greater number of recovered fungal species.

The community of endophytic fungi in *P. polymorpha* was composed of 38 species, with the highest number of species observed in leaves (24 species); greater Shannon and Simpson diversity in stems (H' = 1.70 ± 0.17;

Simpson diversity = 0.76 ± 0.05); and greater uniformity in flowers (J = 0.97 ± 0.01). The heterogeneity in species distribution among organs and the high equity values (close to 1.0) that we observed in our study seem to be a characteristic of the community of cultivable endophytic fungi associated with species from tropical regions (Arnold and Herre, 2003; Arnold et al., 2003). Organs of plant species from tropical regions are known to harbour a great richness and diversity of endophytic fungi (Arnold and Herre, 2003; Arnold et al., 2003; Arnold, 2007; Arnold and Engelbrecht, 2007; Arnold and Lutzoni, 2007; Arnold et al., 2007; Hilarino et al., 2011; Noriler et al., 2018; Yao et al., 2019; dos Reis 2023). For example, it is estimated that each leaf of plant species in tropical forests is colonized by numerous species of endophytic fungi, rather than systemic colonization by a single fungal species (Arnold and Herre, 2003; Arnold et al., 2003). Thus, each leaf can harbour up to one species per millimetre square of tissue and each host leaf can have distinct communities (Arnold et al., 2000).

The alpha diversity metrics reflected a certain degree of heterogeneity in the structure of the endophytic fungal community across organs. In general, the different organs of the same host species can accommodate endophytic fungal communities and the communities in each organ may have certain organizational preferences depending on the organ (Küngas et al., 2020; Wang et al., 2022). These variations can be explained by numerous factors, which include the characteristics of the organ (such as organization and cell types, chemical components, oxygen levels, enzymes, nutrients, half-life, etc.), which acts to form a unique microenvironment (Sun et al., 2008; Li et al., 2016; Ren et al., 2019; Küngas et al., 2020; Wang et al., 2022). This microenvironment, in turn, acts as an 'environmental filter', selecting species that will colonize it and determining the taxonomic structure in the mycobiome in terms of species richness, diversity and evenness (Ren et al., 2019; Küngas et al. al., 2020; Wang et al., 2022).

Beta diversity analysis showed that there was an overlap in the fungal community among different organs. Furthermore, the fungal community in flowers was more dispersed than the community present in leaves and stems. The bipartite network shows that most species and genera of fungi are shared among organs. Flowers shared the greatest number of species with leaves, and stems shared the greatest number of species with leaves. Regarding organ specificity, five species were exclusive to flowers and leaves, and seven species were exclusive to stems. The co-occurrence of species among different organs may be a result of the adaptive capacities of these species and systemic colonization since some species of endophytic fungi can initially colonize a given tissue and migrate to another (Fang et al., 2019). Tissue specificity, in turn, can be caused by variations in the tissue microenvironments of plants, which directly and indirectly affect the composition and dynamics of the fungal community associated with each tissue (Chai et al., 2016; Li et al., 2016; Du et al., 2020). The greater dispersion of the community associated with flowers may be the result of visitation by pollinating insects, which can be fungal spore transport vectors (Aylward et al., 2014; Hedtke et al., 2015; Becchimanzi and Nicoletti, 2022). Thus, the fungal inoculum in flowers tends to be larger and more phylogenetically diverse than in other organs, which may explain the presence of a greater number of classes and greater community dispersion.

Endophytic fungi isolated from *P. polymorpha* were grouped into four primary and secondary functional guilds. The primary dominant functional guild was the "phytopathogen" and the secondary dominant was the "litter saprotroph". The dominance of these functional guilds can be explained by the biological and evolutionary characteristics of the host plant together with the random environmental dynamics that act in the assembly process of the endophytic fungal community (Rodriguez et al., 2009; Fang et al., 2019). For example, the endophytic fungal community present in the different organs of *Ageratina adenophora* depends on the fungi that are present in the surrounding environment, such as air, soil, and rhizosphere (Fang et al., 2019). In our case, *P. polymorpha* is a decumbent subshrub species, which grows close to the soil and litter, which puts it in contact with

fungal species present in these two ecological niches. Therefore, its cultivable mycobiome will be rich in fungal species that present this duality in functional guilds and that present the necessary adaptive capacities to be able to colonize it in an endophytic way.

Our study is one of the first to study the diversity of cultivable endophytic fungi in a native subshrub of the Brazilian Cerrado. Therefore, we provide important data on the pattern of richness, diversity, occurrence, and co-occurrence of cultivable endophytic fungal species in different organs of *P. polymorpha* in the Brazilian Cerrado. In addition, we provide a collection of isolates that represent an important genetic and biotechnological resource that can be better explored in future studies. Our study also reinforces the importance of a greater number of samples and sampling of different plant tissues/organs to obtain a ample diversity of fungal species, which can guide the choice of experimental design/experiments. Our findings also contribute to the estimation of the national and global fungal diversity associated to plants protected in conservation units of the Cerrado in Brazil, highlighting this biome as also a hotspot of mycodiversity.

Declarations

Funding

This study was sponsored by grants from the Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF)– Proc. SEI 00193.00000229/2021-21, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES)– Funding code 001, and the University of Brasília DPG Nº 010/2023.

Data Availability

The internal transcribed spacer region (ITS) of the nrDNA operon and intervening 5.8S nRNA gene amplicon data generated in the article is accessible from NCBI through the gene sequence accession numbers available at: http://www.ncbi.nlm.nih.gov/. The deposit codes are in Table 2.

Contributions Made By Each Author

JBAR idealized the project, collected the samples, carried out the laboratory work, analyzed the data, and wrote the manuscript. JDPB and HMMV reviewed the manuscript. All authors read and approved the final version of the manuscript.

ETHICS DECLARATIONS

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Competing interests

The authors declare no competing interests.

ACKNOWLEDGMENTS

We thank Prof. Danilo Batista Pinho (Department of Phytopathology, University of Brasília) for allowing this work to be carried out in his laboratory. Without the infrastructure of its laboratory, the steps of surface disinfection, isolation, purification of cultures, DNA extraction, and deposit of isolates in the culture collection of the University of Brasília (CCUB) would not be possible. We also thank Clemildo de Souza Queiroz Júnior for his precious collaboration in the laboratory stages. We are grateful to the Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF)–Proc. SEI 00193.00000229/2021-21, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES)–Financing Code 001, and the University of Brasília DPG Nº 010/2023.

References

1. Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90 (6):1829-45. doi: 10.1007/s00253-011-3270-y.
2. Arnold AE and Engelbrecht BMJ (2007) Fungal endophytes double minimum leaf conductance in seedlings of a tropical tree. J Trop Ecol 23: 369–372.
3. Arnold AE, Henk DA, Eells RA, Lutzoni F, Vilgalys R (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental pcr. Mycologia 99: 185–206.
4. Arnold AE and Herre EA (2003) Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in Theobroma cacao (Malvaceae). Mycologia 95: 388–398.
5. Arnold AE and Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots?. Ecology 88: 541–549.
6. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA, (2000) Are tropical fungal endophytes hyperdiverse?. Ecol Lett 3: 267–274.
7. Arnold AE (2007) Understanding the diversity of foliar fungal endophytes: progress, challenges, and frontiers. Fungal Biol Rev 21: 51–66.
8. Aylward J, Dreyer LL, Steenkamp ET, Wingfield MJ, Roets F (2014) Panmixia defines the genetic diversity of a unique arthropod-dispersed fungus specific to Protea flowers. Ecol. Evol. 4 (17): 3444-55. doi: 10.1002/ece3.1149.
9. Badotti F, de Oliveira FS, Garcia CF, Vaz AB, Fonseca PL, Nahum LA, Oliveira G, Góes-Neto A (2017) Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). BMC Microbiol 23;17(1):42. doi: 10.1186/s12866-017-0958-x.
10. Becchimanzi A and Nicoletti R (2022) Aspergillus-bees: A dynamic symbiotic association. Front Microbiol. 7;13:968963. doi: 10.3389/fmicb.2022.968963.
11. Bhunjun CS, Phukhamsakda C, Hyde KD, et al. (2023) Do all fungi have ancestors with endophytic lifestyles? Fungal Divers https://doi.org/10.1007/s13225-023-00516-5.
12. Cardoso MRD, Marcuzzo FFN, and Barros JR (2014) Climatic classification of Köppen-Geiger for the state of Goiás and the Federal District. ACTA Geog 8(6): 40-55.
13. Cavararo R, (2004) Reserva Ecológica do IBGE: Ambiente e plantas vasculares. Estudos e Pesquisas. Informação Geográfica. 3, 1-71.

14. Chai XY, Chai GQ, Xiang YY, Zhang WW, Yin PF (2016) Composition and ecological distribution of endophytic and epiphytic fungi from the foliage of Pteroceltis tatarinowii. Acta Ecol Sin 36(16): 5163–5172.
15. Chi WC, Chen W, He CC, Guo SY, Cha HJ, Tsang LM, Ho TW, Pang KL (2019) A highly diverse fungal community associated with leaves of the mangrove plant Acanthus ilicifolius var. xiamenensis revealed by isolation and metabarcoding analyses. PeerJ 9; 7: e7293. doi: 10.7717/peerj.7293.
16. Dixon P (2003) VEGAN, A Package of R Functions for Community Ecology. J Veg Sci 14(6), 927–930. http://www.jstor.org/stable/3236992.
17. Dormann CF, Bernd G and Jochen F (2008) Introducing the bipartite Package: Analysing Ecological Networks. R News. 8.
18. dos Reis JBA, do Vale HMM and Lorenzi AS (2022a). Insights into taxonomic diversity and bioprospecting potential of Cerrado endophytic fungi: a review exploring an unique Brazilian biome and methodological limitations. World J Microbiol Biotechnol 38, 202. https://doi.org/10.1007/s11274-022-03386-2.
19. dos Reis JBA, Lorenzi AS and do Vale HMM (2022b) Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. Arch Microbiol 204, 675. https://doi.org/10.1007/s00203-022-03283-0.
20. dos Reis JBA, Pappas Junior GJ, Lorenzi AS, Pinho DB, Costa AM, Bustamante MMdC, Vale HMM (2023) How Deep Can the Endophytic Mycobiome Go? A Case Study on Six Woody Species from the Brazilian Cerrado. *J Fungi 9*, 508. https://doi.org/10.3390/jof9050508.
21. Dos Santos IR, Abdel-Azeem AM, Mohesien MT, Piekutowska M, Sheir DH, da Silva LL, da Silva Castro C, Carvalho DDC, Bezerra JDP, Saad HA, Borges LL, Xavier-Santos S (2021) Insights into the Bioprospecting of the Endophytic Fungi of the Medicinal Plant *Palicourea rigida* Kunth (Rubiaceae): Detailed Biological Activities. J Fungi 25; 7(9): 689. doi: 10.3390/jof7090689.
22. Du W, Yao Z, Li J, Sun C, Xia J, Wang B, Shi D, Ren L (2020) Diversity and antimicrobial activity of endophytic fungi isolated from *Securinega suffruticosa* in the Yellow River Delta. Plos One 10; 15 (3): e0229589. doi: 10.1371/journal.pone.0229589.
23. Durán M, San Emeterio L, Canals RM (2021) Comparison of Culturing and Metabarcoding Methods to Describe the Fungal Endophytic Assemblage of *Brachypodium rupestre* Growing in a Range of Anthropized Disturbance Regimes. Biology 29; 10 (12): 1246. doi: 10.3390/biology10121246.
24. Egidi E, Delgado-Baquerizo M, Plett JM, et al. (2019) A few Ascomycota taxa dominate soil fungal communities worldwide. Nat Commun 10, 2369 (2019). https://doi.org/10.1038/s41467-019-10373-z.
25. Eiten G (1972) The Cerrado vegetation of Brazil. Bot Rev 38: 201-341.
26. Eiten G (1994) Vegetação do Cerrado. In Cerrado: Caracterização, Ocupação e Perspectivas. Ed Universidade de Brasília. 17–73.
27. El-Shafey NM, Marzouk MA, Yasser MM, Shaban SA, Beemster GTS, AbdElgawad H, (2021) Harnessing Endophytic Fungi for Enhancing Growth, Tolerance and Quality of Rose-Scented Geranium (*Pelargonium graveolens* (L'Hér) Thunb.) Plants under Cadmium Stress: A Biochemical Study. J. Fungi. 3; 7 (12): 1039. doi: 10.3390/jof7121039.
28. Fang K, Miao YF, Chen L, Zhou J, Yang ZP, Dong XF, Zhang HB (2019) Tissue-Specific and Geographical Variation in Endophytic Fungi of *Ageratina adenophora* and Fungal Associations With the Environment. Front. Microbiol. 18;10:2919. doi: 10.3389/fmicb.2019.02919.

29. Fernandes-Júnior AJ and Konno TUP (2017) **Malvaceae do Parque Estadual do Ibitipoca, Estado de Minas Gerais, Brasil**. Hoehnea 44(4): 505-523, 5.
30. Gamboa MA, Laureano S, Bayman P (2002) Measuring diversity of endophytic fungi in leaf fragments: does size matter? Mycopathologia 156 (1): 41-5. doi: 10.1023/a:1021362217723.
31. Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW (2013) *Diaporthe*: A genus of endophytic, saprobic and plant pathogenic fungi. Persoonia *31*, 1–41.
32. Gong A, Zhou T, Xiao C, Jiang W, Zhou Y, Zhang J, Liang Q, Yang C, Zheng W, Zhang C, (2019) Association between dipsacus saponin VI level and diversity of endophytic fungi in roots of *Dipsacus asperoides*. World J Microbiol Biotechnol 18; 35 (3): 42. doi: 10.1007/s11274-019-2616-y.
33. Hawar SN (2022) Extracellular Enzyme of Endophytic Fungi Isolated from *Ziziphus spina* Leaves as Medicinal Plant. Int J Biomater 6; 2022: 2135927. doi: 10.1155/2022/2135927.
34. Hedtke SM, Blitzer EJ, Montgomery GA, Danforth BN (2015) Introduction of Non-Native Pollinators Can Lead to Trans-Continental Movement of Bee-Associated Fungi. Plos One 23; 10 (6): e0130560. doi: 10.1371/journal.pone.0130560.
35. Hilarino MPA, Silveira FA de O e, Oki Y, Rodrigues L, Santos JC, Corrêa Junior A, et al., (2011) Distribution of the endophytic fungi community in leaves of Bauhinia brevipes (Fabaceae). Acta Bot Bras 25 (4): 815–21. doi: https://doi.org/10.1590/S0102-33062011000400008.
36. Hilário S and Gonçalves MFM (2023) Mechanisms Underlying the Pathogenic and Endophytic Lifestyles in *Diaporthe*: An Omics-Based Approach. *Horticulturae* 9 (4): 423. doi: https://doi.org/10.3390/horticulturae9040423.
37. Hoog GS Gerrits van den Ende AH (1998) Molecular diagnostics of clinical strains of filamentous Basidiomycetes. Mycoses 41(5-6):183-9.
38. Iantas J, Savi DC, Schibelbein RDS, Noriler SA, Assad BM, Dilarri G, Ferreira H, Rohr J, Thorson JS, Shaaban KA, Glienke C (2021) Endophytes of Brazilian Medicinal Plants With Activity Against Phytopathogens. Front Microbiol 1; 12: 714750. doi: 10.3389/fmicb.2021.714750.
39. James TY, Stajich JE, Hittinger CT, Rokas A (2020) Toward a Fully Resolved Fungal Tree of Life. Annu Rev Microbiol 8; 74:291-313. doi: 10.1146/annurev-micro-022020-051835.
40. Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. Front Microbiol 9; 7: 906. doi: 10.3389/fmicb.2016.00906.
41. Kindt R and Coe R (2005) Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi (Kenya). ISBN 92-9059-179-X, http://www.worldagroforestry.org/output/tree-diversity-analysis.
42. Küngas K, Bahram M, Põldmaa K (2020) Host tree organ is the primary driver of endophytic fungal community structure in a hemiboreal forest. FEMS Microbiol Ecol 96fiz199. doi: 10.1093/femsec/fiz199.
43. Li P, Wu Z, Liu T, Wang Y (2016) Biodiversity, Phylogeny, and Antifungal Functions of Endophytic Fungi Associated with *Zanthoxylum bungeanum*. Int J Mol Sci 13;17(9):1541. doi: 10.3390/ijms17091541.
44. Liu X, Zhou ZY, Cui JL, Wang ML, Wang JH (2021) Biotransformation ability of endophytic fungi: from species evolution to industrial applications. Appl Microbiol Biotechnol 105 (19): 7095-7113. doi: 10.1007/s00253-021-11554-x.
45. Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, et al., (2020) Unambiguous identification of fungi: where do we

stand and how accurate and precise is fungal DNA barcoding? IMA Fungus 10; 11: 14. doi: 10.1186/s43008-020-00033-z.

46. Ma X, Nontachaiyapoom S, Jayawardena RS, Hyde KD, Gentekaki E, Zhou S, Qian Y, Wen T, Kang J, (2018) Endophytic *Colletotrichum* species from *Dendrobium* spp. in China and Northern Thailand. MycoKeys 43: 23-57. https://doi.org/10.3897/mycokeys.43.25081
47. Martins F, Mina D, Pereira JA, Baptista P, (2021) Endophytic fungal community structure in olive orchards with high and low incidence of olive anthracnose. Sci Rep 12; 11 (1): 689. doi: 10.1038/s41598-020-79962-z.
48. Morais EM, Silva AAR, Sousa FWA, Azevedo IMB, Silva HF, Santos AMG, Beserra Júnior JEA, Carvalho CP, Eberlin MN, Porcari AM, Araújo FDDS (2022) Endophytic *Trichoderma* strains isolated from forest species of the Cerrado-Caatinga ecotone are potential biocontrol agents against crop pathogenic fungi. Plos One 15; 17(4): e0265824. doi: 10.1371/journal.pone.0265824.
49. Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nat 403, 853–858. doi: 10.1038/35002501.
50. Niu L, Rustamova N, Ning H, Paerhati P, Lu C, Yili A, 2022. Diversity and Biological Activities of Endophytic Fungi from the Flowers of the Medicinal Plant *Vernonia anthelmintica*. Int J Mol Sci 8; 23 (19): 11935. doi: 10.3390/ijms231911935.
51. Noriler SA, Savi DC, Aluizio R, Palácio-Cortes AM, Possiede YM, Glienke C (2018) Bioprospecting and Structure of Fungal Endophyte Communities Found in the Brazilian Biomes, Pantanal, and Cerrado. Front Microbiol 24; 9: 1526. doi: 10.3389/fmicb.2018.01526.
52. Pang B, Yin D, Zhai Y, He A, Qiu L, Liu Q, Ma N, Shen H, Jia Q, Liang Z, Wang D (2022) Diversity of endophytic fungal community in *Huperzia serrata* from different ecological areas and their correlation with Hup A content. BMC Microbiol 5; 22 (1): 191. doi: 10.1186/s12866-022-02605-y.
53. Pietro-Souza W, Mello IS, Vendruscullo SJ, Silva GFD, Cunha CND, White JF, Soares MA, (2017) Endophytic fungal communities of *Polygonum acuminatum* and *Aeschynomene fluminensis* are influenced by soil mercury contamination. Plos One 25; 12 (7): e0182017. doi: 10.1371/journal.pone.0182017.
54. Põlme S, Abarenkov K, Henrik Nilsson R, et al., (2020) FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Divers 105, 1–16 (2020). https://doi.org/10.1007/s13225-020-00466-2.
55. Ren F, Dong W, Yan DH (2019) Organs, Cultivars, Soil, and Fruit Properties Affect Structure of Endophytic Mycobiota of Pinggu Peach Trees. Microorganisms 7: 322. doi: 10.3390/microorganisms7090322.
56. Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol. 182 (2): 314-330. doi: 10.1111/j.1469-8137.2009.02773.x.
57. Sun J, Guo L, Zang W, Ping W, Chi D (2008) Diversity and ecological distribution of endophytic fungi associated with medicinal plants. Sci China C Life Sci 51:751–759. doi: 10.1007/s11427-008-0091-z.
58. Vilgalys R and Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172: 4238–4246.
59. Wang R, Zhang Q, Ju M, Yan S, Zhang Q, Gu P (2022) The Endophytic Fungi Diversity, Community Structure, and Ecological Function Prediction of *Sophora alopecuroides* in Ningxia, China. Microorganisms 22; 10(11): 2099. doi: 10.3390/microorganisms10112099.
60. Wang Y, Gao BL, Li XX, Zhang ZB, Yan RM, Yang HL, Zhu D (2015) Phylogenetic diversity of culturable endophytic fungi in Dongxiang wild rice (*Oryza rufipogon* Griff), detection of polyketide synthase gene and their antagonistic activity analysis. Fungal Biol 119 (11): 1032-1045. doi: 10.1016/j.funbio.2015.07.009.

61. Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, Hu Y, (2022) Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. J Fungi (Basel). 20; 8(2): 205. doi: 10.3390/jof8020205.
62. Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.
63. Yadav M, Yadav A, Kumar S, Yadav JP, (2016) Spatial and seasonal influences on culturable endophytic mycobiota associated with different tissues of *Eugenia jambolana* Lam. and their antibacterial activity against MDR strains. BMC Microbiol 18; 16:44. doi: 10.1186/s12866-016-0664-0.
64. Yang G, Li P, Meng L, Xv K, Dong F, Qiu Y, He L, Lin L (2018) Diversity and communities of culturable endophytic fungi from different tree peonies (geoherbs and non-geoherbs), and their biosynthetic potential analysis. Braz J Microbiol 49 Suppl 1(Suppl 1):47-58. doi: 10.1016/j.bjm.2018.06.006.
65. Yao H, Sun X, He C, Maitra P, Li XC, Guo LD (2019) Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. Microbiome 9;7 (1):57. doi: 10.1186/s40168-019-0671-0.
66. Yao YQ, Lan F, Qiao YM, Wei JG, Huang RS, Li LB (2017) Endophytic fungi harbored in the root of *Sophora tonkinensis* Gapnep: Diversity and biocontrol potential against phytopathogens. Microbiol 6(3): e00437. doi: 10.1002/mbo3.437.
67. Yu J, Wu Y, He Z, Li M, Zhu K, Gao B, (2018) Diversity and Antifungal Activity of Endophytic Fungi Associated with *Camellia oleifera*. Mycobiology 23; 46(2): 85-91. doi: 10.1080/12298093.2018.1454008.

Figures

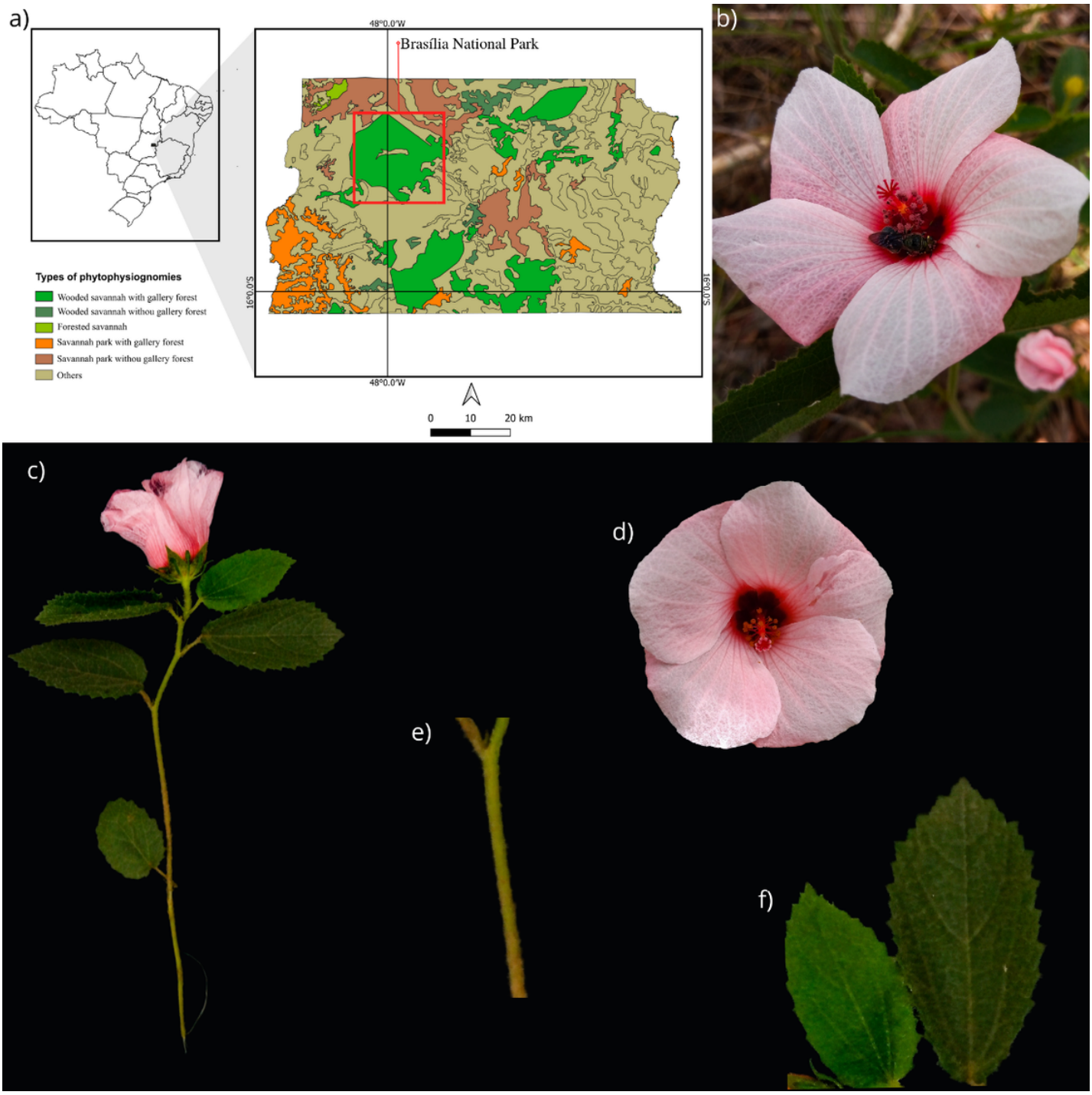

Figure 1

Sampling area and morphology of *Peltaea polymorpha*. a) Geographic location of the Brasília National Park, Federal District, Brazil. b) *P. polymorpha* under natural conditions. c) Morphological details of *P. polymorpha*: d) flower, e) stems, and f) leaves.

Figure 2

Representative morphotypes of endophytic fungi isolated from *P. polymorpha*. The morphotypes were defined based on their morphological characteristics, which included the colour of the colony (verse and obverse), growth rate, the appearance of the mycelium, shape, structure of the hyphae, reproductive structures and colour of exudates.

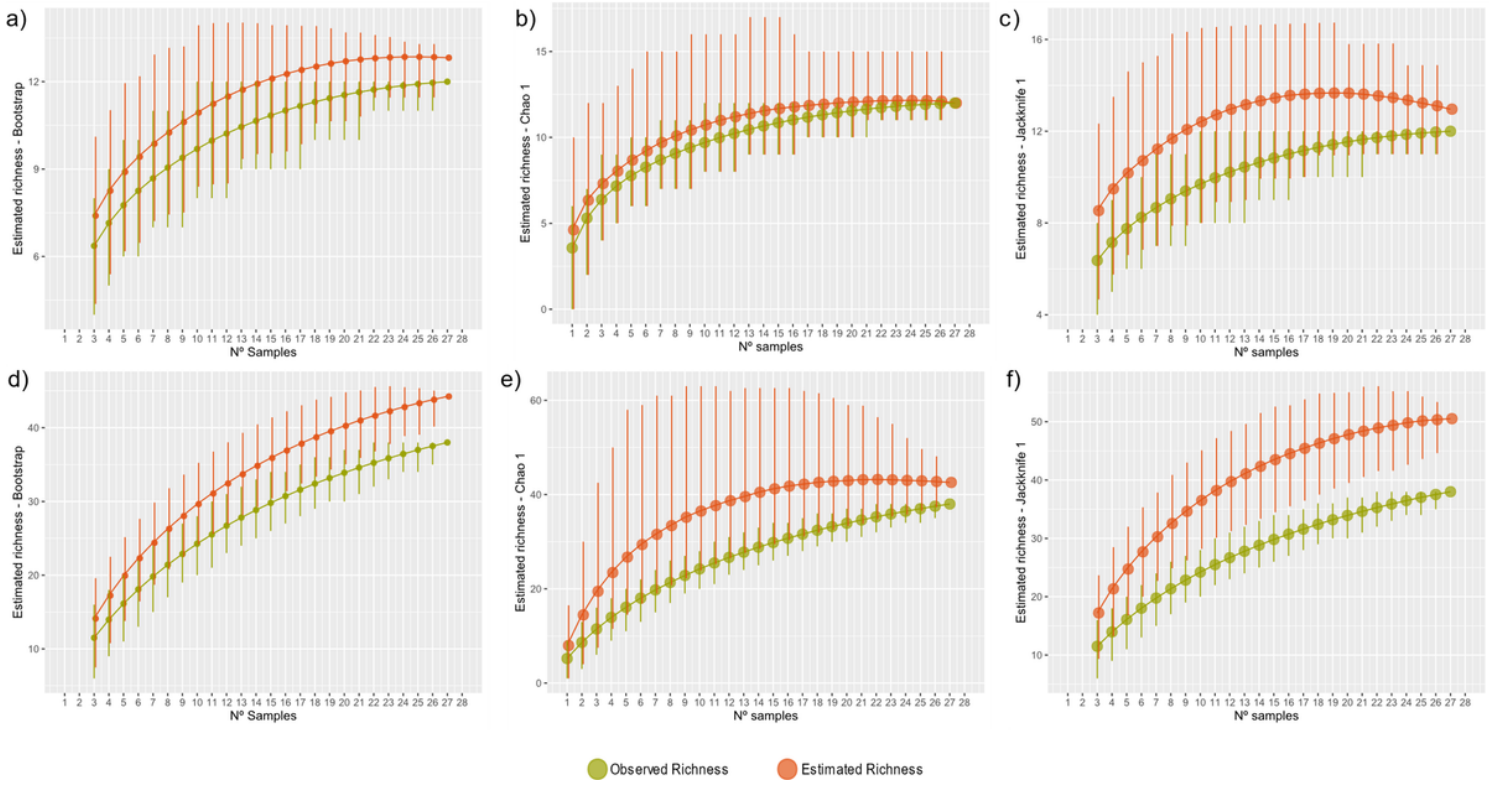

Figure 3

Genus/species richness estimators with 95% confidence intervals of the cultivable endophytic fungal community of *P. polymorpha*, Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil. Genus-level richness estimators a) Bootstrap, b) Chao 1, and c) Jacknife 1. Species-level richness estimators: d) Bootstrap, e) Chao 1, f) Jacknife 1. The richness estimators were calculated with 10,000 permutations.

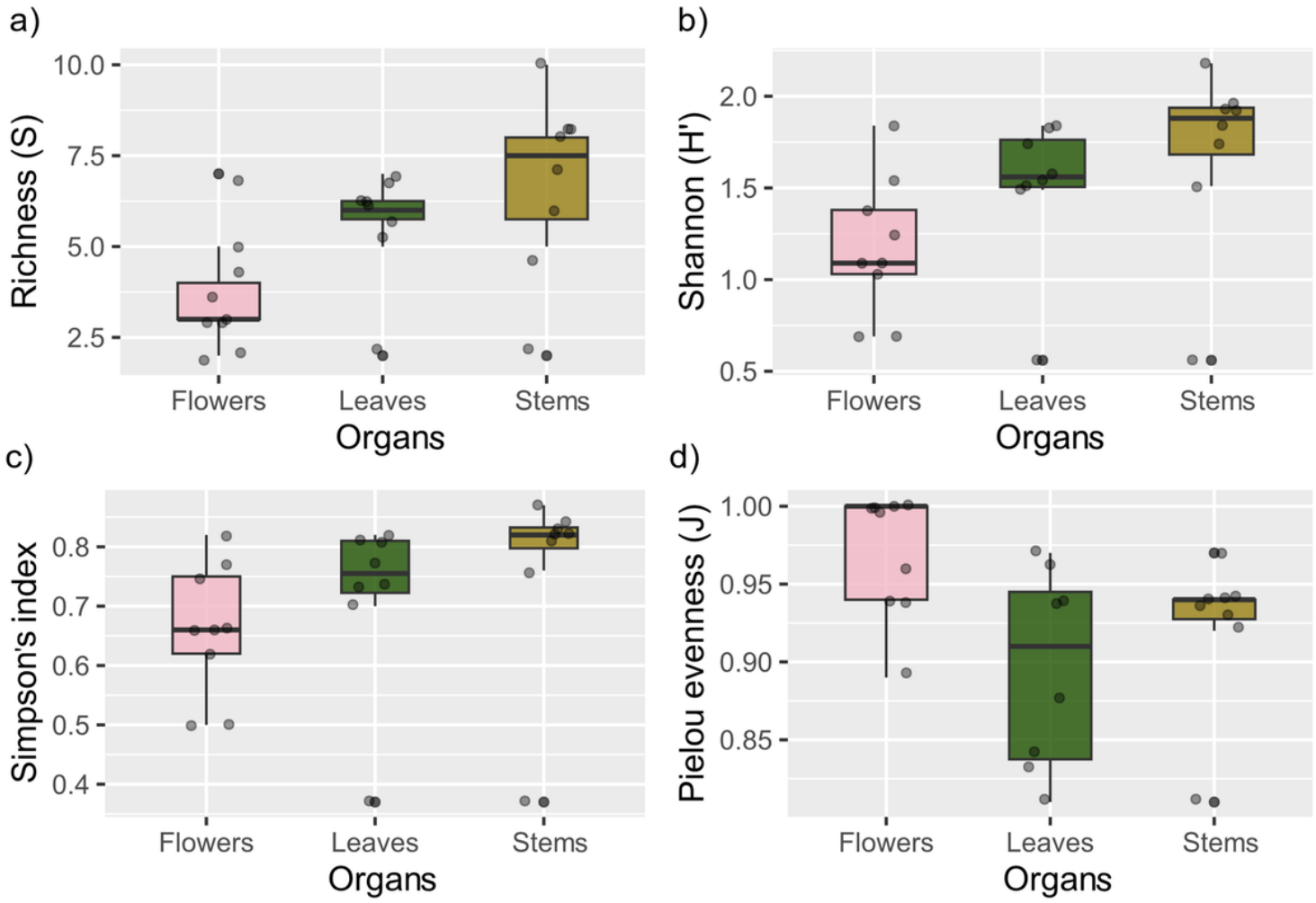

Figure 4

Alpha diversity estimations of cultivable endophytic fungi associated with flowers, leaves, and stems of *P. polymorpha*, Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil . a) species richness (S); b) Shannon diversity 'H'); c) Simps'n's diversity; e) Pielou evenness (J).

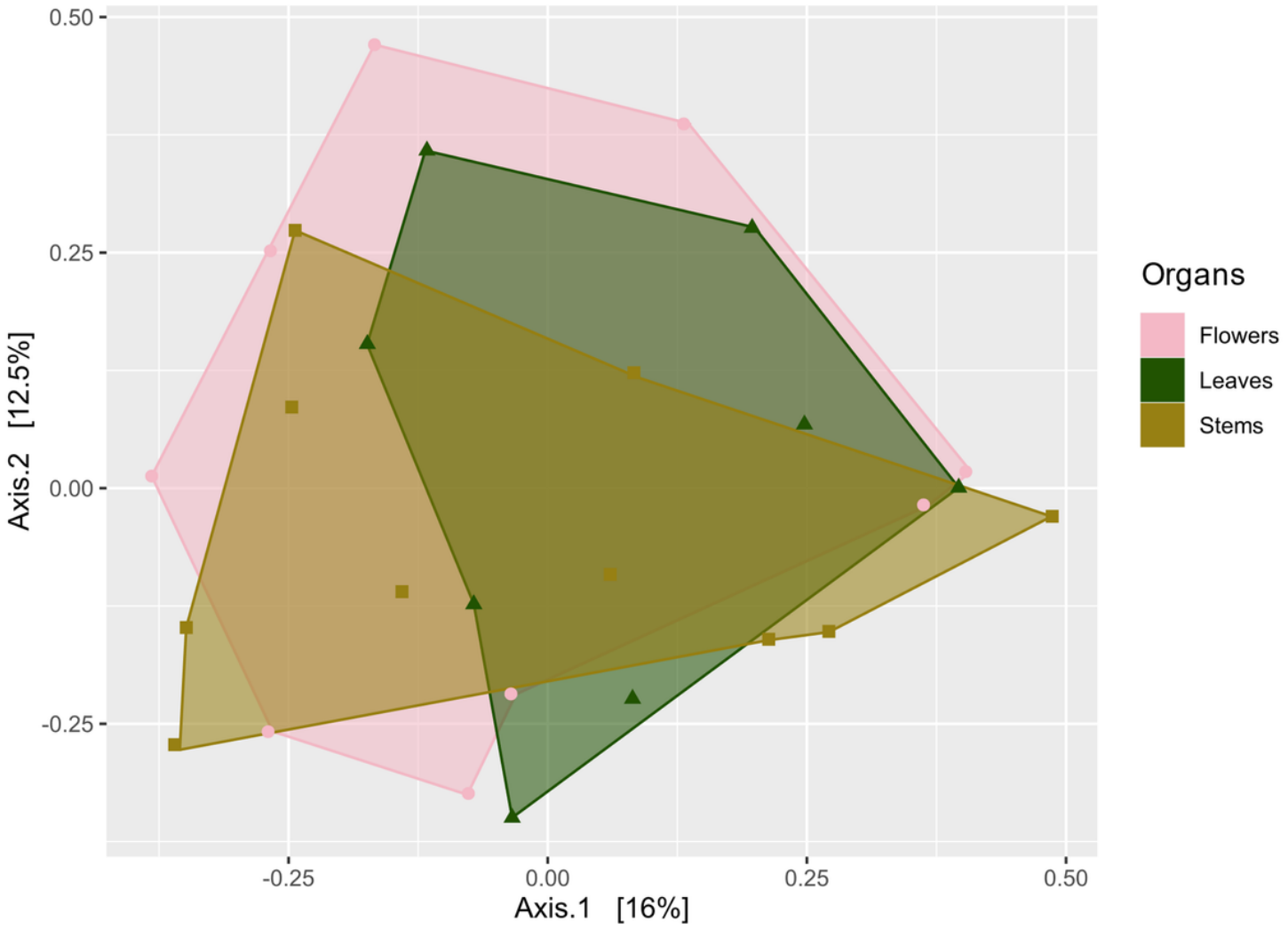

Figure 5

Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity, showing the distance in communities of cultivable endophytic fungi associated with flowers, leaves, and stems of *P. polymorpha,* Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil.

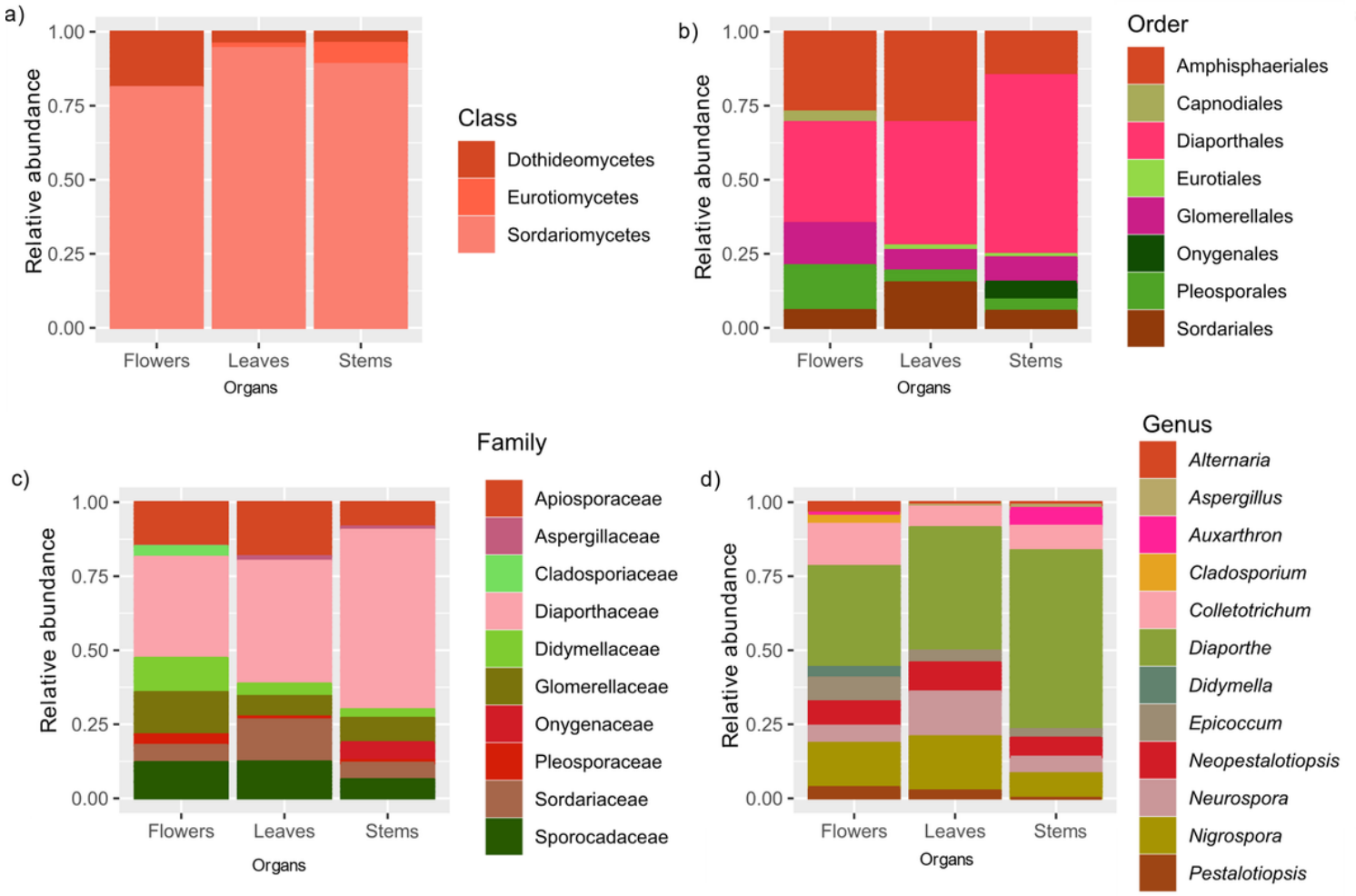

Figure 6

Relative abundance of cultivable endophytic fungi community from flowers, leaves, and stems of *P. polymorpha,* Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil. Relative abundance level of a) class, b) order, c) family, and d) genus.

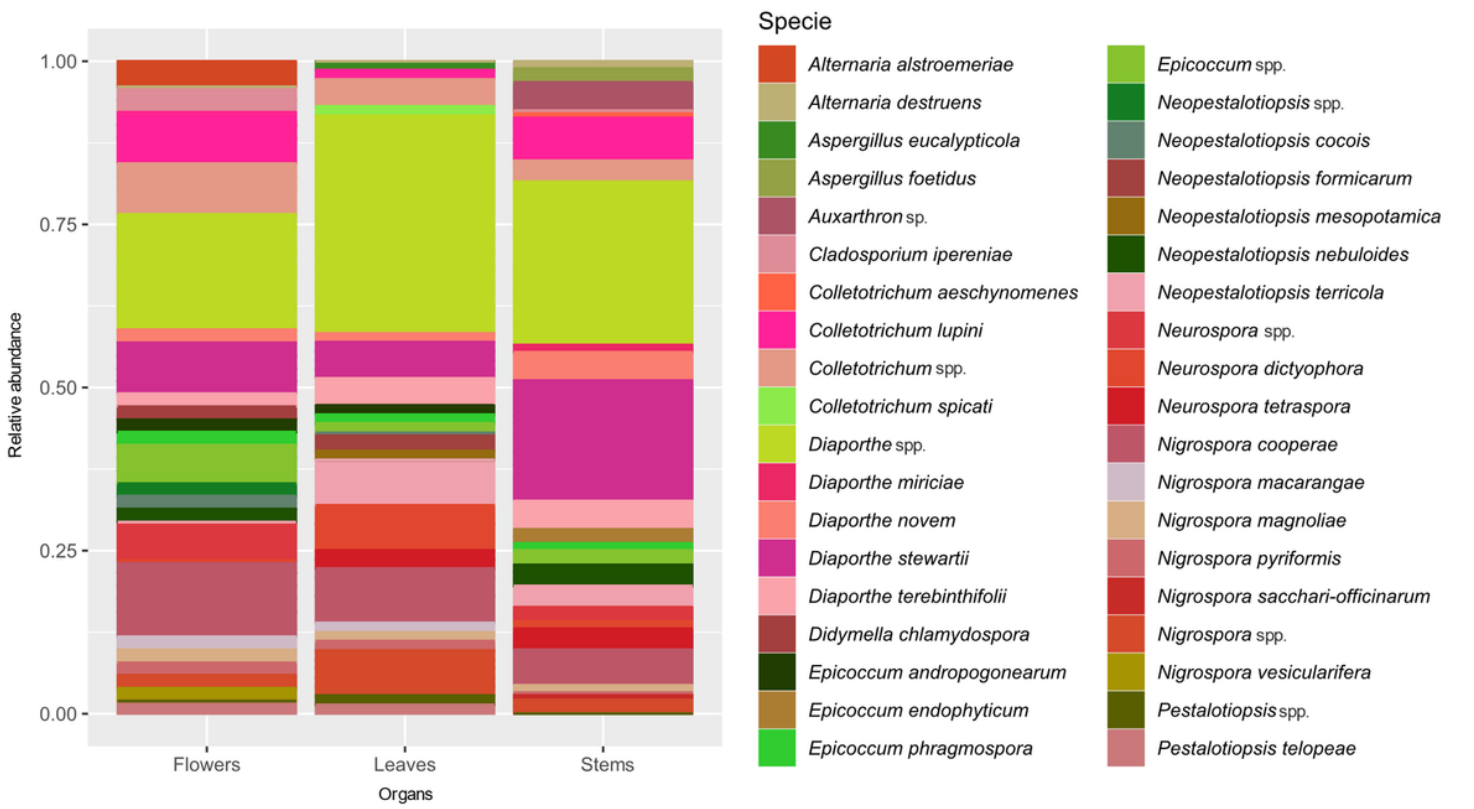

Figure 7

Relative abundance of cultivable endophytic fungus community from flowers, leaves, and stems of *P. polymorpha,* Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil. Relative abundance at taxonomic level of species.

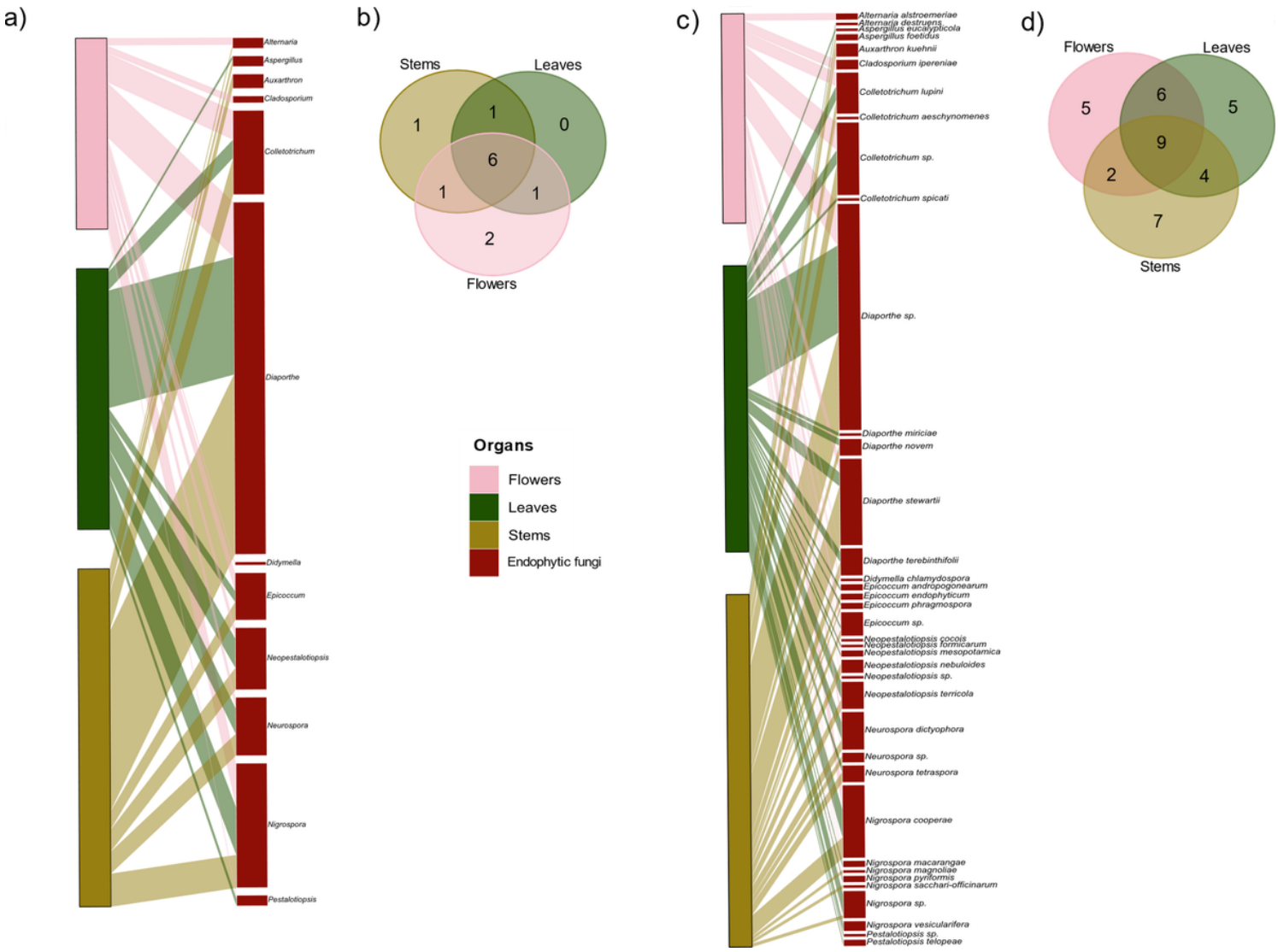

Figure 8

Bipartite networks of co-occurrence of endophytic fungi in flowers, leaves, and stems of *P. polymorpha,* Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil. In a) the co-occurrence network at the genus level and c) at the species level is shown. The left bars in both figures show the different organs of *P. polymorpha*; while the bars on the right refer to the genera/species of isolated endophytic fungi. The length of the bars on the left corresponds to the total number of isolates in each organ, and the length of the bars on the right corresponds to the number of isolates within each genus/species. The lines represent the abundance of each genus/species in each organ. Venn diagrams (b and d) show the number of genera/species shared among different organs, respectively.

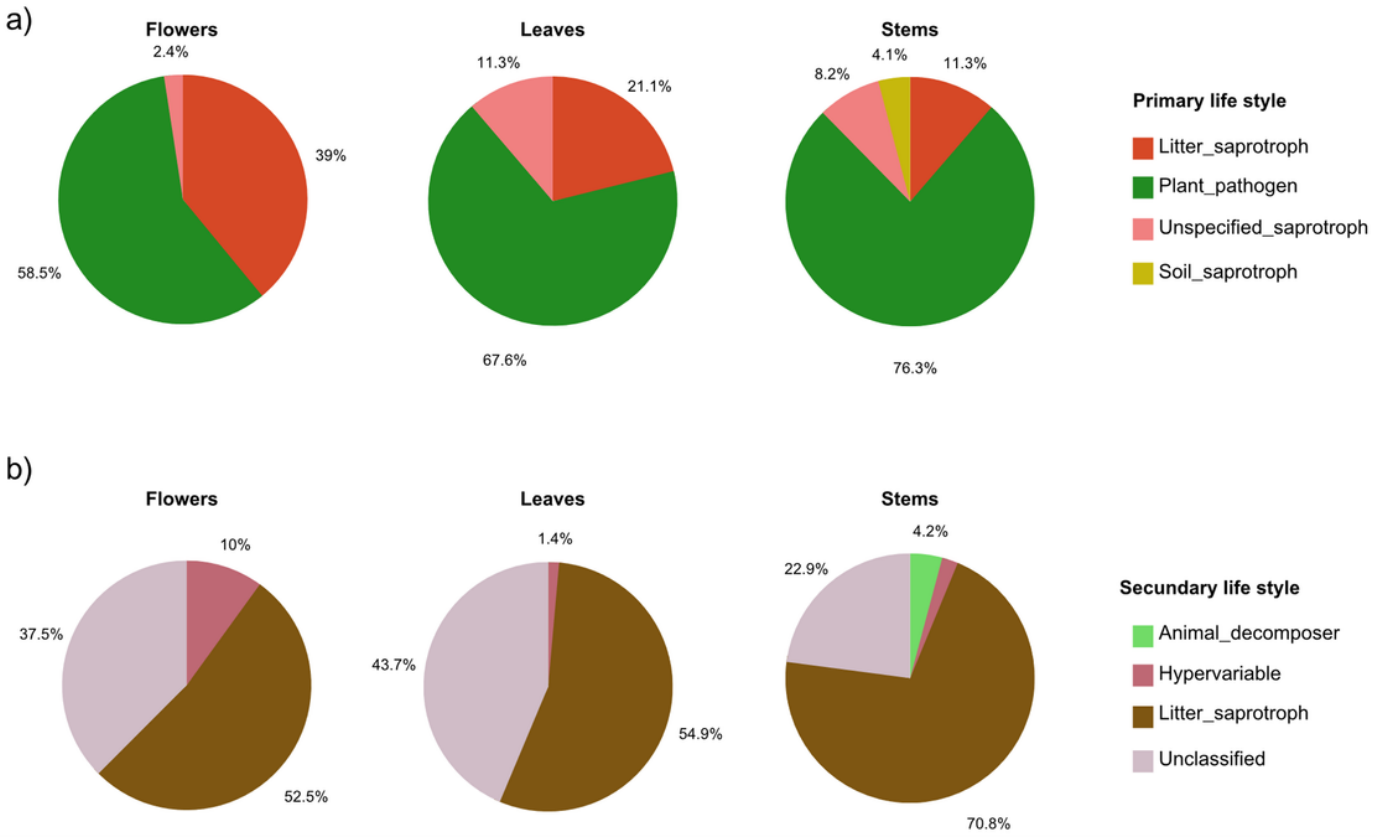

Figure 9

Functional guilds of the cultivable endophytic fungal community associated with *P. polymorpha,* Brazilian savannah (Cerrado), Brasília National Park, Federal District, Brazil. Inferences of fungal lifestyles were made using FungalTraits.