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Diversity of fungal endophytes associated with the Philippine endemic ginger *Vanoverberghia sepulchrei* Merr. (Zingiberaceae)

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Abstract

The pantropical Zingiberaceae family is among the most threatened monocotyledonous plants, with numerous species endemic to the Philippines, and thus, is an ideal host to study fungal endophytes. In this study, we determined the occurrence and diversity of fungal endophytes associated with the Philippine endemic ginger *Vanoverberghia sepulchrei* Merr. All major plant organs, i.e., leaf, pseudostem, rhizome, and root, were surface-sterilized to isolate *Vanoverberghia*-associated fungal endophytes (VFE). A combination of morphological characteristics and phylogenetic analyses of DNA sequences derived from ITS were used to identify selected isolated fungi. Our results showed the isolation of twenty fungal morphospecies, and these were identified as belonging to the genera *Bjerkandera*, *Cladosporium*, *Colletotrichum*, *Cosmospora*, *Diaporthe*, *Fusarium*, *Leptographium*, *Mucor*, *Nigrospora*, *Perenniporia*, *Phomopsis*, *Phyllosticta*, *Phytophthora*, *Pseudopithomyces*, *Rhizopus*, and *Trichoderma.* The results of this study paved the way for the first report of fungal endophytes from the Philippine endemic ginger.

Keywords – biodiversity – endemic plants – fungal phylogeny – ginger – Philippine flora

Introduction

The hundreds of endemic plant species and the top levels of global plant endemism make Southeast Asia one of the world's most biodiverse landscapes (Harrison & Griffin, 2020). The Philippine archipelago, for example, is known for over 9,000 plant species with more than 6,000 indigenous or endemic species (Myers et al. 2000). The Zingiberaceae, also known as the ginger family, is among the most prominent monocotyledonous family in the Order Zingiberales and comprises roughly 53 genera and over 1,500 species (Taylor et al. 2009). These perennial, rhizomatous, usually aromatic herbs are pantropical in the Malesian floristic region comprising Brunei, Indonesia, Papua New Guinea, Peninsular Malaysia, the Philippines, and Singapore as having the highest concentration of genera and species (Zhou et al. 2018). Unfortunately, most species are found in tropical forests and are threatened by habitat loss, deforestation, and invasive alien species.

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One of the most intriguing genera in the Zingiberaceae family is the *Vanoverberghia,* named in honor of Father Maurice (Morice) Vanoverbergh (Docot et al. 2019). The type species of the genus, *V. sepulchrei* Merr., is restricted to northern Luzon provinces of Mountain Province (type locality), Benguet and Ifugao (Funakoshi & Ohashi 2000). This aromatic terrestrial herb is locally known in the Philippines as *agbab*/*akbab* (in Bontoc) and *barapat*/*chakchakil*/*paddapad* (in Igorot) and falls under the other threatened species **(**OTS**)** category due to the ongoing conversion of their natural habitats into alienable and disposable land**.** Locals in the Mountain Province enjoy the fruits of *V. sepulchrei*, which are said to have a sweet and sour taste (Docot et al**.** 2016). *Vanoverberghia* presently comprises only five species native to Taiwan and the Philippines. In the Philippines, so far, only two species have been described: *V. rubrobracteata* Docot & Ambida and *V. sepulchrei* Merr (Docot et al. 2019).

Endophytism is a unique association between a plant and a bacterium or a fungus in which the microorganism colonizes the healthy tissues of the plant without causing any visible disease. This endophytic relationship is almost universal among plants studied thus far (Chakraborty et al. 2019). These diverse endophytic fungi also have significant effects on the plant communities, either by enhancing plant fitness, providing tolerance to both abiotic and biotic stresses, promoting growth, or by negatively impacting fitness by altering resource allocation. Fungal endophytes in plants are described as fungi that live symbiotically within the interior tissues of their hosts for all or part of their life cycles without presenting any visible pathogenic symptoms (Song et al. 2017) but can become saprobes during the host senescence (Rodriguez et al. 2008). Fungal endophytes from plants in unusual or extreme environments have been shown to impact the host's ecological adaptation (Lata et al. 2018, Pan et al. 2018). Fungal endophytes also help plants develop faster and survive biotic and abiotic challenges (Potshangbam et al. 2017). They are advantageous to plants due to the production of secondary metabolites, which can protect the host plant against diseases and infestation (Estrada et al. 2013). The metabolites are with complex structures (Hamed et al. 2015) and varied biological activities (dela Cruz et al. 2020), including antimicrobial (Moron et al. 2018, Ramirez et al. 2020), anticancer (Chen et al. 2016), antidiabetic (Rondilla et al. 2022), antifungal (De Mesa et al. 2020, Pecundo et al. 2021), anti-HIV (Zhang et al. 2016), antioxidant (Eskandarighadikolaii et al. 2015) and cytotoxic activities (Apurillo et al. 2019, dela Cruz et al. 2023a).

Most research on fungal endophytes associated with the Philippine flora are on *Ficus* (Solis et al. 2016), *Pandanus amaryllifolius* Roxb. (Bungihan et al. 2011, 2013a, b), and some endemic plants such as *Canarium ovatum* (Torres & dela Cruz 2015), *Cinnamomum mercadoi* (Marcellano et al. 2017), and *Uvaria grandiflora* (Notarte et al. 2019). To add to this growing list of indigenous Philippine plants, this study isolated and identified fungal endophytes associated with the endemic ginger plant, *V. sepulchrei*. We hypothesized distinct assemblages of fungal endophytes exist in relation to the plant parts.

Materials & Methods

Collections of host plant

The Philippine endemic ginger plant *Vanoverberghia sepulchrei* was collected from a terraced mountain area in Brgy. Amganad, Banaue, Ifugao (16°53'9" N, 121°3'51" E) at an elevation of 1,243.8 masl (meters above sea level) (Fig. 1) and used to isolate VFE in this study**.** The stunning, pendulous inflorescence distinguishes the leafy shoot members by their crimson coriaceous ligules and flabellate leaf apices (Fig. 1). Symptomless and mature *Vanoverberghia sepulchrei* were gathered for the isolation of fungal endophytes and preparation of herbarium voucher specimen for species identification of the host plant.

Isolation of fungal endophytes

Fungal endophytes were isolated from the leaf, pseudostem, rhizome, and root of *V. sepulchrei*, following the protocol described by Hallmann et al. (2006). Briefly, the plant organs were thoroughly washed with running tap water to eliminate soil particles and then dried at room temperature on sterile

Whatman® filter papers. Each plant organ was cut separately, i.e., into 1 cm long segments for the pseudostems and roots, 2 cm^2 for the rhizomes, and 6-mm diameter for leaves. Thirty explants for each plant organ were surface sterilized by immersing in 70% EtOH for 1 minute, then in 5.3% NaOCl for 5 minutes, and finally in 70% EtOH for 30 seconds before finally rinsing three times in sterile distilled water. All explants were blot-dried on sterile Whatman® filter papers for 6 hrs, and five explants were placed in a Petri plate containing Potato Dextrose Agar (Condalab, Spain) supplemented with 50 mg/l tetracycline (Sigma) and 80 mg/l streptomycin (Sigma) as a bacterial growth suppressor, resulting in a total of six plates per organ (= 30 explants). To check for the effectiveness of the surface sterilization, 1 ml of the water from the final rinse was plated on PDA media to check for any microbial growth. All culture plates were incubated for seven days at room temperature and inspected every two days for fungal growth. To obtain pure cultures, the mycelia from the fungal colonies emerging from the edges of the explants were sub-cultured in a freshly prepared PDA medium free of antibiotics and incubated for seven days at room temperature. Fungal colonies were isolated and purified following further subcultures on new PDA growth media.

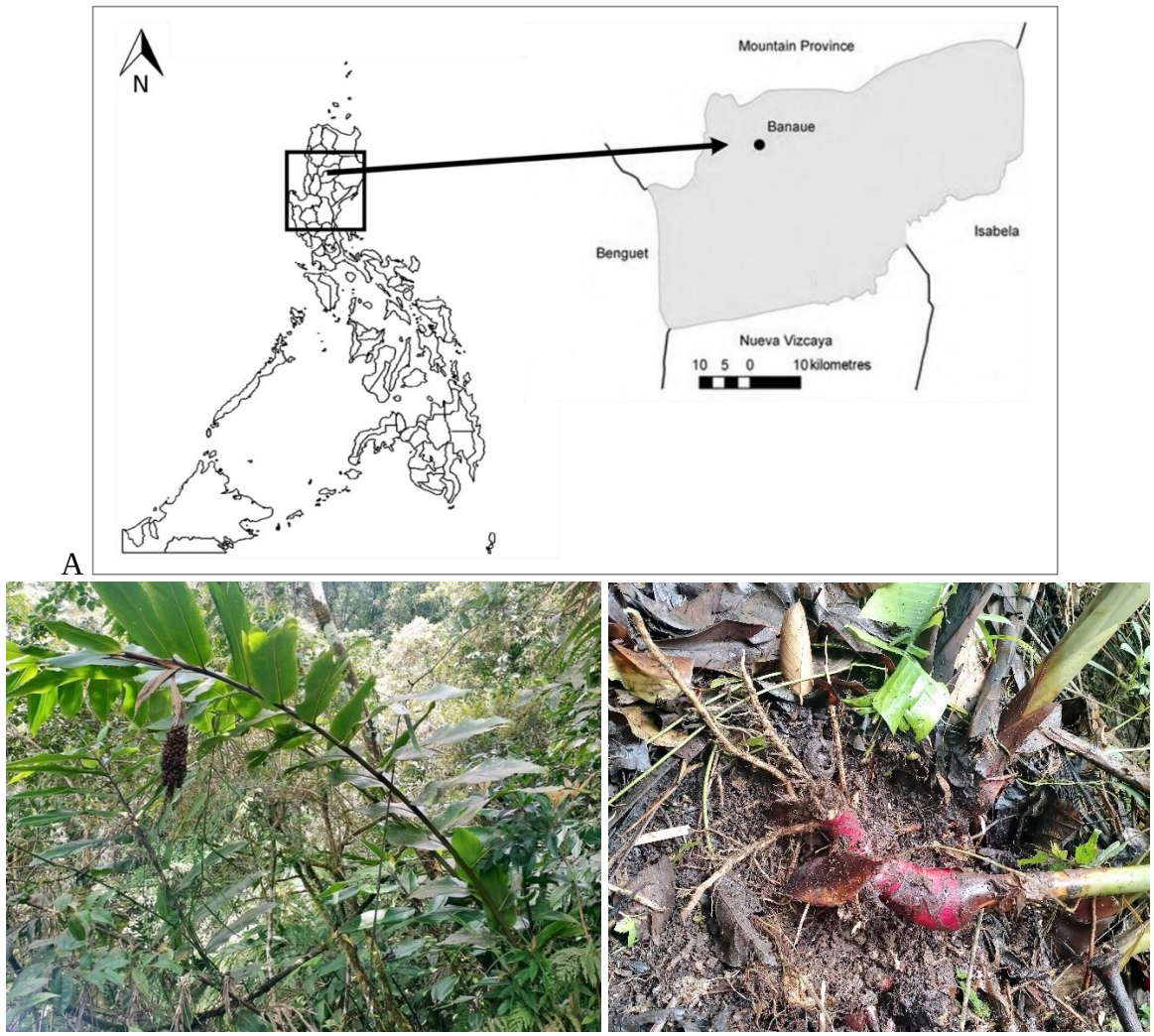

Fig. 1 – A The map of the collection site in Banaue, Ifugao in Northern Philippines. B *Vanoverberghia sepulchrei* Merr. with infructescence in its natural habitat and the roots and rhizomes**.**

Grouping into morphospecies

Vanoverberghia fungal endophytes, here designated as VFE, were cultured on PDA for seven days at room temperature. Following incubation, colony, hyphal, and spore morphologies were

examined under a compound (Yujie YJ-701BN-T) and stereomicroscope (Euromex ZE.1624). Comparison of the colonial growth and spore morphologies grouped the isolates into morphospecies.

Identification of fungal endophytes

DNA Extraction

To confirm species identity, representative isolates from each of the morphotypes were initially grown on PDA for at least seven days. Mycelia were lysed by homogenizing ~10 mg biomass in 500 µl 2% CTAB solution at 65°C. Removal of water-insoluble contaminants was carried out by organic extraction in a 1:1 volume of 24:1 chloroform-isoamyl alcohol. Further purification was done by adding 20% polyethylene glycol solution to a final concentration of 10% and washing of genomic DNA precipitate with 80% ethanol.

PCR Amplification

Fragments of the nuclear ribosomal internal transcribed spacer (ITS) region were amplified for all morphospecies with ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') fungal universal primer pairs (White et al. 1990). PCR was carried out using a thermal cycler for 35 cycles with an initial denaturation at 95°C for 5 min, cyclic denaturation at 95°C for 1 min, annealing at 55°C for 30 seconds, and extension at 70°C for 1 min with the final extension of 10 min at 72°C (hold at 4°C). The PCR products were validated visually by Agarose Gel Electrophoresis and were then sent to the Philippine Genome Center DNA Sequencing Core Facility for sequencing.

Sequence Processing, Alignment and Constructing Phylograms

BioEdit v.7.0.0 was used to assess the quality of the ITS gene sequences**.** DNA Baser was then used to generate consensus sequences for each isolate, which were loaded to the NCBI website using the Basic Local Alignment Search Tool (BLAST) search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare with related sequences and identify the isolates**.** These were used to look for published backbone trees of related taxa**.** The sequences were aligned with these reference sequences by using MAFFT (Multiple Sequence Alignment based on Fast Fourier Transform) software v7**.**0 (Katoh & Standley 2013) and BMGE **(**Block Mapping and Gathering with Entropy**) (**Criscuolo & Gribaldo 2010) at NGPhylogeny**.**fr (https://ngphylogeny.fr) (Lemoine et al. 2019). The Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway software v3.3 (https**:**//www**.**phylo**.**org/) (Miller et al**.** 2010) was used to generate maximum likelihood trees**.** Statistical support was computed from 1,000 bootstrap replicates by using RAxML v8.0. Finally, the generated phylogenetic trees were visualized and re**-**rooted with FigTree 1.4.4 (http**:**//tree**.**bio**.**ed**.**ac**.**uk/software/figtree/). Branches that received bootstrap support (BS) ≥ 50% for maximum likelihood were considered as significantly supported**.** Our gene sequences are finally uploaded to the NCBI Gene Bank (Table 1).

Ecological analyses

To analyze the ecological patterns, data on the occurrence of VFE on leaves, pseudostems, rhizomes, and roots were examined. Initially, the frequencies of VFE were assessed by counting the number of explants with isolates. Using these counts, colonization rate (CR) and isolation rate (IR) were computed as follows (Solis et al. 2010):

$$Colonization\ Rate\ (CR) = \frac{total\ number\ of\ explants\ with\ VFE\ per\ plant\ part}{total\ number\ of\ explants\ per\ plant\ part} x\ 100$$

$$Isolation\ Rate\ (IR) = \frac{total\ number\ of\ VFE\ isolates\ per\ plant\ part}{total\ number\ of\ explants\ per\ plant\ part} x\ 100$$

The composition and diversity of fungal endophytes between the different plant parts were compared based on species richness (S), Shannon-Wiener (H') index, and Fisher's-Alpha (FA)

diversity indices using the Paleontological statistics software PAST (version 4.03) (Hammer et al. 2001). The formula for each diversity indices is listed below:

S = number of species found in a particular plant part

$H' = -\Sigma i\ (p_i \ln p_i)$
where p_i = The total number of individuals in ith species

$FA = a*\ln(1+n/a)$
where S = number of taxa
n = number of individuals
a = Fisher's alpha

The taxonomic diversity index, represented as the ratio of species over genus (S/G), was also determined. To assess the differences in diversity, the analysis of variance ($p < 0.05$) was also conducted using PAST (version 4.03). The datasets obtained from the study aimed to provide insights into the overall community composition and structure of fungal endophytes associated with the endemic ginger plant. The data were represented as a Venn diagram to visually represent the number of species that are shared between different plant parts and the number of unique taxa found within each plant part.

Results

In this study, 123 VFE were isolated from mature leaves (n = 35), pseudostems (n = 30), rhizomes (n = 28), and roots (n = 30) (Table 1). The findings of this study show that the endemic ginger *V. sepulchrei* is a favorable host for various fungal species. The isolated VFE were distributed among four phyla, four classes, 11 orders, and 15 families. The records are enumerated in alphabetical and hierarchical order (Table 1). The fungal endophytes that were successfully isolated from the endemic ginger belong to the Phylum Ascomycota (88.62%), Basidiomycota (4.88%), Mucoromycota (4.07%), and Oomycota (2.44%). The most frequently isolated Ascomycota belong to the class Sordariomycetes, order Glomerellales, and class Glomerellaceae (23.58%). The percentage of explants colonized by VFE was high, ranging from 87% to 97%. Specifically, the VFE isolated from the leaves exhibited a high isolation frequency (28%) compared to VFE isolated from the pseudostems, roots, and rhizomes, ranging only from 23–24 %.

However, the TDI of VFE isolated from each part displayed no difference (Wilcoxon, $p = 0.125$). A comparative analysis of species diversity indices between the different plant parts showed the highest values for the leaves (H' = 2.56, FA = 9.94), followed by rhizomes (H' = 2.02, FA = 5.59) (Table 2). The pseudostems exhibited a lower diversity value (H' = 1.92, FA = 3.57) than that of the leaves and rhizomes, though the least diversity was observed in roots (H' = 1.74, FA = 2.87). However, the differences in species diversity values between plant parts were not statistically significant (Kruskal Wallis, $p = 0.2529$), indicating that the taxonomic diversity or species richness was similar across the different plant parts being compared.

The phylogenetic trees based on the maximum likelihood of the selected 20 VFE morphospecies are presented in Figs 2–16. These confirm the identities of our isolated *Vanoverberghia* fungal endophytes. Figs 17 and 18 show the colony growth and spore morphologies of selected VFE on the PDA medium after five days of incubation.

Table 1 Abundance and diversity of fungal endophytes isolated from different plant parts of *Vanoverberghia sepulchrei*.

Taxonomic Rank				Fungal Endophytes	Number of Isolates				Total no. of isolates	GenBank Accession No.
					Leaf	Pseudostem	Rhizome	Root		
Phylum	Class	Order	Family							
Ascomycota	Dothideomycetes	Botryosphaeriales	Phyllostictaceae	*Phyllosticta fallopiae*	1	1	1	1	4	OR052164
Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae	*Cladosporium chasmanthicola*	1	-	1	-	2	OR052150
Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae	*Cladosporium oxysporum*	1	-	-	-	1	OR052151
Ascomycota	Dothideomycetes	Pleosporales	Didymosphaeriaceae	*Pseudopithomyces palmicola*	1	-	-	-	1	OR052166
Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe litchiicola*	3	-	-	5	8	OR052156
Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae	*Diaporthe millettiae*	-	3	-	-	3	OR052157
Ascomycota	Sordariomycetes	Diaporthales	Valsaceae	*Phomopsis mahothocarpi*	-	-	4	-	4	OR052163
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum aeschynomenes*	2	1	1	-	4	OR052152
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum gloeosporioides*	10	7	6	-	23	OR052153
Ascomycota	Sordariomycetes	Glomerellales	Glomerellaceae	*Colletotrichum yulongense*	2	-	-	-	2	OR052154
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma longibrachiatum*	1	-	6	5	12	OR052168
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Cosmospora khandalensis*	-	-	-	2	2	OR052155
Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium ramigenum*	2	9	7	4	22	OR052158
Ascomycota	Sordariomycetes	Ophiostomatales	Ophiostomataceae	*Leptographium gibbsii*	5	3	1	-	9	OR052159
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	*Nigrospora aurantiaca*	-	-	-	12	12	OR052161
Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae	*Bjerkandera ecuadorensis*	1	-	-	-	1	OR052149
Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae	*Perenniporia subtephropora*	-	5	-	-	5	OR052162
Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor fusiformis*	1	1	1	1	4	OR052160
Mucoromycota	Mucoromycetes	Mucorales	Rhizopodaceae	*Rhizopus azygosporus*	1	-	-	-	1	OR052167
Oomycota		Peronosporales	Peronosporaceae	*Phytophthora palmivora*	3	-	-	-	3	OR052165

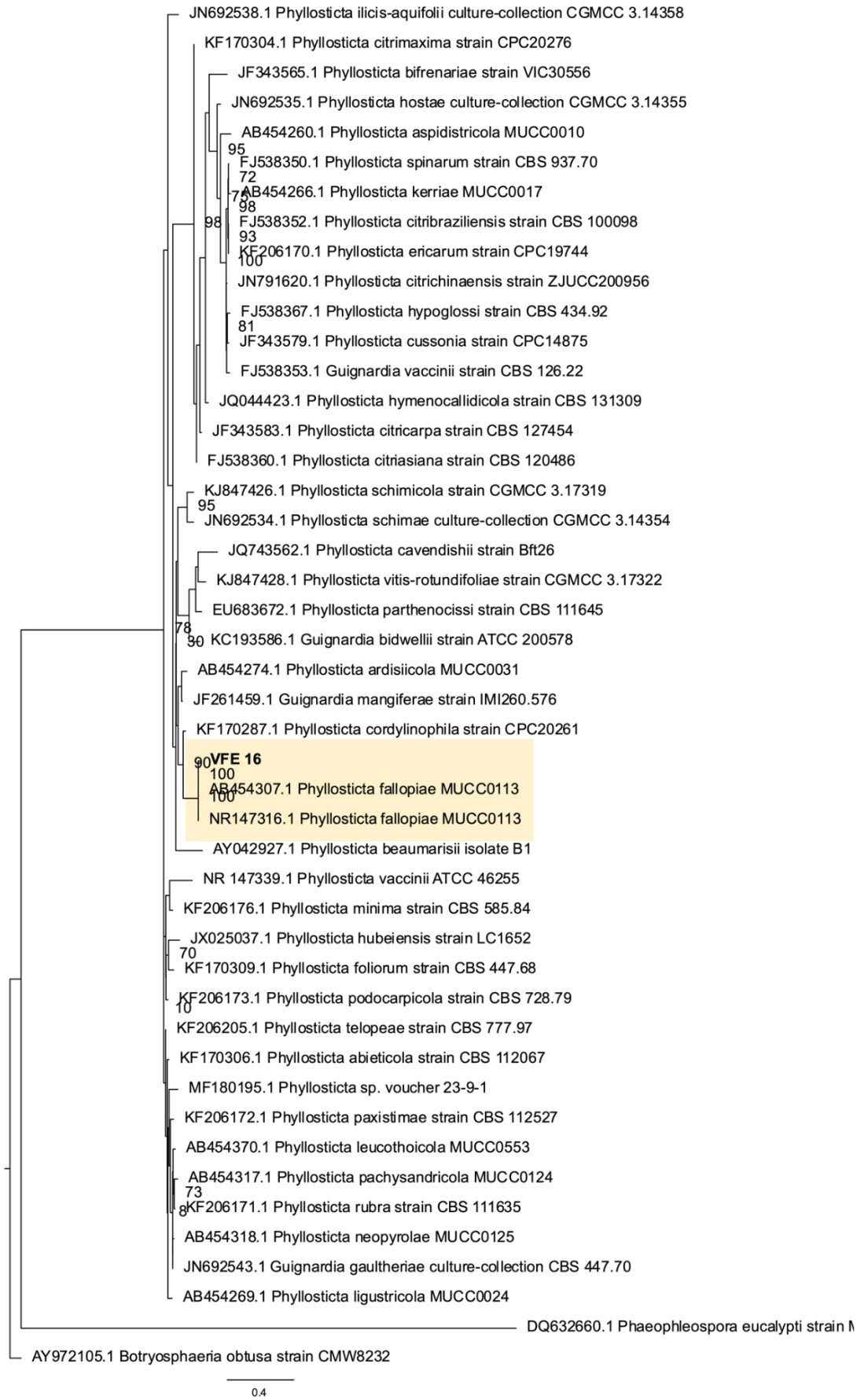

Fig. 2 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Phyllosticta.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to *Botryosphaeria obtusa* (CMW8232). Bootstrap support values for RAxML greater than 70% are given at each node**.**

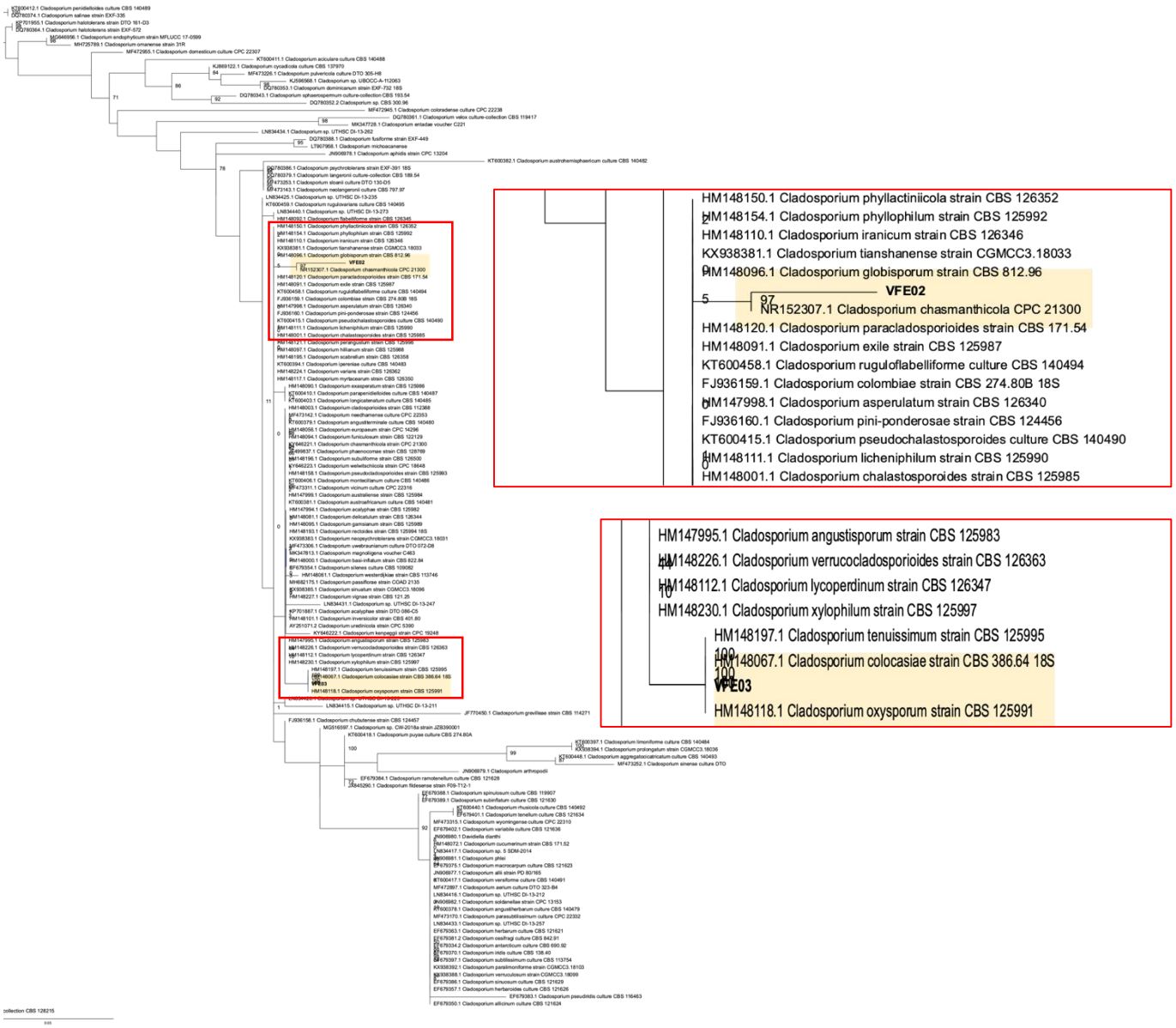

Fig. 3 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Cladosporium.* Tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to *Toxicocladosporium banksiae* (CBS 128215). Bootstrap support values for RAxML greater than 70% are given at each node.

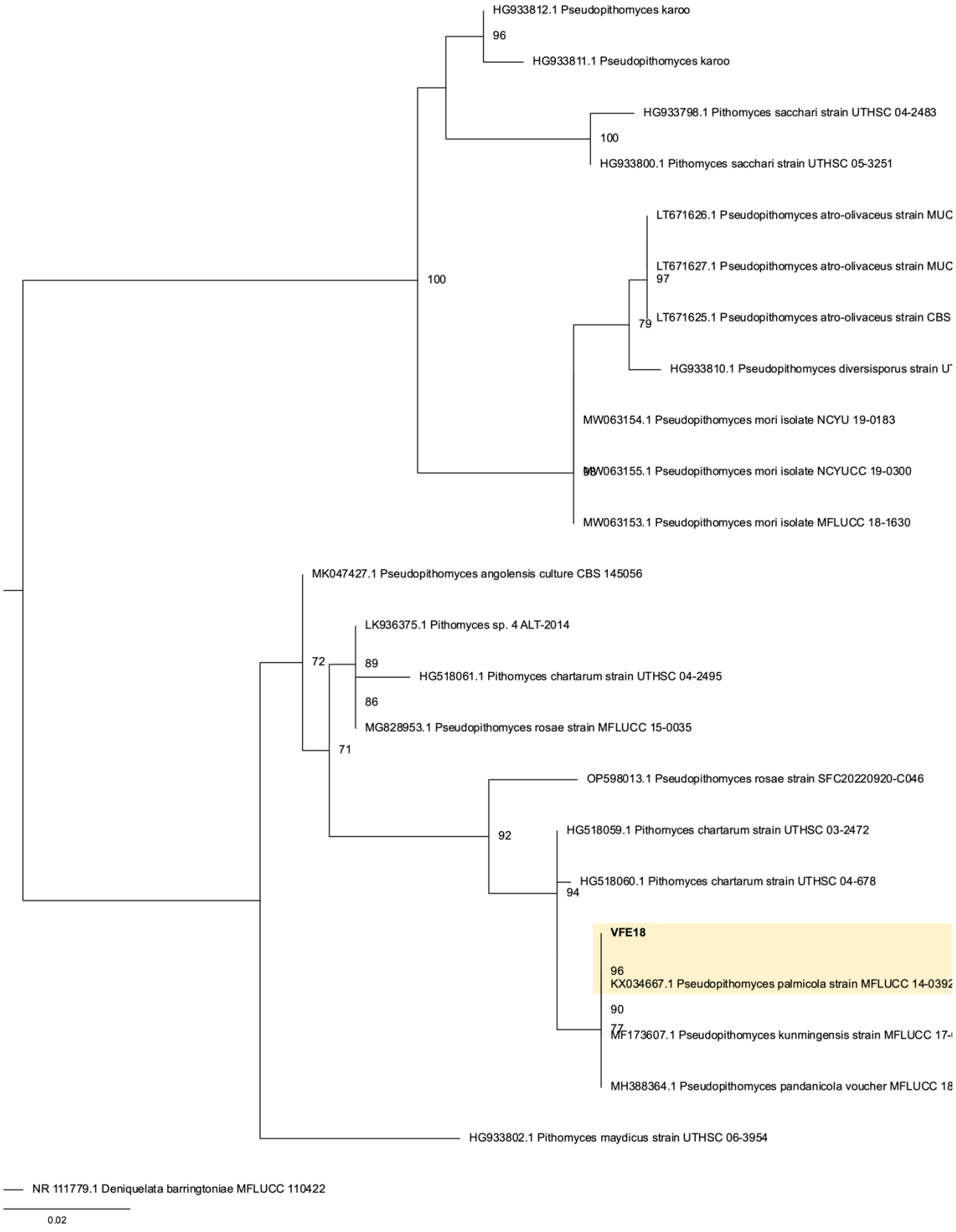

Fig. 4 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Pseudopithomyces.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to NR111779.1 *Deniquelata barringtoniae* (MFLUCC 110422). Bootstrap support values for RAxML greater than 70% are given at each node.

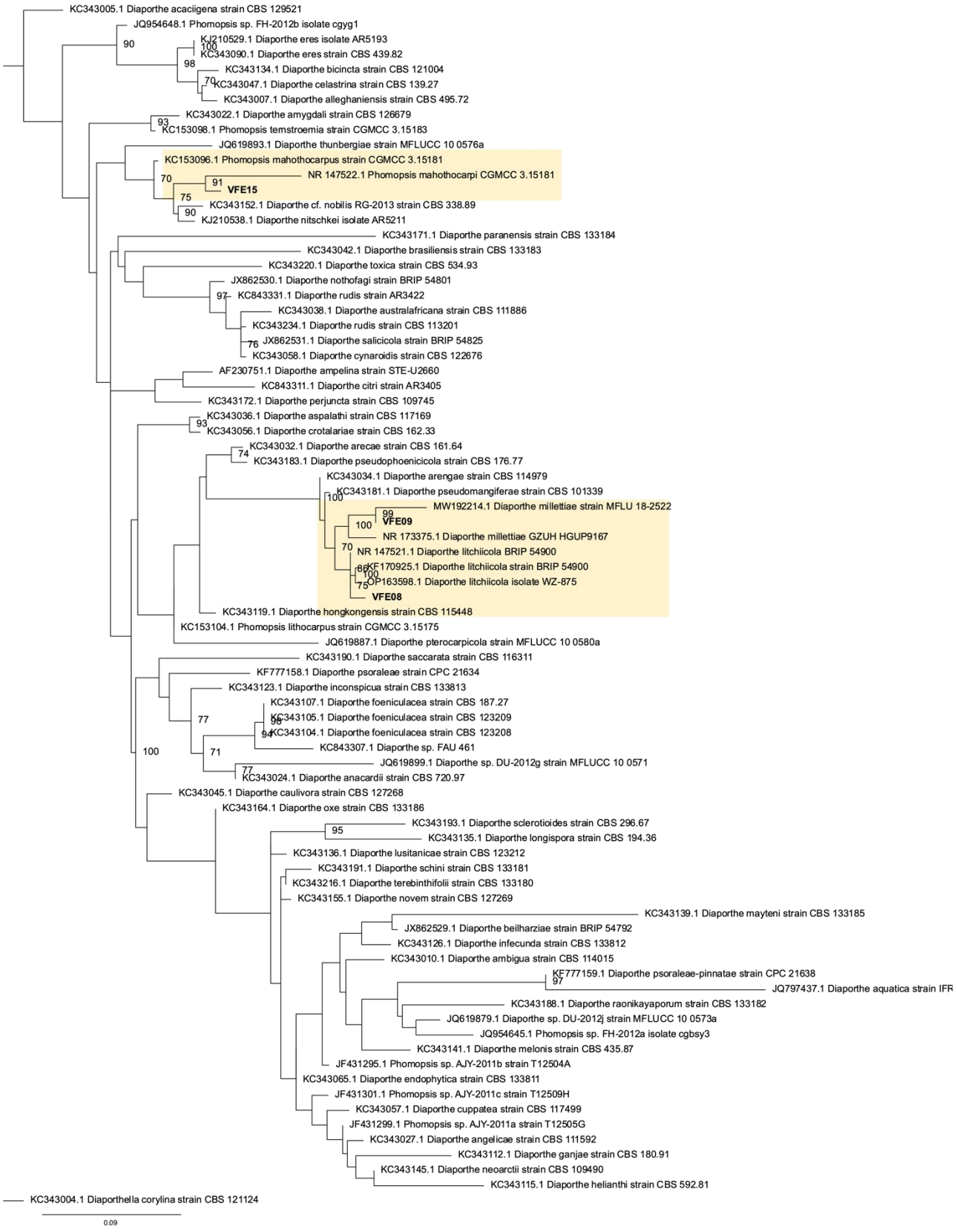

Fig. 5 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Diaporthe.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to GU174589.1 *Valsa mali* var. *pyri* (GSZY113). Bootstrap support values for RAxML greater than 70% are given at each node.

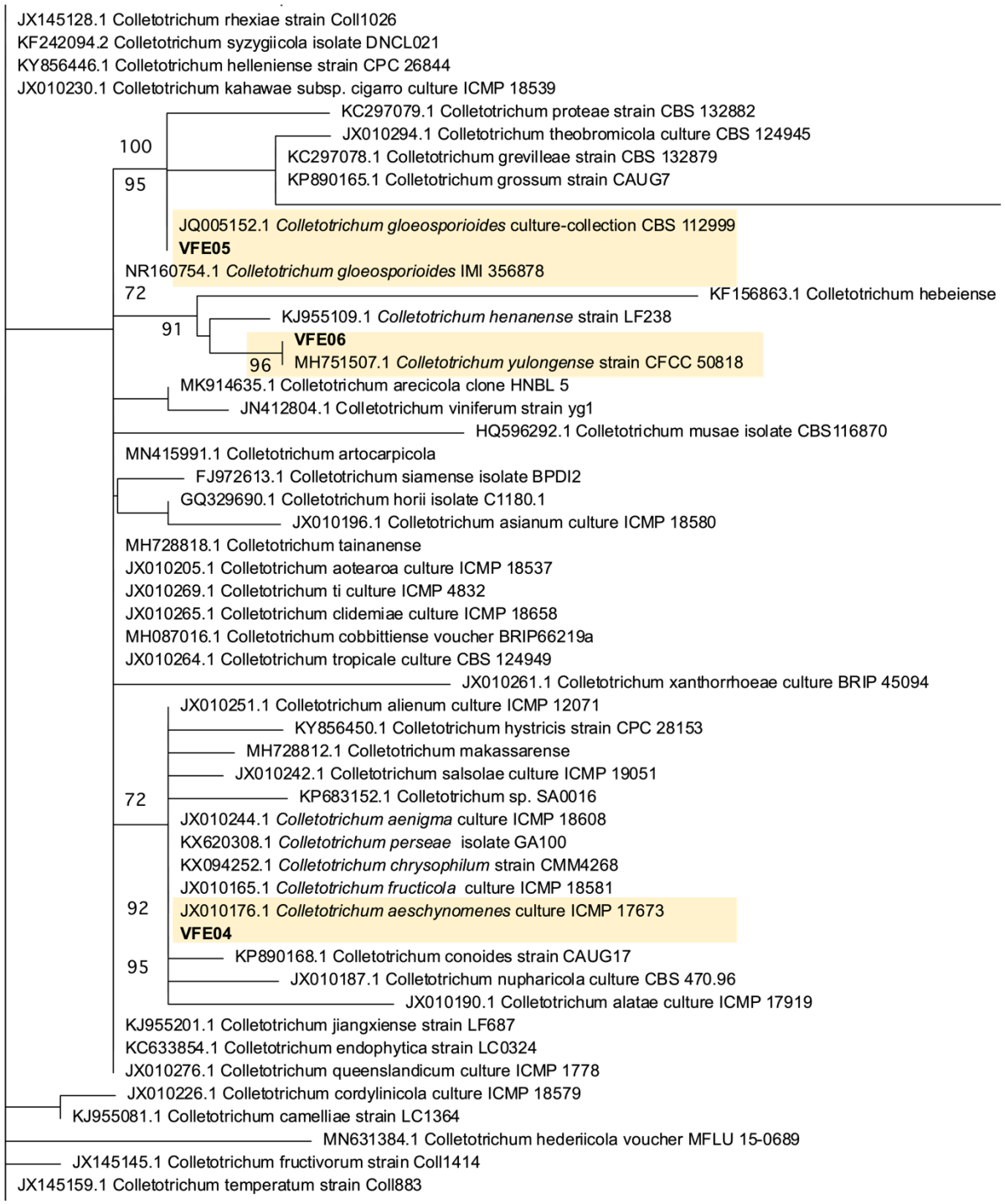

Fig. 6 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Colletotrichum.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

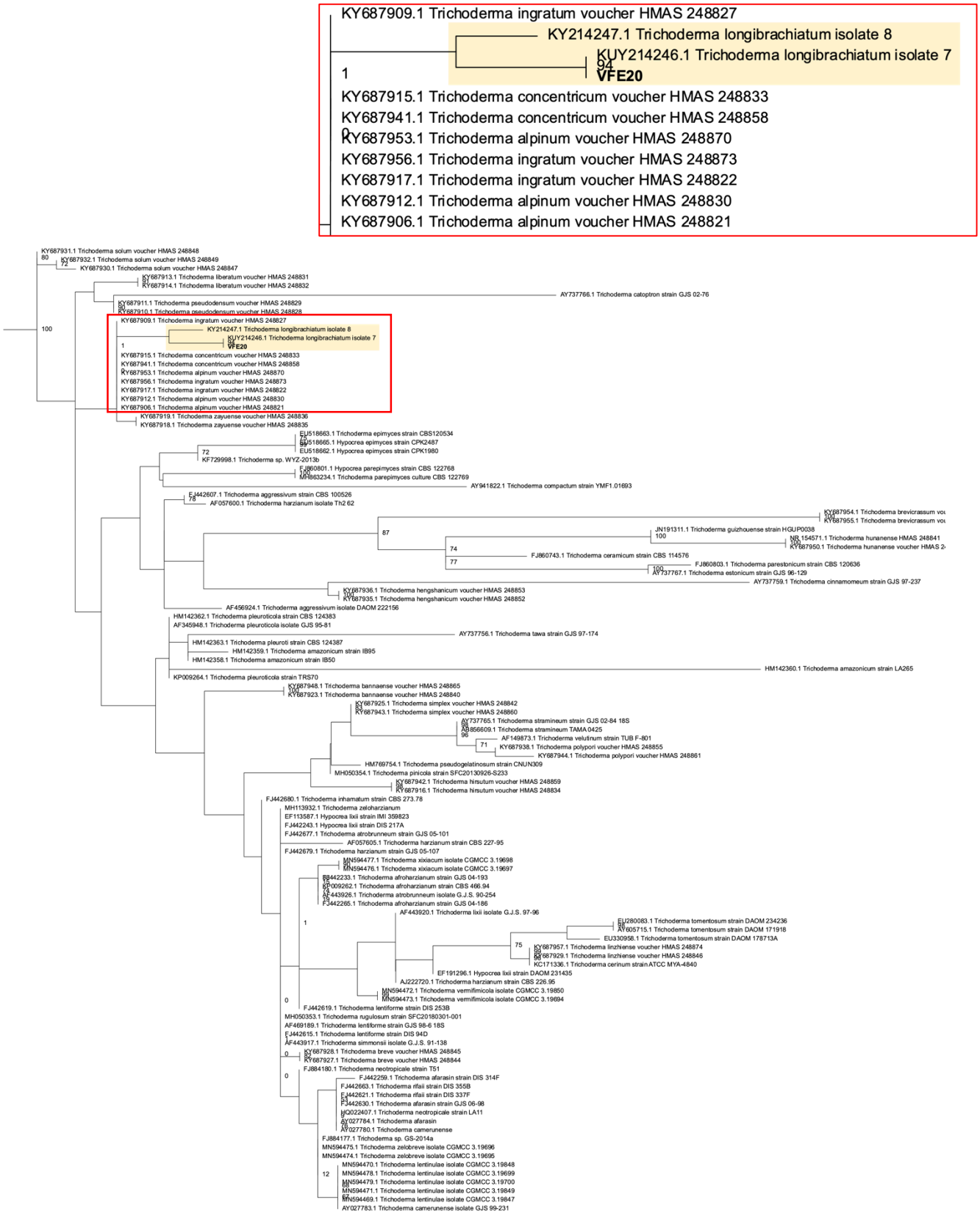

Fig. 7 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Trichoderma***.** The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to DQ825982.1 *Gliocladium cibotii* (CBS 147.44). Bootstrap support values for RAxML greater than 70% are given at each node.

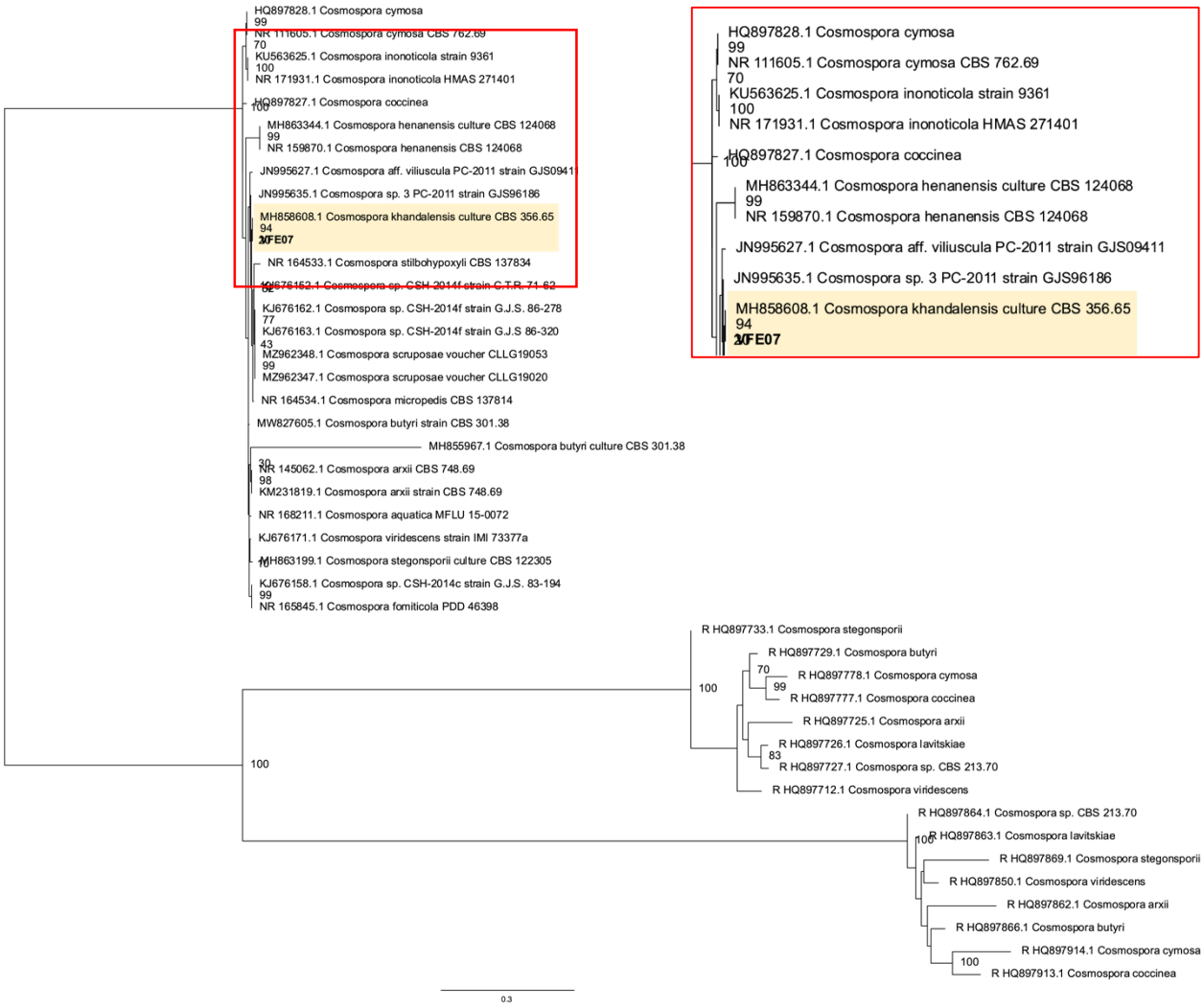

Fig. 8 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Cosmospora.* Tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

Table 2 Records and diversity of fungal endophytes associated with the host *Vanoverberghia sepulchrei*.

Records of Fungal Endophytes	Sample Source			
	Leaf	Pseudostem	Rhizome	Root
R	35	30	28	30
G	12	7	8	7
S	15	8	9	7
TDI	1.25[a]	1.14[a]	1.13[a]	1.00[a]
D	0.89[a]	0.83[a]	0.85[a]	0.79[a]
H’	2.56[a]	1.92[a]	2.02[a]	1.74[a]
J	0.86[a]	0.85[a]	0.84[a]	0.81[a]
FA	9.94[a]	3.57[a]	4.59[a]	2.87[a]
CR (%)	97	93	87	90
IR (%)	28	24	23	24

R = individuals/records, G = number of genera, S = number of species, TDI = Taxonomic Diversity Index, D = Simpson diversity index, H’ = Shannon diversity index, J = evenness, FA = Fisher’s Alpha diversity index, CR = colonization rate, IF = isolation frequency. Values followed by the same letter are not significantly different, $p < 0.05$.

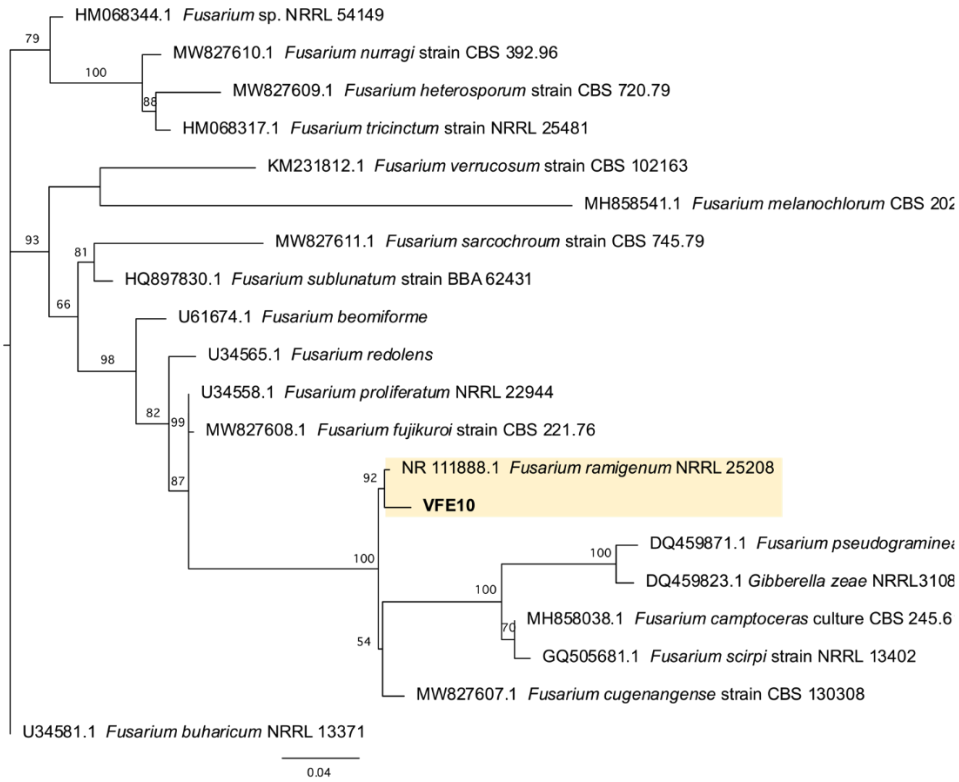

Fig. 9 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Fusarium.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

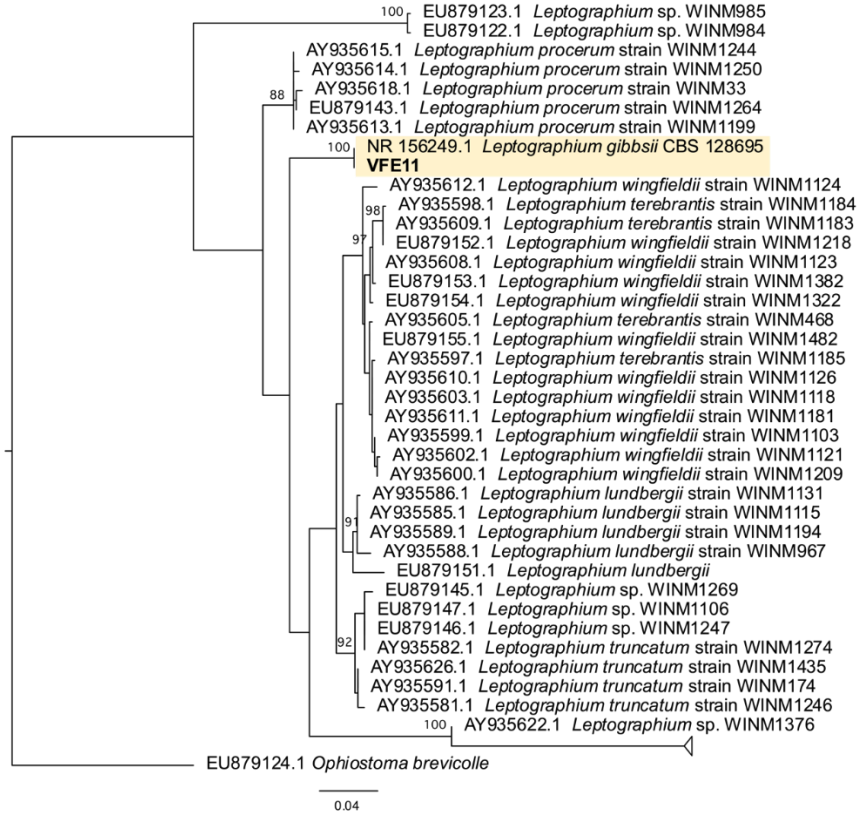

Fig. 10 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Leptographium.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to EU879124.1 *Ophiostoma brevicoll*e. Bootstrap support values for RAxML greater than 70% are given at each node.

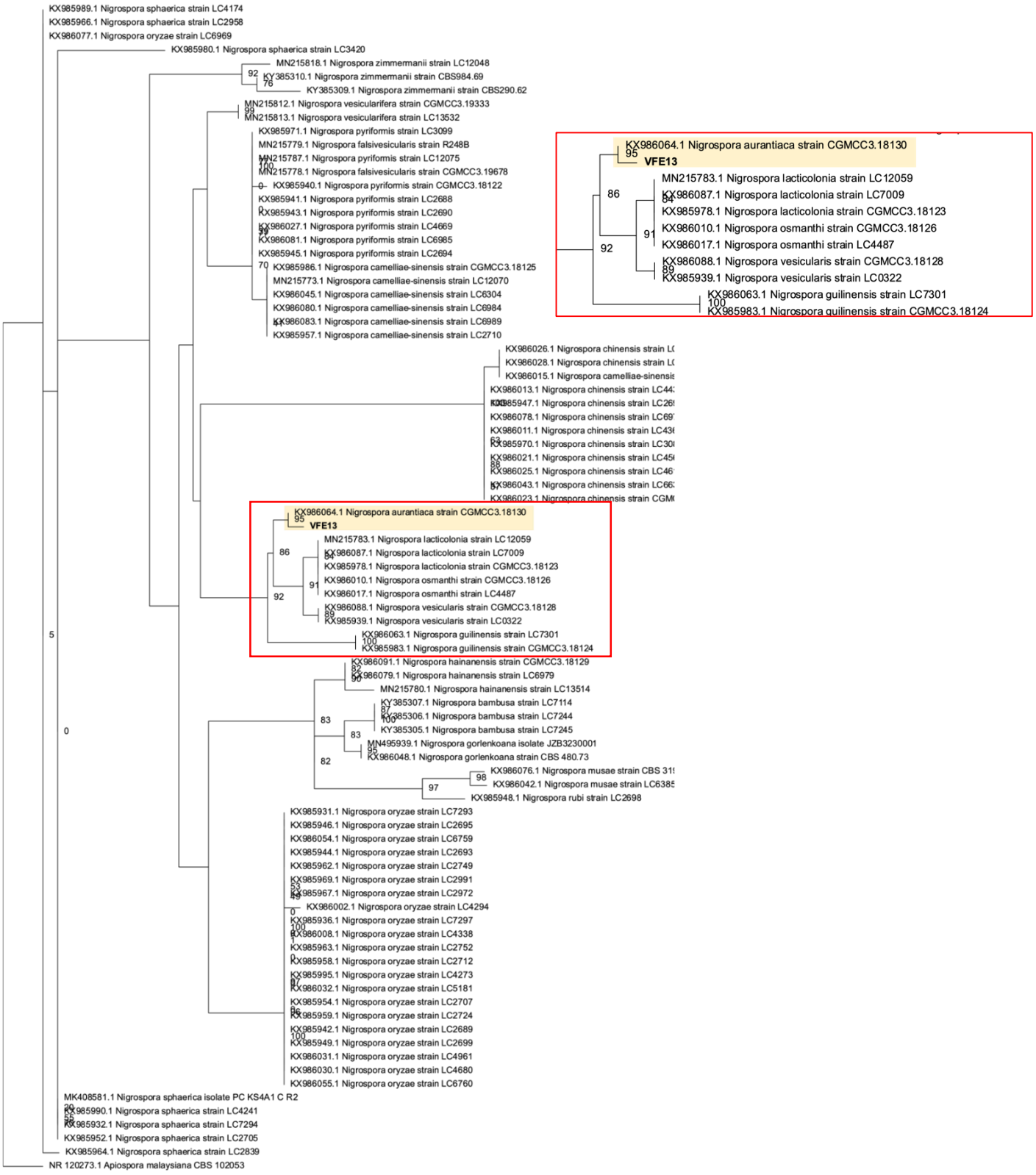

Fig. 11 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Nigrospora.* The tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to

NR120273.1 *Apiospora malaysiana* (CBS 102053). Bootstrap support values for RAxML greater than 70% are given at each node.

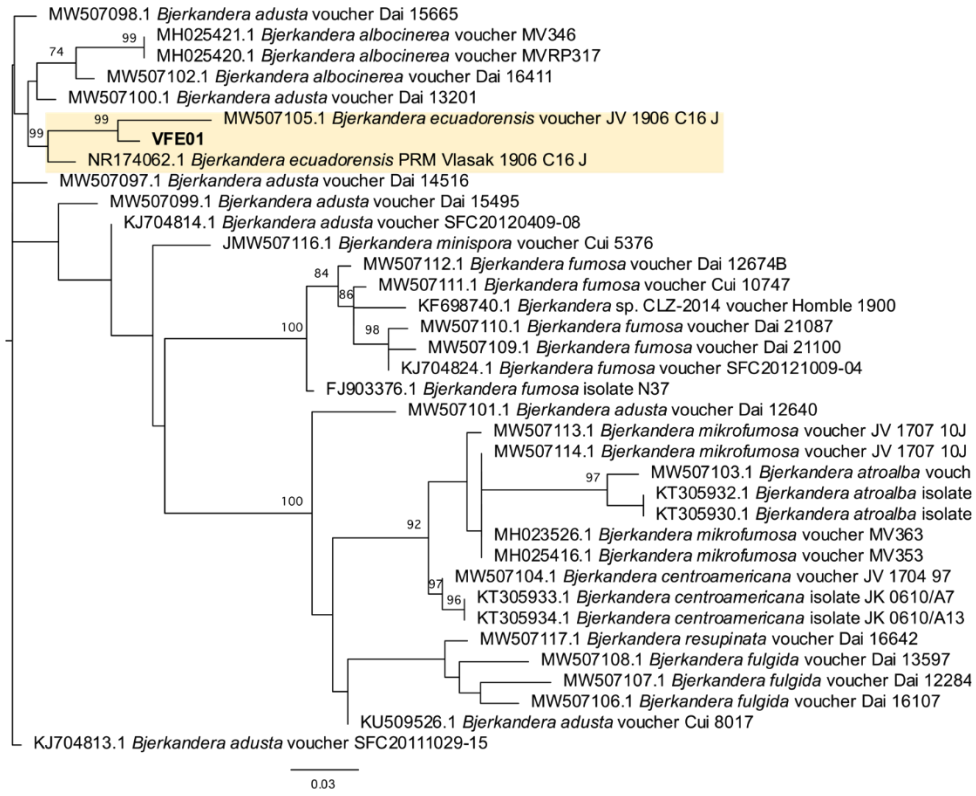

Fig. 12 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Bjerkandera.* Tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

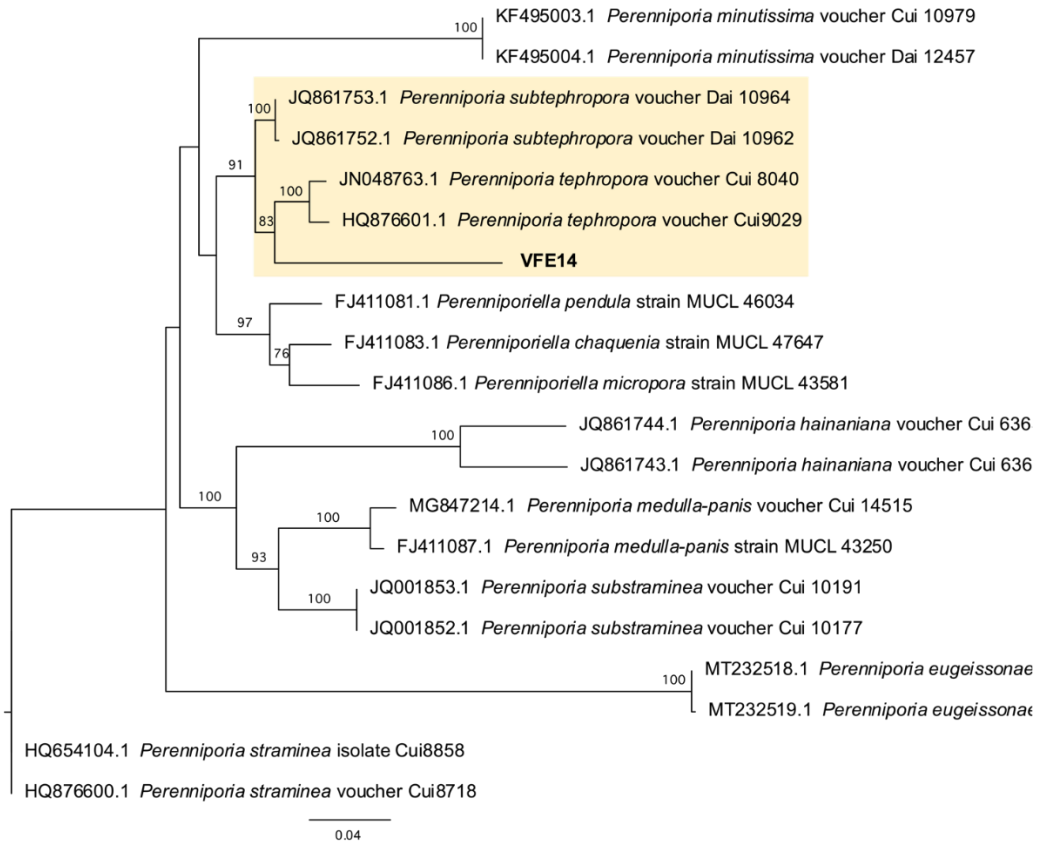

Fig. 13 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Perenniporia***.** Tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

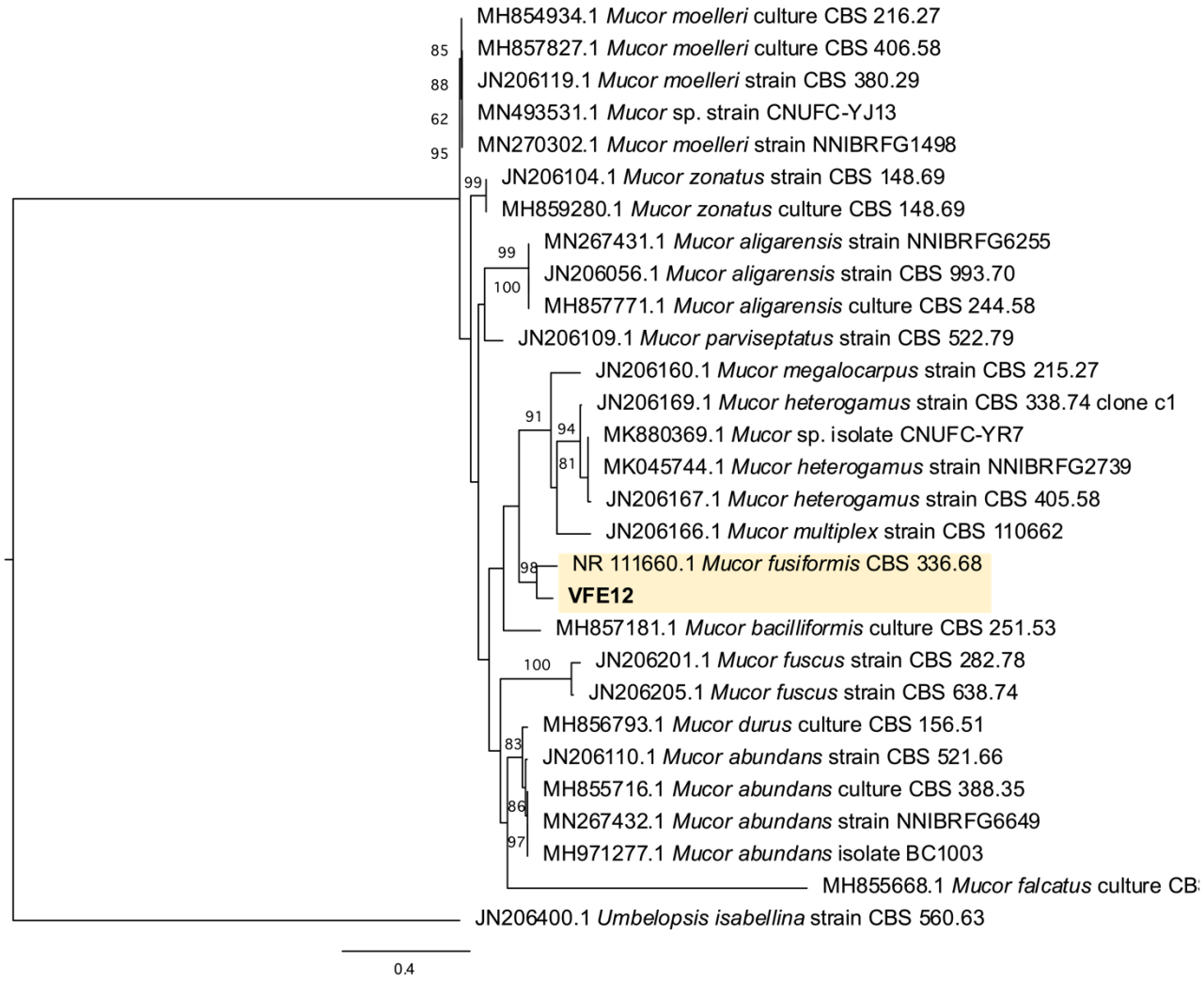

Fig. 14 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Mucor***.** Tree topology of the RAxML is similar to that of the maximum parsimony analysis. The tree is rooted to JN206400.1 *Umbelopsis isabellina* (CBS 560.63). Bootstrap support values for RAxML greater than 70% are given at each node.

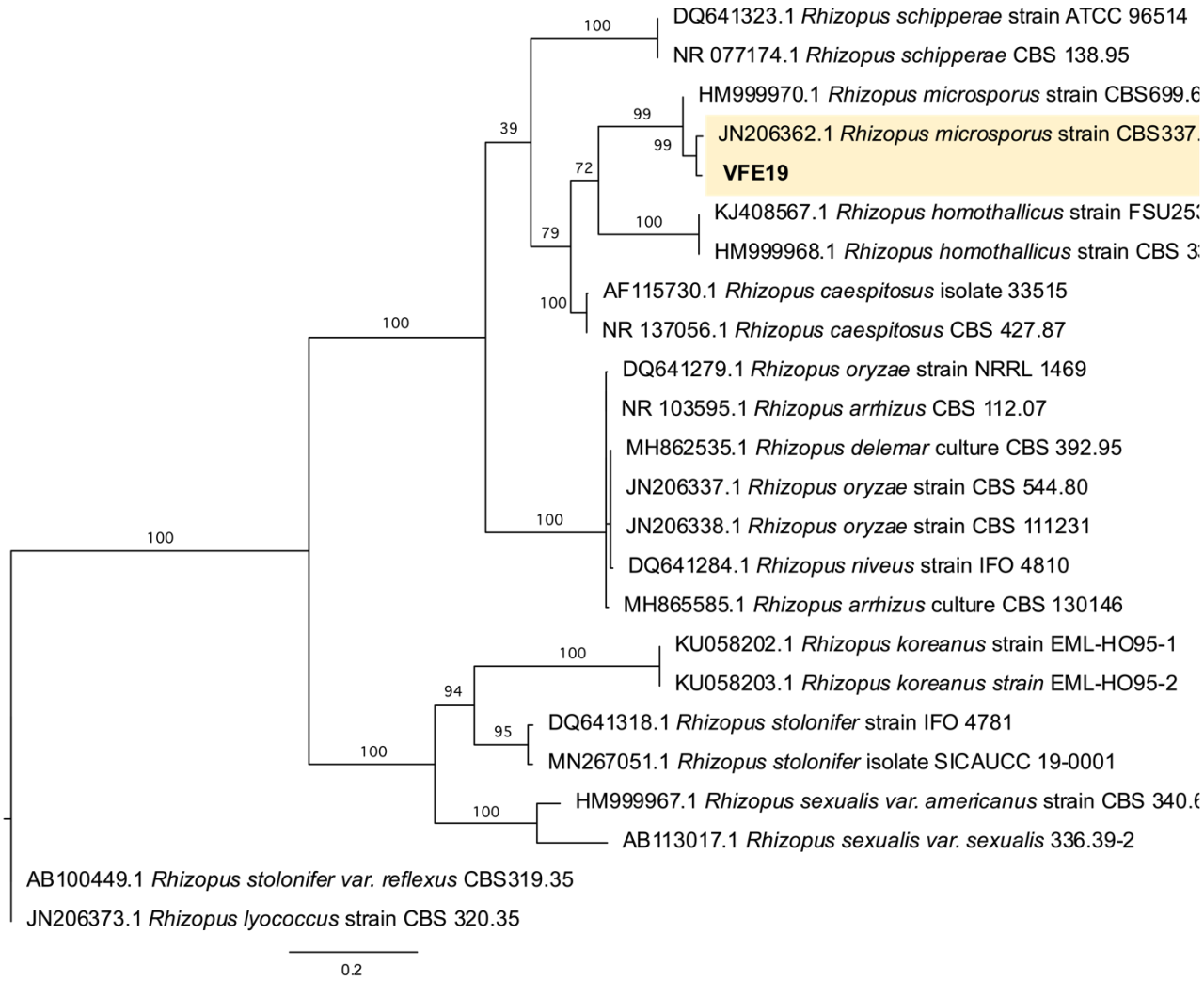

Fig. 15 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Rhizopus***.** The tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

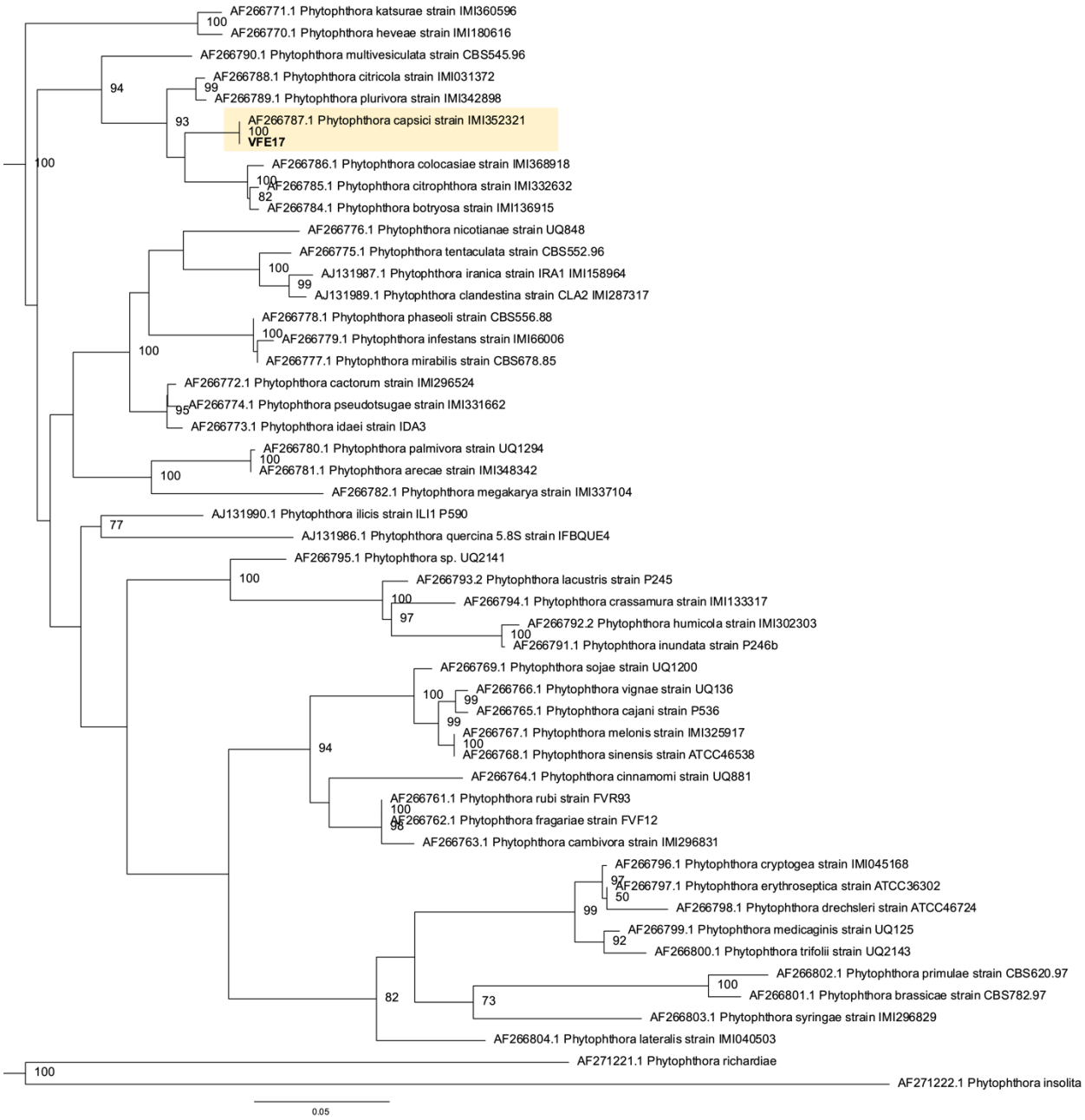

Fig. 16 – Phylogenetic tree based on RAxML analysis of ITS dataset of *Phytophthora***.** Tree topology of the RAxML is similar to that of the maximum parsimony analysis. Bootstrap support values for RAxML greater than 70% are given at each node.

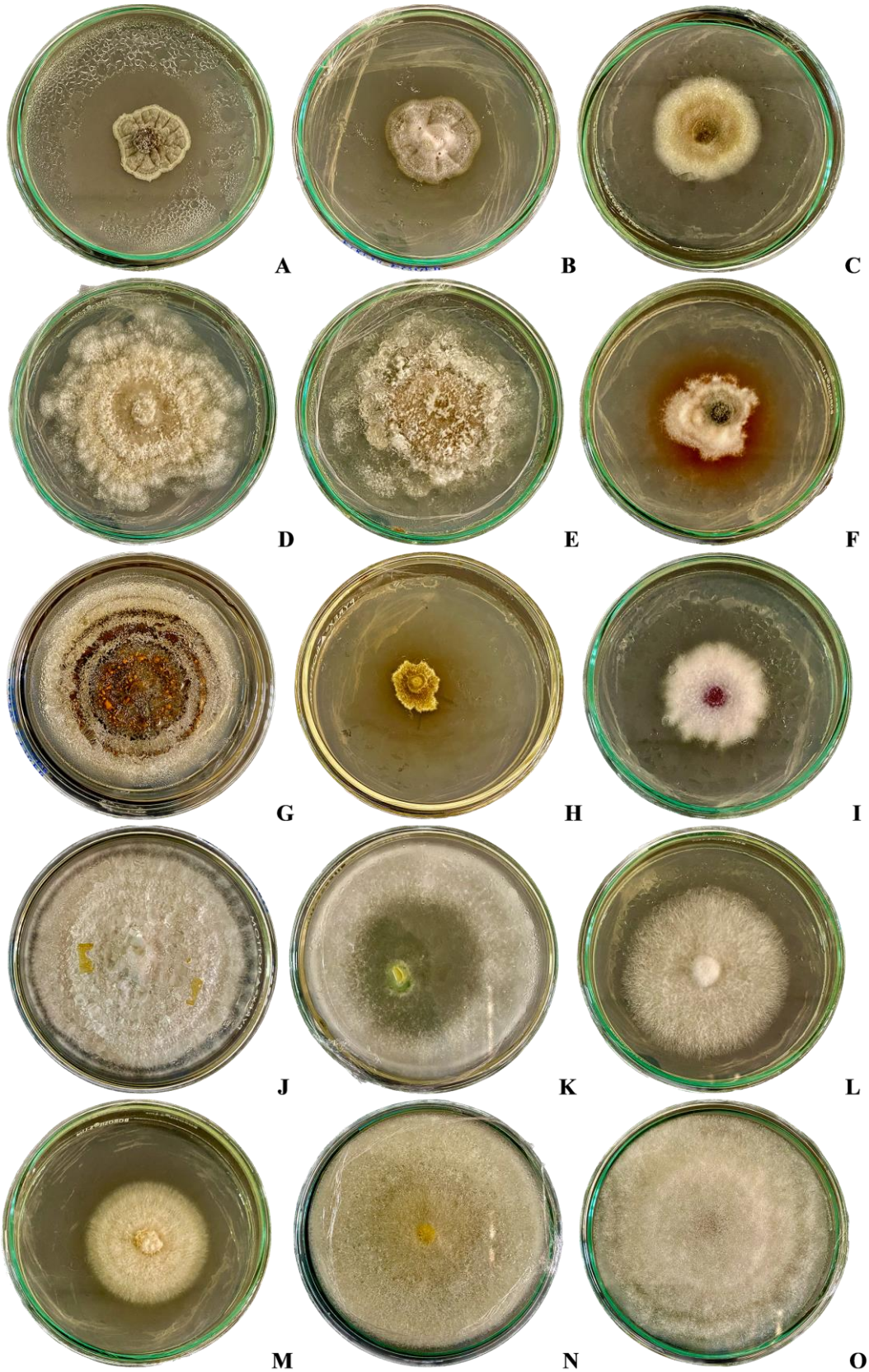

Fig. 17 – Colony growth of selected VFE on PDA media after 5 days of incubation. A *Phyllosticta fallopiae*. B *Cladosporium chasmanthicola.* C *Pseudopithomyces palmicola.* D *Diaporthe litchiicola.* E *Diaporthe millettiae.* F *Phomopsis mahothocarpi.* G *Colletotrichum aeschynomenes.* H *Cosmospora khandalensis.* I *Fusarium ramigenum.* J *Leptographium gibbsii.* K *Nigrospora aurantiaca.* L *Bjerkandera ecuadorensis.* M *Perenniporia subtephropora.* N *Mucor fusiformis.* and O *Rhizopus azygosporus*.

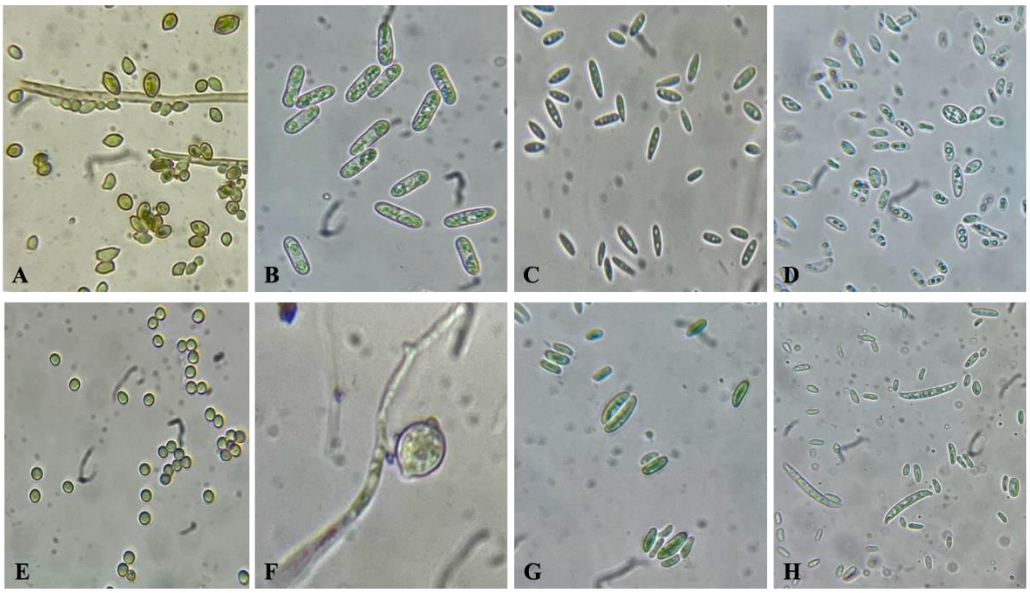

Fig. 18 – Spore morphologies of selected VFE. Fungal isolates were grown on PDA and observed after 5 days of incubation. A *Cladosporium chasmanthicola*. B *Colletotrichum aeschynomenes*. C *Phomopsis mahothocarpi*. D *Perenniporia subtephropora*. E *Rhizopus azygosporus*. F *Phytophthora palmivora*. G *Fusarium ramigenum*. and H *Diaporthe millettiae*.

We also compared the assemblages of fungal endophytes and illustrated the number of shared species in a Venn diagram **(**Fig 19**),** and it was found to be higher in the leaves of the endemic ginger. Only three fungal taxa were shared among the plant parts: *Fusarium ramigenum*, *Mucor fusiformis*, and *Phyllosticta fallopiae.* Some VFEs exclusively reside in above-ground plant organs, and other VFEs inhabit solely below plant parts. However, there were other fungal species that were shared in more than two plant parts. For example, *Colletotrichum gloeosporioides*, *C. aeschynomenes* and *Leptographium gibbsii* were found across all plant parts except in roots. On the other hand, *Cosmospora khandalensis* and *Nigrospora aurantiaca* were exclusively reported in roots. *Trichoderma longibrachiatum* was not recorded in the pseudostem, whereas *Diaporthe millettiae* and *Perenniporia subtephropora* were exclusively recorded in this plant part. Moreover, *Phomopsis mahothocarpi* was the only species observed in the rhizome of the endemic ginger.

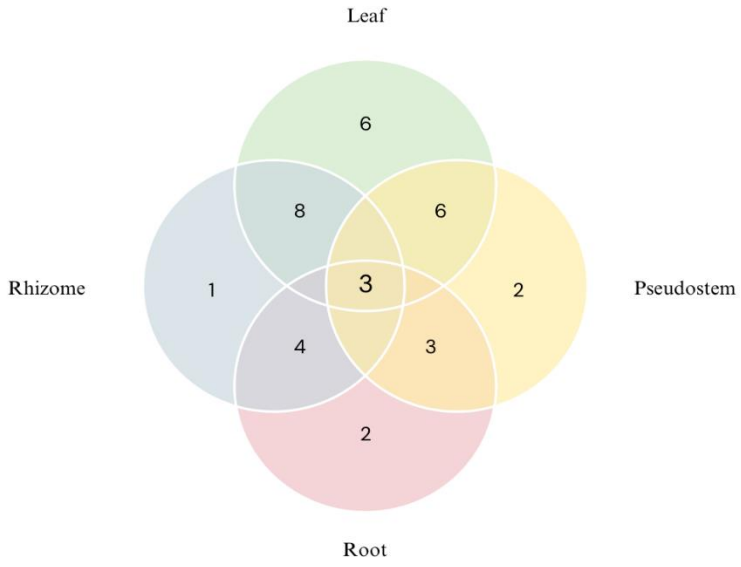

Fig. 19 – Community analysis illustrates the number of shared species and unique taxa**.** A comparison was carried out between plant parts**.**

Discussion

Vanoverberghia sepulchrei is host to a variety of fungal endophytes. We observed a slightly higher isolation frequency from above-ground (52.85%) than below-ground (47.15%) plant parts. This contrasts with the previous study of Ginting et al. (2013), where fungal isolates derived from the root, stem, and leaf of *Zingiber officinale* all hosted an equal number of isolates, with each plant organ hosting eight fungi. The selectivity of fungal endophytes in colonizing different plant tissues is still a subject of research, as there are multiple factors that can influence their colonization (Mane et al. 2018). However, it is important to note that fungal endophytes are ubiquitously present in all plant tissues, with variations in frequency and diversity, which are likely based on their symbiotic capacity with the host plant (Selim et al. 2012, Fesel & Zuccaro 2016).

When endophytes invade and proliferate within the tissues of a host plant, they encounter defensive responses from the host. The ability of endophytes to establish asymptomatic growth within their host relies on their capacity to maintain a delicate balance in interaction with the host and other competing microorganisms (Schulz et al. 2015). They also possess the capability to withstand and adapt to the host's defense mechanisms, primarily for the purpose of infecting and establishing themselves within the plant tissues. In cases of pathogenic interaction, the host's defense mechanisms are overcome, thereby resulting in disease symptoms. There are two main groups of fungal endophytes: non-clavicipitaceous endophytes (NC-endophytes) and clavicipitaceous endophytes (C-endophytes), as grouped by Rodriguez et al. (2009). C-endophytes primarily infect grasses and have specialized interactions with their host, including vertical transmission through seed infections. They have been reported to enhance drought tolerance, increase plant biomass, and produce chemicals toxic to animals (Saikkonen et al. 2006). Forest tree endophytes belong to the NC-endophytes, which mainly infect host plants horizontally, i.e., between individuals. These fungal endophytes are further categorized. Class 2 NC-endophytes comprise a diverse range of species found in above- and below-ground tissues but with limited diversity within individual host plants. They are known to confer stress tolerance to host plants (Rodriguez et al. 2008). Class 3 NC-endophytes are restricted to above-ground tissues and often form localized infections. They belong to the fungal group Dikarya, mainly ascomycetes. They are found in virtually all plant leaves and show high diversity within host plant tissues and different forest stands (Rodriguez et al. 2009). Lastly, Class 4 NC-endophytes are restricted to host roots and extensively colonize these tissues. They belong to different taxonomic groups, mainly in the phylum Ascomycota, including the dark septate endophytes (DSE). DSE can be found in various terrestrial plants and are characterized by the formation of specialized structures in host roots. Other non-DSE species in Class 4 include *Cryptosporiopsis*, *Cylindrocarpon*, *Fusarium*, *Gibberella*, *Ilyonectria*, *Microdochium*, *Neonectria*, and *Sebacinales*. However, the ecological roles and functions of Class 4 NC-endophytes are still not well understood (Sieber 2002).

In this study, fungal colonization of the Philippine endemic ginger plant is more prevalent in leaves, less in pseudostems and roots, and only occasionally in rhizomes. This study confirmed the presence of 13 genera and 15 species of VFE in leaves. Notably, six genera were exclusively identified in leaves. This indicates that the composition of endophytic fungal communities varied between plant organs. However, it is important to note that it is not conclusive that a particular taxon is limited to a specific organ, as many fungi can disperse through the air and opportunistically infect plants through various means, such as spores landing on soil or leaves. Furthermore, the extent to which endophytic fungi are isolated may depend on factors like time and growth medium, making it challenging to precisely determine their distribution at the current level. However, the distribution of fungal endophytes within the host *Vanoverberghia sepulchrei* follows a pattern similar to that of the non-clavicipitaceous endophytes. There are species which are confined to the pseudostems, as seen in the case of *Diaporthe millettiae* and *Perenniporia subtephropora*, and those restricted to the roots such as *Cosmospora khandalensis* and *Nigrospora aurantiaca*, intercellular in both roots and shoots, as observed in *Fusarium ramigenum*, *Mucor fusiformis*, and *Phyllosticta fallopiae*, and adapted to grow within the rhizome like *Phomopsis mahothocarpi*. Interestingly, these fungal endophytes were also isolated from the rhizomes of other Zingiberaceous plant species, which indicates non-host

specificity. Species of *Cladosporium* and *Mucor* were isolated from *Curcuma zedoaria*, species of *Colletotrichum* and *Phomopsis* in *Zingiber officinale*, and species of *Fusarium* in *Curcuma xanthorrhiza* (Praptiwi et al. 2016). Consistently, dominant fungal endophytes on *Amomum siamense* included *Colletotrichum gloeosporioides*, *Fusarium* spp., *Phomopsis* spp., and *Phyllosticta* spp., though most taxa displayed a preference for either leaf tissue or pseudostems (Bussaban et al. 2001). While it is commonly believed that colonization of above-ground organs is mainly localized, extensive endophytic growth within the roots has also been frequently observed. Root colonization can occur in inter- and intracellular spaces, with hyphae often forming intracellular coils, as seen in the case of DSE. Different endophytic fungal communities in different plant organs enhance the plant's phenotypic plasticity, perhaps an advantage in unpredictable environments.

Fungal endophytes in plants have been shown to contribute to the antifungal properties of the rhizome of ginger. For instance, *Colletotrichum gloeosporioides* and species of *Trichoderma* have been correlated with the antifungal activities of ginger (Golinska et al. 2015, Yan et al. 2019). Similarly, in this study, these two species were isolated from the rhizome and perhaps could also contribute to the antifungal properties of this ginger plant. They produce bioactive compounds that contribute to the defense mechanisms of the ginger plant against fungal pathogens, thus enhancing its resistance to fungal infections. The anti-fungal property of the rhizome not only protects the plant but also influences the diversity of associated microbial communities by shaping the composition and abundance of fungi and other microorganisms within the plant's tissues (Qu et al. 2020). This intricate interplay between endophytes, anti-fungal properties, and diversity highlights the importance of understanding plant-microbe interactions for the sustainable management of plant health and ecosystem functioning.

Finally, we identified our VFE through a combined morpho-cultural characterization and analysis of the ITS genes as the DNA barcoding marker. This study confirmed the presence of four phyla, four classes, 11 orders, 15 families, 16 genera, and 20 species of VFE. Our morphometric data concur with our molecular identification based on the ITS gene marker with high bootstrap support of 92 and above, thereby confirming the identities of the isolated VFE. The ITS gene was also successfully used to confirm the identities of fungi in support of other phenotype-based ID methods, e.g., ITS confirmed the identities of *Trichoderma*, which were initially identified through protein profiling using MALDI-TOF MS (dela Cruz et al. 2023b). In the study of Pecundo et al. (2021), fungal endophytes associated with the coralloid roots of *Cycas* were also identified using the ITS phylogeny coupled with morphological characterization. However, we still recognized the limitation of the use of ITS gene marker for species identification, particularly between closely related taxa, e.g., species of *Cladosporium*, *Colletotrichum*, *Diaporthe*, and *Fusarium*. For example, Apurillo et al. (2019) used four gene markers to identify species of *Diaporthe* and *Phomopsis* and six gene markers for *Colletotrichum*. It is therefore suggested to use other gene markers such as beta-tubulin (*tub*), translation elongation factors 1-alpha (*tef*), histone H3, calmodulin (*cal*), and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) genes, to fully ascertain species identities.

In summary, this study reported the Philippine endemic ginger *Vanoverberghia sepulchrei* as a viable host to various fungal endophytes. The differences in the isolated taxa showed the importance of developing a strategy that will investigate the different plant parts and endemic host plants. The Philippines harbors numerous ginger species, of which 79% are said to be endemic. These all represent many unique potential hosts for fungal endophytes, which could lead to the discovery of unique groups of cultivable fungal communities living inside the hosts. Our paper calls for more research on fungal endophytes associated with local indigenous plant communities.

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Accessibility of data

Data are available from the first author upon request.

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