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ENDOPHYTIC FUNGI ASSOCIATED WITH FOUR ENDEMIC WILD COFFEE SPECIES (*Mascarocoffea*) IN MADAGASCAR

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ABSTRACT

Mascarocoffea are wild coffee plants endemic to Madagascar. These plants produce diverse, often specific biomolecules that are not found in cultivated coffee plants. Production of these compounds could be due to interactions between the endophytes and the host plant. Few studies have been carried out on the richness and diversity of microorganisms associated with these coffee plants. The objective of this study was to identify endophytic fungi isolated from the leaves of species of *Mascarocoffea* by morphological and molecular methods. Fifteen taxa were morphologically identified among the 30 isolated. These included *Phyllosticta* sp., *Colletotrichum* sp., *Daldinia* sp., *Diaporthe* sp., *Cladosporium* sp., *Fusarium* sp.01, *Fusarium* sp. 02, *Fusarium* sp. 03, *Monilinia* sp., *Trichoderma* sp., *Alternaria* sp, *Penicillium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Nigrospora* sp. The phylum Ascomycota was the most represented, with 14 taxa and 1 taxon (*Rhizopus* sp.) belonging to the phylum of Zygomycota. Molecular characterisation confirmed of the identity of these 15 taxa and those of the morphologically Unidentified (NI) mycotaxa including *Colletotrichum karstii*, *Colletotrichum siamense*, *Neofusicoccum parvum*, *Colletotrichum siamense, Punctularia strigosozonata*, *Stemphylium solani*, *Phoma multirostrata*, *Calophoma complonata, Daldinia vanderguchtiae, Phoma exigua* and *Boremia exigua*. This study allowed us to identify the endophytic fungi isolated from *Mascarocoffea* leaves from Madagascar.

Key Words: Diversity, endophytic fungi, Madagascar, *Mascarocoffea*

RÉSUMÉ

Les *Mascarocoffea* sont des caféiers sauvages endémiques de Madagascar. Ces plantes produisent des biomolécules diversifiées, souvent spécifiques et inexistantes chez les caféiers cultivés. La production de ces composés pourrait être due aux interactions des microorganismes endophytes et la plante hôte. Peu d'étude a été réalisée sur la richesse et la diversité des microorganismes associés à ces caféiers. L'objectif de cette étude est d'identifier les champignons endophytes isolés à partir des feuilles de quatre espèces de *Mascarocoffea* par la méthode morphologique et moléculaire. Quinze taxons ont pu être identifiés morphologiquement parmi les 30 isolés. Ce sont : *Phyllosticta* sp., *Colletotrichum* sp., *Daldinia* sp., *Diaporthe* sp., *Cladosporium* sp., *Fusarium* sp.01, *Fusarium* sp.02, *Fusarium* sp. 03, *Monilinia* sp., *Trichoderma* sp., *Alternaria* sp., *Penicillium* sp., *Aspergillus niger*, *Rhizopus* sp. et *Nigrospora* sp. Le phylum des *Ascomycota* est le mieux représenté avec 14 taxons et 1 taxon (*Rhizopus* sp.) appartient au phylum de *Zygomycota*. La caractérisation moléculaire a permis de confirmer l'identité de ces 15 taxons et celles des mycotaxons morphologiquement Non Identifiées (NI) dont *Colletotrichum karstii, Colletotrichum siamense, Neofusicoccum parvum, Colletotrichum siamense, Punctularia strigosozonata, Stemphylium solani, Phoma multirostrata, Calophoma complonata, Daldinia vanderguchtiae, Phoma exigua* et *Boremia exigua*. Cette étude nous a permis d'identifier les champignons endophytes des feuilles de *Mascarocoffea* de Madagascar.

Mots Clés : Champignons endophytes, diversité, Madagascar, *Mascarocoffea*

INTRODUCTION

The term *Mascarocoffea* was used by Chevalier (1938) to group wild coffee species endemic to Madagascar and neighbouring islands. These coffee trees are characterised by the absence of caffeine (Deng *et al*., 2017) and the presence of various specific phenolic and diterpene derivatives in their organs (Hamon *et al.,* 2015). Previous studies demonstrated the involvement of fungal endophytes associated with *Mascarocoffea* in the synthesis of these metabolites (Ratsimbazafy, 2011; Andriamialiharisoa, 2011).

The diversity of endophytic fungi and their distribution in natural environments has been the subject of much work over the past decades. The majority of endophytic fungi isolated belong to the Ascomycota phylum and other taxa belong to the Zygomycota, Deuteromycota, Basidiomycota and the Oomycota phyla (Selim *et al.,* 2017); with an estimated of 1.5 million species and an average of about 50 endophytic species per plant species (Vasundhara *et al.,* 2019).

Most of the fungi described are species identified on the basis of colony morphology, hyphae and asexual and sexual reproductive structures (Rana *et al.,* 2019). However, this identification is very complex (Hyde and Soytong, 2007) and is a hindrance to the progress of studies on these fungi. Due to this complexity or inefficiency, use of alternative studies such as molecular trait analysis has proven to be more efficient and reliable (Seifert, 2006). The objective of this study was to identify endophytic fungi isolated from the leaves of species of *Mascarocoffea* by morphological and molecular methods.

MATERIALS AND METHODS

Isolation and purification. Mature and healthy leaves of four Malagasy wild coffee species, *Coffea perrieri, Coffea kianjavatensis, Coffea vianneyi* and *Coffea millotii*, distributed in different phytogeographic areas, were collected (Table 1). Before being used for seeding, the collected leaves were washed gently with soapy water, rinsed thoroughly under tap water and then

TABLE 1. Populations and species studied

Scientific names	Number of population	Geographical description		
		Site of origin	Phytogeography	Climate
C. kianjavatensis	A 213	Kianjavato	Eastern Domain	Wet
C. perrieri	A305	Ihosy	Southern Domain	Dry
C. vianneyi	A20	Nosyvarika	Eastern Domain	Wet
C. millotii	A721	Nosyvarika	Eastern Domain	Wet

with sterile distilled water to remove epiphytes and/or contaminants lining the leaf blade surface (Nefzi *et al.,* 2018). They were then disinfected (70% ethanol for 1 minute, then in sodium hypochlorite (NaOCl, 3%) for 4 minutes; and in 70% ethanol for 30 seconds (Suman *et al.,* 2016b). Finally, the leaves were rinsed four times with sterile distilled water for one minute each and then dried on sterile filter paper under a laminar flow host (Khan *et al*., 2017).

Isolation was done according to the method described by Huang *et al.* (2007a). Sterilised leaves were cut into fragments of a few millimetres, at a rate of 5 to 6 segments per dish, and then placed in petri dishes containing a standard Potato Dextrose Agar (PDA) culture, previously autoclaved at 121 °C for 15 minutes, and supplemented with 150 mg l^{-1} chloramphenicol to inhibit bacterial growth. The cultures were incubated in an oven at 25°C (Hazalin *et al.,* 2009; Khan *et al*., 2010). Cultures were monitored daily for 15 days; each fungus developed on the twig fragments was isolated, purified by successive subculturing for identification and preserved on PDA pellet at 4°C and transplanted every 2 months (Pimentel *et al*., 2006). The percentage of colonisation was calculated in order to evaluate the rate of infection of the fragments by these microorganisms, using the following formula:

$$\text{Colonisation (\%)} = \frac{\text{Total number of samples yielding}}{\text{Total number of samples in trial}} \times 100$$

Morphological characterisation of endophytic fungi strains. Macroscopic identification was performed based on cultural characteristics (growth rate, thallus texture, colony colour, culture reverse, daylight medium and exudate). The fungi were also cultured on two other media, Czapek Yeast extract Agar (CYA) and Malt Extract Agar (MEA), at 25°C for seven days.

Microscopic identification of the endophytic fungal strains was done based on the morphological characters of hyphae: septation, coloration and reproductive forms: fruiting bodies, spore shapes and colours (Kim *et al.,* 2013), and also referring to the identification the key of Barnett and Hunter (1998). Cultures were placed under extreme photon and thermal conditions to stimulate sporulation and facilitate identification. In the absence of sporulation, the strains were coded as Unidentified Fungi (NI) and the characteristics of the cultures such as general colony surface appearance, texture and hyphal pigmentation were taken into consideration (Suryanarayanan *et al.,* 2002).

The abundance rate is calculated according to the following formula:

$$\text{Abundance} = \frac{\text{Number of fungal isolate taken x Number of fruiting fragments}}{\text{Total number of fragments inoculated}} \times 100$$

Molecular identification of isolated endophytic fungi strains. DNA was extracted from young mycelium of isolated endophytic fungal strains according to the method

developed by Moller *et al.* (1992) and amplified by the PCR method. The ITS (Internal Transcribed Spacer) region of the rDNA was amplified using a universal primer pair for ITS1/ITS4 fungi (Wali, *et al.,* 2007). Their nucleotide sequences were respectively $^{5'}$TCCGTAGGTGAACCTGCGG$^{3'}$ and $^{5'}$GCTGCGTTCTTCATCGATGC$^{3'}$. The amplification reaction was performed in a 50 µl reaction volume containing 25 µl of 2X Taq DNA polymerase staining buffer, 1 µl of forward primer (ITS1), 1 µl of reverse primer (ITS2) and 22 µl sterile water. The mixture was made for the total number of isolates and then split at 23 µl per tube. Two ìl of DNA (50 ng µl^{-1}) was added last to the reaction mixture. DNA amplification was performed according to the following procedure (Sedra *et al.,* 1998): pre-denaturation at 95°C for 3 min followed by 35 consecutive cycles of denaturation at 98 °C for 15 sec, specific hybridisation of the primers with the DNA template at 59 °C for 60 sec and elongation at 72 °C for 2 min. Finally, the last elongation phase was done at 72 °C for 10 min.

The amplified products were sequenced by Macrogen Inc. using the same primers. The identity of the fungal sequences was determined by calculating similarity using the BLAST tool (Altschul *et al.,* 1997). A similarity of 95% with a 90% overlap was selected for a sequence to appear in the results. The remaining non-sequenced isolates were grouped into taxa based on their morphological characters.

RESULTS

***Mascarocoffea* colonisation.** Out of the 600 fragments of *Mascarocoffea* leaves inoculated, only 399 fragments were infected by endophytic fungi. A low infection rate of 57.34% was recorded in *C. vianneyi* and this rate did not vary throughout the culture. On the other hand, an infection rate of 82.67% was observed in *C. perrieri* on the third day of cultivation, while *C. kianjavatensis* and *C. millotii* species showed a colonisation rate of around 63% (Fig. 1). These results suggest that the frequency of colonisation varies from one species to another.

Morphological identification of isolated species. Among the 399 infected fragments, thirty different mycotaxons were identified macroscopically and microscopically. These observations allowed the identification of 15 mycotaxons belonging to two different phyla: Ascomycota (14) and Zygomycota (1).

Among the Ascomycota, four classes were represented; namely Sordariomycetes,

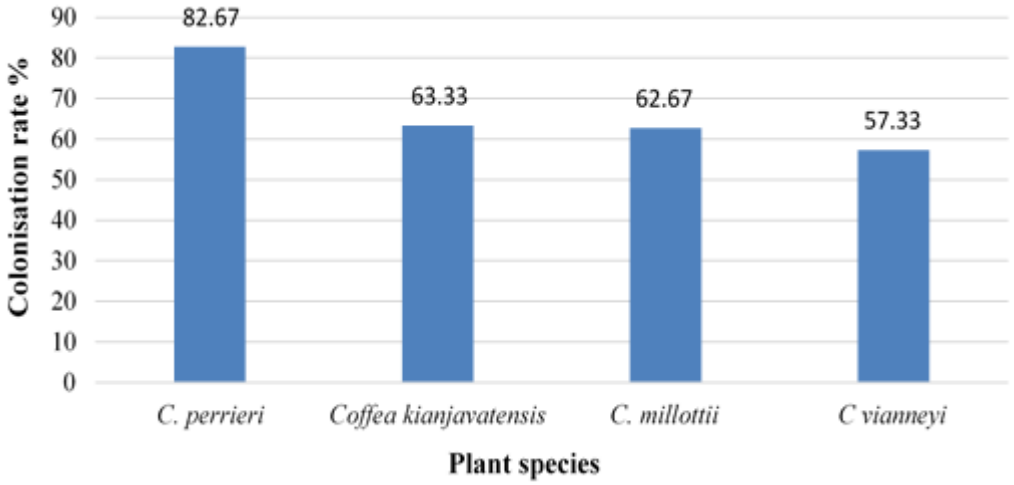

Figure 1. Colonisation rate of fragments of four collected species.

Dothideomycetes, Eurotiomycetes and Leotiomycetes. The results obtained indicated that Sordariomycetes were the most abundant, represented by 6 genera: *Fusarium* (111 isolates), *Trichoderma* (39 isolates), *Colletotrichum* (33 isolates), *Daldinia* (18 isolates), *Diaporthe* (17 isolates) and *Nigrospora* (15 isolates). The Dothideomycetes were represented by 3 genera: *Phyllosticta* (43 isolates), *Cladosporium* (20 isolates) and *Alternaria* (8 isolates). Then, the Eurotiomycetes with two species *Aspergillus niger* and *Penicillium* sp. with, respectively 29 and 19 isolates. Finally, the Leotiomycetes were only represented by the genus *Monilinia* (6).

The Zygomycota were the least dominant and were represented by the genus *Rhizopus* (21 isolates). Fifty four isolates were not identified due to the difficulty encountered during microscopic examination (Table 2).

The study of the diversity of endophytes in the four species of coffee trees showed that *Colletotrichum* sp., *Fusarium* sp., *Trichoderma* sp., *Aspergillus niger* were present in each of them.

In *C. kianjavatensis*, the results showed the absence of *Fusarium* sp. 03 and *Nigrospora* sp. In the case of *C. perrieri*, three strains were completely absent, namely *Cladosporium* sp., *Monilinia* sp. and *Rhizopus* sp. Finally, an absence of four strains among the fifteen identified was observed in *C. millotii* (*Daldinia* sp., *Diaporthe* sp., *Fusarium* sp. 02, *Alternaria* sp.) and in *C. vianneyi* (*Phyllosticta* sp., *Fusarium* sp. 03, *Monilinia* sp., *Penicillium* sp.). It should also be noted that genus *Fusarium* was very abundant, it included three different species according to the result obtained: *Fusarium* sp.01, Fusarium sp. 02 and *Fusarium* sp. 03. *Fusarium* sp. 01 had the highest abundance of *C. perrieri* and *C. millotii;* while for *C. vianneyi, Fusarium* sp.02 was the most abundant. Unidentified isolates (UI) were found in all species studied (Table 3).

Molecular identification of isolates. Table 4 gives details of the fungal profiles obtained by morphological and molecular characterisation of 30 representative mycotaxa isolated from four wild coffee trees in Madagascar. Molecular analysis confirmed the identity of 10 isolated strains; namely *Phyllosticta capitalensis*, *Colletotrichum Karstii, Diaporthe eres, Monilinia fructigena, Daldinia vanderguchtiae, Cladosporium asperulatum, Penicillim* sp, *Alternaria alternata, Fusarium tricinctum* and *Trichoderma* sp.

The morphological identification of 06 strains, namely: *Trichoderma* sp., *Fusarium* sp. 02, *Nigrospora* sp., *Rhizopus* sp., *Fusarium* sp. 01 and *Aspergillus niger,* could not be confirmed molecularly.

Morphologically Unidentified (NI) strains were identified they included 14 species, namely: *Colletotrichum karstii* (2 species), *Colletotrichum boninense, Colletotrichum siamense* (3 species), *Neofusicoccum parvum, Punctularia strigosozonata, Stemphylium solani, Phoma multirostrata, Calophoma complonata, Daldinia vanderguchtiae, Phoma exigua* and *Boremia exigua.*

DISCUSSION

The results obtained confirmed the presence of endophytic fungi on the leaves of four species studied (Table 3). The importance of the presence of endophytic fungi on coffee leaves and more precisely on *C. perrieri* has already been demonstrated by Ratsimbazafy (2011). Hyde and Soytong (2008) showed that endophytes are ubiquitous in all plant species. These authors also showed that endophytes are best adapted to superficial tissues before colonising other plant tissues. They can conquer and grow in all leaf compartments individually, but often in community (Whipps *et al*., 2008).

According to the results obtained, the colonisation rates of endophytic fungi were

TABLE 2. Taxonomic classification of endophytic fungi isolated from the leaves of four wild coffee species

Taxon	Endophytic mycotaxa	Total number of isolates	Abundance (%)	Classification of endophytic Mycotaxa			
				Phylum	Class	Order	Family
1	*Phyllosticta* sp.	43	7.15	Ascomycota	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae
2	*Colletotrichum* sp.	33	5.49	Ascomycota	Sordariomycetes	Phyllachorales	Phyllachoraceae
3	*Daldinia* sp.	18	2.99	Ascomycota	Sordariomycetes	Xylariales	Hypoxylaceae
4	*Diaporthe* sp.	17	2.83	Ascomycota	Sordariomycetes	Diaporthales	Diaporthaceae
5	*Cladosporium* sp.	20	3.32	Ascomycota	Dothideomycetes	Capnodiales	Davidiellaceae
6	*Fusarium* sp. 01	62	10.3	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae
7	*Fusarium* sp. 02	46	7.64	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae
8	*Fusarium* sp. 03	3	0.49	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae
9	*Monilinia* sp.	6	0.99	Ascomycota	Leotiomycetes	Heloliales	Sclerotinaceae
10	*Trichoderma* sp.	39	6.49	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae
11	*Alternaria* sp.	8	1.33	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae
12	*Penicillium* sp.	19	3.16	Ascomycota	Eurotiomycetes	Pleosporales	Pleosporaceae
13	*Aspergillus niger*	29	4.83	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae
14	*Rhizopus* sp.	21	3.49	Zygomycota	Incertea sedis	*Mucorales*	Rhizopodaceae
15	*Nigrospora* sp.	15	2.49	Ascomycota	Sordariomycetes	Trichosphaeriales	Trichosphaeriaceae
16	NI	54	8.97	-	-		- -

TABLE 3. Abundance rate of endophytic fungi for each *Mascarocoffea* species

Taxon	Endophytic Mycotaxa	Abundance (%)			
		Coffea perrieri	*Coffea kianjavatensis*	*Coffea millotii*	*Coffea vianneyi*
01	*Phyllosticta* sp.	19.42	14.8	5.55	0
02	*Colletotrichum* sp.	13.87	3.7	8.32	4.62
03	*Daldinia* sp.	8.32	2.77	0	5.55
04	*Diaporthe* sp.	3.7	10.17	0	1.85
05	*Cladosporium* sp.	0	2.77	13.87	1.85
06	*Fusarium* sp. 01	28.67	6.47	15.72	6.47
07	*Fusarium* sp. 02	16.65	2.77	0	23.12
08	*Fusarium* sp. 03	1.85	0	0.92	0
09	*Monilinia* sp.	0	4.62	0.92	0
10	*Trichoderma* sp.	10.17	3.7	0.92	21.27
11	*Alternaria* sp.	5.55	0.92	0	0.92
12	*Penicillium* sp.	5.55	7.4	4.62	0
13	*Aspergillus niger*	8.32	11.1	1.85	5.55
14	*Rhizopus* sp.	0	3.7	12.95	2.77
15	*Nigrospora* sp.	3.7	0	7.4	2.77
16	NI	12.95	19.42	13.87	3.7

different between host plants. This difference could be related to the problems of cultivation conditions (nutrient medium, temperature, surface sterilisation) that influence the colonisation of endophytic fungi (Gong and Guo; 2009) and/or by the nature of the plant species and the sampling area. In addition, the variation in daily atmospheric microflora concentration, as well as precipitation and strong winds vary from region to region and may be responsible for the difference in colonisation of endophytic fungi (Whipps *et al.,* 2008). Physiological and/or biochemical factors such as salt stress, nutrient stress, and water stress must also be taken into consideration. Indeed, many authors show that the symbiotic association of the plant with endophytic fungi could be fundamental for plant survival, by helping to adapt to different stress conditions (Malinowski *et al.*, 2006; Radhakrishnan *et al.,* 2013; Ikram *et al.,* 2020).

Morphological identification yielded a collection of 30 leaf-associated endophytic fungal isolates of the four different *Mascarocoffea* species. Among these 30 isolates, only 15 isolates could be identified. Indeed, the analysis of morphological criteria of the isolated microorganisms by macroscopic and microscopic observations is complex (Hyde and Soytong, 2007), generally due to the morphological similarities and especially the sterility of the endophytic fungi (Yeh and Kirschner, 2019). This last reason explains the non-identification of the other 15 isolates in this study.

Confirmation of the identity of endophytic fungal strains isolated from wild coffee leaves based on molecular analysis had not yet been the subject of previous work (Paulino De Moraes and Luchese, 2003; Martins *et al.,* 2003; Taniwaki *et al.,* 2003), which led to an incomplete identification of these species. In contrast with morphological identification, the

TABLE 4. Comparison of the identification of endophytic fungi by morphological and molecular criteria

Number	Morphological identification	Molecular identification					
		Species	Nucleotide number	Query ID	Total score	Accession number (GenBank)	Percentage of similarity
1	*Phyllosticta* sp.	*Phyllosticta capitalensis*	599	Query_14921	1101	KP900294.1	100%
2	*Colletotrichum* sp.	*Colletotrichum Karstii*	534	Query_15373	968	KY940478.1	99,44%
3	*Diaporthe* sp.	*Diaporthe eres*	*524*	Query_36069	942	MH931269.1	99,24%
4	NI	*Colletotrichum karstii*	*535*	Query_14095	944	MK569286.1	98,69%
5	*Monilinia* sp	*Monilinia fructigena*	488	Query_45297	902	LT615193.1	100%
6	NI	*Colletotrichum karstii*	535	Query_14095	944	MK569286.1	98,69%
7	NI	Colletotrichum boninense	*531*	Query_7337	950	KM520011.1	98,88%
8	NI	*Colletotrichum siamense*	510	Query_56155	933	MK984285.1	99,61%
9	NI	*Colletotrichum siamense*	*510*	Query_20799	907	MK984285.1	98,64%
10	NI	*Neofusicoccum parvum*	511	Query_14635	856	MN180877.1	96.42%
11	NI	*Colletotrichum siamense*	520	Query_36049	952	MK984285.1	99.62%
12	*Trichoderma* sp. 01	*Trichoderma* sp.01					
13	NI	*Punctularia strigosozonata*	597	Query_50615	1981	MH558554.1	97,12%
14	*Daldinia* sp.	*Daldinia vanderguchtiae*	534	Query_48581	965	MH862910.1	99,25%
15	*Cladosporium* sp	*Cladosporium asperulatum*	491	Query_22861	891	MN202774.1	100%
16	*Penicillium* sp.	*Penicillium* sp.	514	Query_12621	937	GU270552.1	99%
17	*Fusarium* sp. 02	*Fusarium* sp. 02					
18	NI	*Stemphylium solani*	533	Query_54953	976	KX025107.1	99,81%
19	*Nigrospora* sp.	*Nigrospora* sp.					
20	*Alternaria* sp.	*Alternaria alternata*	516	Query_23723	924	KX988016.1	99,41%
21	*Fusarium* sp. 03	*Fusarium tricinctum*	507	Query_8727	928	MN907441.1	100%
22	NI	Phoma multirostrata	487	Query_65437	893	AY943053.1	99,79%
23	NI	Calophoma complonata	561	Query_11861	935	JN624891.1	96,80%
24	NI	*Daldinia vanderguchtiae*	540	Query_15601	983	MH862910.1	99,63%

TABLE 4. Contd.

Number	Morphological identification	Molecular identification					
		Species	Nucleotide number	Query ID	Total score	Accession number (GenBank)	Percentage of similarity
25	*Rhizopus* sp	*Rhizopus* sp.					
26	*Trichoderma* sp. 02	*Trichoderma* sp. 02	579	Query_31585	1064	MK870330.1	100%
27	*Fusarium* sp. 01	*Fusarium* sp 01.					
28	*Aspergillus niger*	*Aspergillus niger*					
29	NI	*Phoma exigua*	501	Query_31073	917	KJ396074.1	99,60%
30	NI	*Boremia exigua*	536	Query_12295	976	MH858126.1	99,44%

molecular method allowed us to characterise more precisely the taxa composing the fungal communities (Alamouti *et al.,* 2007). The molecular identification of the present study allowed us to highlight the presence of 24 taxa of endophytic fungi from the leaves of four *Mascarocoffea* species. These are *Colletotrichum karstii, Colletotrichum siamense, Fusarium tricinctum*, *Trichoderma* sp., *Phyllosticta capitalensis, Cladosporium asperulatum, Monilinia fructigena, Colletotrichum boninense, Calophoma complonata, Neofusicoccum parvum, Punctularia strigosozonata, Diaporthe eres, Daldinia vanderguchtiae, Penicillim* sp, *Alternaria alternata, Phoma multirostrata, Phoma exigua, Boremia exigua*, and *Stemphylium solani*. All these fungal species have already been recorded as isolated endophytes in coffee leaves and roots (Oliveria *et al.,* 2014; Saucedo-Garcia *et al.,* 2014; Roberta *et al.,* 2020). These taxa belong mainly to the phylum Ascomycota. This predominance of Ascomycetes has been reported as a characteristic of root and leaf endophyte communities (Angelini *et al.,* 2012; Jha, 2019).

According to Senequier-Grozet and Canard (2016) regarding the general classification of endophytes, our strains would belong to the classes of endophytes 2-4 of the Non-Clavicipitaceae. These classes are characterised by their ability to grow in tissues above and below the soil, thus conferring tolerance to different types of environmental stress to infested plants (Rodriguez *et al.,* 2008). In addition, the genera of endophytic fungi grouped in these classes have different types of interactions with plants, ranging from pathogenicity to symbiosis (Rodriguez *et al.,* 2009), which explains the interest of their presence in Malagasy coffee leaves.

CONCLUSION

The present study has provided a more complete data on the diversity of endophytes associated with four species of wild coffee trees endemic to Madagascar. All selected

Mascarocoffea plant species were harboured by endophytic fungi. The identification of these endophytes, based on morphological criteria, allowed the identification of the genus *Phyllosticta* sp., *Colletotrichum* sp., *Daldinia* sp., *Diaporthe* sp., *Cladosporium* sp., *Fusarium* sp.01, *Fusarium* sp. 02, *Fusarium* sp. 03, *Monilinia* sp, *Trichoderma* sp., *Alternaria* sp., *Penicillium* sp., *Aspergillus niger*, *Rhizopus* sp. and *Nigrospora* sp. The molecular techniques used allowed the identification most of the endophytic fungi species isolated. The majority of the isolates described in this study belong to the Ascomycota. Our study suggests that the ITS1 region of the rDNA is accurate for the identification of different species of endophytic fungi.

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