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Fungal endophyte diversity in coffee plants from Colombia, Hawai'i, Mexico and Puerto Rico

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ABSTRACT

Coffee (*Coffea arabica*) plant tissues were surface-sterilized and fungal endophytes isolated using standard techniques, followed by DNA extraction and sequencing of the internal transcribed spacer region (ITS). A total of 843 fungal isolates were recovered and sequenced (Colombia, 267; Hawai'i, 393; Mexico, 109; Puerto Rico, 74) yielding 257 unique ITS genotypes (Colombia, 113; Hawai'i, 126; Mexico, 32; Puerto Rico, 40). The most abundant taxa were *Colletotrichum*, *Fusarium*, *Penicillium*, and Xylariaceae. Overall, 220 genotypes were detected in only one of the countries sampled; only two genotypes were found in all four countries. Endophytes were also isolated from *Coffea canephora*, *Coffea congensis*, *Coffea liberica*, *Coffea macrocarpa*, *Coffea racemosa*, and *Coffea stenophylla* in Hawai'i. The high biodiversity of fungal endophytes in coffee plants may indicate that most of these are "accidental tourists" with no role in the plant, in contrast to endophytes that could be defined as "influential passengers" and whose role in the plant has been elucidated. This study, the most comprehensive analysis of fungal endophytes associated with a single host species, demonstrates that coffee plants serve as a reservoir for a wide variety of fungal endophytes that can be isolated from various plant tissues, including the seed, and illustrates the different fungal communities encountered by *C. arabica* in different coffee-growing regions of the world.

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Introduction

The genus *Coffea* (Rubiaceae) comprises 103 species from tropical Africa, Madagascar, and the Mascarene Islands (Davis *et al.* 2006). Two species, *Coffea arabica* and *Coffea canephora* (also known as *robusta*) make coffee the second largest export commodity in the world after petroleum products, with an estimated annual retail sales value of US $70 billion (Lewin *et al.* 2004). *C. arabica* is endemic to Ethiopia, SE Sudan, and northern Kenya, while *C. canephora* is endemic to various countries throughout tropical Africa (Davis *et al.* 2006). Coffee is planted in more than 10 million hectares in over 50 countries (http://faostat.fao.org), and approximately 125 million people in Latin America, Africa, and Asia are dependent on coffee for their subsistence (Osorio 2002; Lewin *et al.* 2004).

While a diversity of fungal pathogens associated with coffee has been recorded in the literature (e.g., Muller *et al.* 2004), there is a paucity of information regarding nonpathogenic, symbiotic fungi such as endophytes associated with these economically important plants. Fungal endophytes have been defined in many ways (Schulz & Boyle 2006; Hyde & Soytong 2008), however, broadly defined, fungal endophytes are ''fungi … which for all or part of their life cycle invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues, but cause no symptoms of disease'' (Wilson 1995). Recent studies have shown that some fungal endophytes can protect host plants against pathogens and herbivores (e.g., Freeman & Rodriguez 1993; Arnold *et al.* 2003; Arnold & Lewis 2005; Schulz & Boyle 2005) and in some cases include entomopathogenic species (Arnold & Lewis 2005; Vega *et al.* 2008b). Neither the ecological importance nor economic applications of most endophytes have been resolved, however, in part because very few plant species have been comprehensively surveyed for endophytic fungi. Given the tremendous economic importance of coffee, the wide geographic range of the genus, and the importance of the plant in sustainable agroforestry and conservation efforts (Wintgens 2004; Perfecto *et al.* 2007), there is growing enthusiasm to examine the endophyte communities associated with *Coffea*.

Rayner (1948), working in Kenya, published the first paper on coffee endophytes after surface-sterilizing healthy leaves, pedicels, stems, and green berries with a mercuric chloride–saponin solution. That study recovered the causal agent of coffee berry disease, *Colletotrichum coffeanum*, (currently known as *Colletotrichum kahawae* Waller & Bridge), and species of *Phoma* and *Phomopsis*. Santamaría & Bayman (2005) reported *Botryosphaeria*, *Colletotrichum*, *Guignardia*, and *Xylaria* species as fungal endophytes in coffee plants from Puerto Rico. Vega *et al.* (2006) identified 13 *Penicillium* species as endophytes in *C. arabica*, *Coffea congensis*, *Coffea dewevrei* and *Coffea liberica*, including *Penicillium coffeae*, a new species described by Peterson *et al.* (2005). In a study describing the introduction of the fungal entomopathogen *Beauveria bassiana* as an endophyte in coffee plants, Posada *et al.* (2007) reported the presence of more than 35 fungal endophytes from coffee seedlings purchased at a plant nursery in Maryland; they hypothesized that the presence of these endophytes might have prevented *B. bassiana* from becoming established in the plants. Vega *et al.* (2008a) also detected seven genera of fungi as endophytes in green coffee seeds from Colombia, Guatemala, India, Kenya, Papua New Guinea, Puerto Rico, and Vietnam. Several genera of entomopathogenic fungi in coffee plants were also recovered from various locations in a different study (Vega *et al.* 2008b). Similarly, Vega *et al.* (2005) reported the presence of 19 genera of bacterial endophytes in coffee plants from Colombia, Hawai'i, and Mexico. To date, however, comprehensive studies assessing the geographic heterogeneity of *Coffea* endophytes among different coffee-growing regions of the world, the similarity of endophyte communities among different sympatric species of *Coffea*, and the tissue specificity of endophytes inhabiting *C. arabica* have not been conducted.

In this paper, we report the first results of a survey of fungal endophytes associated with various asymptomatic tissues of coffee plants in Colombia, Hawai'i, Mexico, and Puerto Rico.

Materials and methods

Sampling sites

Coffee plants (*C. arabica*) were sampled for endophytic fungi in 2002 and 2003. Sampling sites included one location in Colombia (National Coffee Research Center (CENICAFÉ), Chinchiná, Caldas; Jul. 2003); 10 locations throughout Hawai'i (Jan. 2003); six locations in Chiapas, Mexico (Sep. 2002); and one location in Puerto Rico (Jun. 2002; Table 1). To facilitate discussion, Colombia, Hawai'i, Mexico, and Puerto Rico are referred to as ''countries'' throughout the paper. At various Hawai'i locations, other *Coffea* species were also sampled: *C. canephora*, *C. congensis*, *C. liberica*, *C. macrocarpa*, *C. racemosa*, and *C. stenophylla* (Table 1).

Endophyte isolation

Various asymptomatic parts of coffee plants were sampled, including leaves, roots, stems, and berries. Berries were divided into various sections: crown, sections of the berry itself, seeds, and the peduncle. Mature leaves were collected from the middle section of each plant at the approximate midpoint of the branch. Age of plants could not be determined, and collection sites varied in levels of shading from fully shaded to full sun. No attempts were made to collect only under one condition. Not all tissues were sampled in each location.

Plant parts were washed in running tap water and sectioned into small pieces under sterile conditions with a sterile scalpel (see Posada *et al.* 2007 for standard isolation methods). Sections were surface-sterilized by dipping in 0.5 % sodium hypochlorite for 2 min and 70 % ethanol for 2 min (Arnold *et al.* 2003), and rinsed in sterile distilled water before surface-drying on sterile paper. Sections were plated on yeast malt agar (YMA; Sigma Aldrich Co., St. Louis, MO) to which 0.1 % stock antibiotics was added (see Vega *et al.* 2005). Plates were kept at room temperature for several months, and any fungal growth was subcultured onto individual YMA plates for subsequent DNA extraction. Because the vast majority of

fungi did not sporulate in culture, we characterized all isolates using molecular sequence data.

DNA extraction, amplification, and sequencing

Endophytes were grown in potato dextrose broth (Difco, Becton Dickinson, Sparks, MD) at 125 rpm on an Innova 4000 Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 25 °C for one week. Fungal tissue was then harvested, lyophilized, and stored at −80 °C. Lyophilized tissues were also intended for re-growth of the isolates and subsequent deposit in culture collections. Unfortunately, most tissues were not viable, except for several *Penicillium* and *Aspergillus* species that have been deposited in the NRRL collection (Table 1). For DNA extraction, ca. 50 mg of lyophilized mycelium were placed in a 2 ml microcentrifuge tube with ca. 0.2 ml 1.0 mm zirconia-glass beads (Cat # 1107911-0z, BIOSPEC, Bartlesville, OK). The mycelium was crushed with a plastic pestle and further ground in a Fast-Prep-120 sample grinder (Q-BIOgene, Irvine, CA) for 3 sec at the maximum speed setting of 6. The powdered mycelium was suspended in 700 μL detergent solution (2 M NaCl, 0.4 % w/v deoxycholic acid–sodium salt, 1 % w/v polyoxyethylene 20 cetyl ether) and then agitated for 14 s in the Fast-Prep at maximum speed. Vials were incubated for 5 min at 55 °C in a heat block and then centrifuged at 7 000 *g* for 5 min followed by emulsion with 700 μl chloroform/isopropyl and centrifugation at 7 000 *g* for 5 min. The aqueous phase was transferred to a clean tube to which an equal volume of 6 M guanidinium thiocyanate was added. Fifteen microliters of silica powder were gently mixed with the solution while incubating at room temperature for 5 min, followed by 3 s centrifugation, after which the supernatant was discarded. The glass powder was rinsed twice by suspending in 750 μL ethanol buffer (10 mM Tris–HCl, pH 8.0, 0.1 mM EDTA, 50 % ethanol) with a disposable transfer pipette, then collected by centrifugation. The supernatant was discarded, and the glass powder pellet was dried on a heat block at 55 °C for 5–10 min. The glass powder was re-hydrated with 105 μL ultra-pure water and genomic DNA eluted by incubating on a heat block at 55 °C for 5–10 min. Following vortexing and centrifugation, 100 μL of the aqueous supernatant was transferred to a clean tube. In a few instances, DNA extractions were made directly from fungal cultures grown on potato dextrose agar (Difco, Becton Dickinson, Sparks, MD). In those cases, approximately 2–4 mm^2 of mycelium was aseptically removed from the growing edge of the colony and extracted with the UltraClean Plant DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA) as per the manufacturer's instructions.

Primers ITS1-F (fungal-specific) (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990) were used for both PCR-amplification and sequencing of the internal transcribed spacer region (ITS) of the nuclear rDNA repeat for each isolate. PCRs were done in 25 μL reaction volumes with 12.5 μL of PCR Master Mix (Promega Corp., Madison, WI), 1.25 μL each of 10 μM primers, and 10 μL of diluted (10- to 100-fold) DNA template. Amplification was done with an initial denaturation step of 5 min at 94 °C; 35 cycles of 30 s at 94 °C, 45 s at 50 °C, and 45 s at 72 °C; and a final extension of 7 min at 72 °C. PCR products were purified with Montage PCR Centrifugal Filter Devices (Millipore Corp., Billerica, MA) according to the manufacturer's protocol. Elongation factor-1 alpha (TEF) was also amplified for clavicipitaceous isolates 57 and 59 as described by Rehner & Buckley (2005). Purified PCR products were sequenced with BigDye Terminator sequencing enzyme v.3.1 (Applied Biosystems, Foster City, CA) using 2 μL of diluted BigDye in a 1:3 dilution of BigDye:dilution buffer (400 mM Tris pH 8.0, 10 mM $MgCl_2$), 0.3 μL of 10 μM primer, 10–20 ng of cleaned PCR template, and H_2O to 5 μL total reaction volume. Cycle sequencing parameters consisted of a 2 min denaturation step at 94 °C, then 35 cycles of 94 °C for 39 s, 50 °C for 15 s, and 60 °C for 4 min. Reaction products were cleaned by ethanol precipitation and sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing reads were edited and contiguous sequences for each isolate were assembled and edited in Sequencher v.4.1.4 (Gene Codes Corp., Ann Arbor, MI).

To assign genotype groups, sequences from all 843 isolates were compared to each other in Sequencher at the 99 % homology level. Each contig was then edited by eye to remove any prior editing errors, disassembled, and a final assembly of all sequences was conducted at the 100 % homology level to yield a total of 257 unique sequences. DNA sequences have been deposited in GenBank; in most cases, only one sequence was deposited when different isolates yielded identical sequences (Table 1). Diversity and similarity indices (following Arnold *et al.* 2003, Arnold & Lutzoni 2007) were calculated using genotype groups as operational taxonomic units. Diversity was calculated using Fisher's alpha, which is robust to differences in sampling intensity (see Arnold *et al.* 2007). Similarity indices included two indices based on presence/absence data only (Jaccard's index, Sørensen's index), and one based on abundance data (Morisita–Horn index). All indices were calculated using nonsingleton genotypes only (genotypes recovered more than once) and range from 0 (no similarity) to 1 (full similarity of endophyte communities).

Identification of isolates

Initial identification of all sequences was obtained by BLAST analysis using BLASTn (http://www.ncbi.nlm.nih.gov/BLAST). *Penicillium* and *Aspergillus* species were then further identified using additional sequences in our reference database (S.W. Peterson, unpublished). Exact matches to the sequences from ex-type cultures were considered reliable identifications for these genera. Where genealogical concordance multilocus phylogenetic studies have been published, those concepts were used to corroborate the identity of isolates (Taylor *et al.* 2000).

Identification of other isolates was derived by interpreting a combination of the first 100 BLAST matches and the distance tree results produced from BLAST-generated pairwise alignments. In general, taxonomic identification based on BLAST was applied cautiously (i.e., at the genus level or above, and with caution given the occurrence of misidentified sequences in GenBank; see Vilgalys 2003; Arnold & Lutzoni 2007). Sequences with high identity (98–100 %) to multiple isolates of a given genus, and that also fell within that genus in distance analyses, were assigned to that genus. Sequences with ~92–100 % identity to more than one genus within a single family, and that also fell within that family in distance analyses, were

Table 1 – Fungal endophyte genotypes isolated from various surface-sterilized, asymptomatic coffee tissues in Colombia, Hawai'i, Mexico and Puerto Rico

Fungal ID	GenBank[1]	COLOMBIA[2]								HAWAI'I[3]								MEXICO[4]						PUERTO RICO[5]						
		Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	*SUM*
Agaricomycetes sp. 1	EU002896								0	1[d]							1						0		1				1	2
Agaricomycetes sp. 2	EF694649	1		1					2								0						0						0	2
Agaricomycetes sp. 3	EF687930	1							1								0						0						0	1
Agaricomycetes sp. 4	EF672294				1				1								0						0						0	1
Agaricomycetes sp. 5	EF672293								0						1[g]		1						0						0	1
Agaricomycetes sp. 6	EF694648								0							1[g]	1						0						0	1
Agaricomycetes sp. 7	EU009975								0								0						0	1					1	1
Agaricomycetes sp. 8	EU009976								0				1[a]				1						0						0	1
Agaricomycetes sp. 9	EU009979								0				1[d]				1						0						0	1
Ascomycota sp. 1	EF672301								0								0						0				1		1	1
Ascomycota sp. 2	EF694665	1					1		2								0						0						0	2
Ascomycota sp. 3	EF672299						1		1								0						0						0	1
Ascomycota sp. 4	EF672300	1							1								0						0						0	1
Ascomycota sp. 5	EU002914	1							1								0						0						0	1
Ascomycota sp. 6	EU002910			1	1				2								0						0						0	2
Ascomycota sp. 7	EF672291	1							1								0						0						0	1
Ascomycota sp. 8	EF672295								0							2[g]	2						0						0	2
Ascomycota sp. 9	EF687939								0	2[a,d]			1[a]				3						0						0	3
Ascomycota sp. 10	EF687937								0	1[i,q]							1						0						0	1
Ascomycota sp. 11[6]	EF687938								0				1[h,q]				1						0						0	1
Ascomycota sp. 12	EU002909								0	1[a]							1						0						0	1
Aspergillus sp. 1	EF687940								0								0						0		1				1	1
Aspergillus sp. 2	EF687941								0								0						0		1	1	1		3	3
Aspergillus sp. 3	EF672306								0	1[i]							1				1[p]		1						0	2
Aspergillus sp. 4	EF672304						2		2								0						0						0	2
Aspergillus sp. 5	EF672305								0					1[g]	1[g]		2						0						0	2
Aspergillus sp. 6	EF672303						1		1								0						0						0	1
Aspergillus fumigatus	EF634383 (35190)	2							2								0						0						0	2
Aspergillus niger	EF634375 (35172)								0								0		1[m]			5[m,p]	6						0	6
Aspergillus oryzae	EF591304 (35191)	1							1								0						0						0	1
Aspergillus phoenicis	EF634380 (35179)								0			1[a]					1						0						0	1
Aspergillus pseudodeflectus	DQ778908		1						1		1[d]					2[g]	3						0						0	4
Aspergillus cf. *sclerotiorum*	EF672302								0								0						0				1		1	1
Aspergillus tamarii	EF634378 (35176)								0								0					1[p]	1						0	1
Aspergillus westerdijkiae	EF634412 (35437)								0				4[g]				4						0						0	4
Beauveria sp.	EF672308	2							2								0						0						0	2
Beauveria bassiana	EF672309	2	1	6	1	2			12	1[d]							1						0						0	13
Beauveria brongniartii	EF672310								0	1[a]		1[a]					2						0						0	2
Biscogniauxia sp.	EU009960								0	1[i,q]							1						0						0	1
Botryosphaeria sp.	EF672312			2					2	2[b]							2						0						0	4
Cercospora sp.	EF672313		1						1								0						0						0	1
Cladosporium sp. 1	DQ299297								0								0						0	1					1	1
Cladosporium sp. 2	DQ299300		1						1								0						0						0	1
Cladosporium sp. 3	DQ299298	1	2	1					4	1[d]							1						0						0	5
Cladosporium sp. 4	DQ299299	1							1								0						0						0	1
Cladosporium sp. 5	DQ299302								0	2[g]							2						0						0	2

(*continued on next page*)

Table 1 – (*continued*)

Fungal ID	GenBank[1]	COLOMBIA[2]								HAWAI'I[3]								MEXICO[4]						PUERTO RICO[5]							
		Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	*SUM*
Cladosporium sp. 6	DQ299301								0					1[g]			1						0						0	1
Cladosporium sp. 7	DQ299303								0		1[i]		2[i]	2[i]			5						0						0	5
Cladosporium sp. 8	EF672315								0				1[f]				1						0						0	1
Clavicipitaceae sp.	DQ682571								0								0						0		3				3	3
Clonostachys cf. *rosea*	DQ287243	2							2								0						0						0	2
Colletotrichum sp. 1	EF687921								0								0						0	1					1	1
Colletotrichum sp. 2	EF672288	11		6	3		5		25	6[d,g,h]							6	6[p]		1[k]			7						0	38
Colletotrichum sp. 3	EF672327								0								0						0		1				1	1
Colletotrichum sp. 4	EF672317								0	3[d,g,h]							3						0		1				1	4
Colletotrichum sp. 5	EF672286	1							1	1[h]							1						0						0	2
Colletotrichum sp. 6	EF672287	1							1								0						0						0	1
Colletotrichum sp. 7	EF672328	1							1								0						0		1	1			2	3
Colletotrichum sp. 8	EF672284	2		2			1		5								0						0						0	5
Colletotrichum sp. 9	EF694637	1							1								0						0						0	1
Colletotrichum sp. 10	EF687924						1		1								0						0						0	1
Colletotrichum sp. 11	EF672322				2				2								0						0		1				1	3
Colletotrichum sp. 12	EF687920			3					3								0						0						0	3
Colletotrichum sp. 13	EF672290				1				1								0						0						0	1
Colletotrichum sp. 14	EF687923	2							2								0						0						0	2
Colletotrichum sp. 15	EF687925	1							1								0						0						0	1
Colletotrichum sp. 16	EF672324	4							4								0						0						0	4
Colletotrichum sp. 17	EF672325	1							1								0						0						0	1
Colletotrichum sp. 18	EF687922			1			1		2								0						0						0	2
Colletotrichum sp. 19	EF672318	1							1								0						0						0	1
Colletotrichum sp. 20	EF694640	1							1	1[d]							1	1[p]					1						0	3
Colletotrichum sp. 21	EF672326	4			1		4		9	3[d,h,i,r,t]							3						0	1		3			4	16
Colletotrichum sp. 22	EF687927						2		2								0						0						0	2
Colletotrichum sp. 23	EF687928						2		2	3[h]							3	1[p]					1						0	6
Colletotrichum sp. 24	EF694638	12							12	27[a,b,d,g,i,j,q,t]			3[i]				30	1[p]					1						0	43
Colletotrichum sp. 25	EF672329	4					6		10	1[g]			1[g]		1[g]		3			1[o]			1						0	14
Colletotrichum sp. 26	EF672282								0						1[g]		1						0						0	1
Colletotrichum sp. 27	EF694639								0	1[g]							1						0						0	1
Colletotrichum sp. 28	EU049287								0	29[a,d,e,i,g,q,s]	1[i]			1[i]			31	1[p]					1						0	32
Colletotrichum sp. 29	EF672330	4							4	7[d,g,h,s]							7	1[p]					1						0	12
Colletotrichum sp. 30	EF672283								0	6[g,j]							6						0						0	6
Colletotrichum sp. 31	EF687919	4	2	3	4				13								0						0						0	13
Colletotrichum sp. 32	DQ682572								0	2[d]							2						0						0	2
Colletotrichum sp. 33	EF672285								0								0	1[p]					1						0	1
Colletotrichum sp. 34	EF672320	1							1								0						0						0	1
Colletotrichum sp. 35	EF672321								0	1[g]							1						0						0	1
Colletotrichum sp. 36	EF687918								0	17[b,c,d,g,j,q,s]							17						0						0	17
Colletotrichum sp. 37	EF672323								0	1[i]							1						0						0	1
Colletotrichum sp. 38	EF672289								0	1[e]							1						0						0	1
Colletotrichum sp. 39	EF672281								0						1[d]		1						0						0	1
Colletotrichum sp. 40	EF687926								0	2[d]							2						0						0	2

Cryptococcus sp.	EF687931								0								0			1^{n}			1						0	1
Engyodontium sp.	EF687942								0							1^{j}	1						0						0	1
Exobasidiomycetes sp.	EU009983		1	1					2								0						0						0	2
Fusarium sp. 1	EF687954								0								0						0	1					1	1
Fusarium sp. 2	DQ778914					1			1								0						0		1	4	1	2	8	9
Fusarium sp. 3	EF687949								0								0						0		1	1			2	2
Fusarium sp. 4	EF687914								0								0				1^{p}		1			1			1	2
Fusarium sp. 5	EF687948								0								0					3^{k}	3						0	3
Fusarium sp. 6	EF687911								0								0						1			1			1	2
Fusarium sp. 7	EF687910								0								0						0			2			2	2
Fusarium sp. 8	EF694669								0								0						0			1			1	1
Fusarium sp. 9	EF687915								0								0					3^{m}	3						0	3
Fusarium sp. 10	EF687952								0	1^{g}		2^{i}					3						0						0	3
Fusarium sp. 11	EU049288								0	1^{b}				3^{i}			4						0						0	4
Fusarium sp. 12	EF687917								0						1^{g}	1^{g}	2						0						0	2
Fusarium sp. 13	DQ778913				1		1		2			1^{i}			3^{g}	1^{g}	5		1^{o}	1^{o}			2						0	9
Fusarium sp. 14	EF687947								0					1^{g}	4^{g}	3^{g}	8						0						0	8
Fusarium sp. 15	EF687912								0								0		1^{o}	$4^{k,p}$			5						0	5
Fusarium sp. 16	EF687916	5			2		6		13					1^{b}		2^{g}	3	$2^{p,m}$	$16^{k,m,n,p}$	$10^{k,n,o,p}$	$6^{k,m}$	$14^{k,m,n,o,p}$	48						0	64
Fusarium sp. 17	EF687951							1	1								0						0						0	1
Fusarium sp. 18	DQ682580		2	7	2	1	1		13	$7^{d,q}$							7	1^{p}	1^{o}			3^{o}	5		2	6			8	33
Fusarium sp. 19	DQ682578								0							5^{g}	5						0						0	5
Fusarium sp. 20	EF694636								0	3^{g}			1^{d}				4						0						0	4
Fusarium sp. 21	EF694633								0				1^{d}				1						0						0	1
Fusarium sp. 22	EF687950								0	$6^{d,u}$				1^{b}			7						0						0	7
Fusarium sp. 23	EF687913								0	2^{d}				$1^{d,s}$			3						0						0	3
Fusarium sp. 24	EF687955								0					$1^{i,v}$			1						0						0	1
Fusarium sp. 25	EF694635								0	$1^{d,t}$				1^{j}			2						0						0	2
Hymenochaetaceae sp.	EF694647			1					1								0						0						0	1
Hypocreales sp. 1	EF694654			1					1								0						0						0	1
Hypocreales sp. 2	DQ287230								0							1^{g}	1						0						0	1
Irpex sp. 1	EF694660								0								0						0		1	1	1		3	3
Irpex sp. 2	EF694658								0							1^{j}	1						0						0	1
Irpex sp. 3	EF694659								0	1^{g}							1						0						0	1
Leptosphaeriaceae sp.	EF694661								0					$1^{i,q}$			1						0						0	1
Leptosphaerulina sp.	EF694653								0	1^{g}					1^{g}		2						0						0	2
Meira sp. 1	EF694663								0				1^{b}				1						0						0	1
Meira sp. 2	EF694664								0	1^{g}				1^{d}			2						0						0	2
Mycosphaerella sp.	EF694668								0	3^{d}		1^{d}					4						0		1				1	5
Mycosphaerellaceae sp.	EF694667								0	1^{d}							1						0						0	1
Nemania sp.	EF694670								0	1^{i}							1						0						0	1
Neosartorya sp.	EF694671	1							1	1^{e}							1						0						0	2
Nodulisporium sp.	EF694672								0					$1^{d,q}$			1						0						0	1
Paecilomyces sp. 1	DQ287248								0							1^{g}	1						0						0	1
Paecilomyces sp. 2	EU049286				1				1								0		1^{o}				1						0	2
Paecilomyces sp. 3	DQ682588								0								0						0			1			1	1
Penicillium sp. 1	EU002901						1		1								0			$8^{o,p}$			8						0	9
Penicillium sp. 2	DQ778916						1		1								0						0			1			1	2
Penicillium sp. 3	EF694646	1							1								0						0						0	1
Penicillium sp. 4	EF694623				1				1								0						0						0	1
Penicillium sp. 5	EF694624						1		1								0						0						0	1
Penicillium sp. 6	EF694625								0				1^{i}				1						0						0	1

(continued on next page)

Table 1 – (*continued*)

Fungal ID	GenBank[1]	COLOMBIA[2]								HAWAI'I[3]								MEXICO[4]						PUERTO RICO[5]							
		Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	Leaf	Berry	Crown	Peduncle	Seed	*TOTAL*	*SUM*
Penicillium sp. 7	EF694626								0		1[i]						1						0						0	1
Penicillium sp. 8	EF694627								0						1[g]		1						0						0	1
Penicillium sp. 9	DQ682589	4		5	2		1		12	5[b,d,i,j]		1[d]	1[j]				7	1[p]					1		1		2		3	23
Penicillium sp. 10	EF694628								0				1[d]				1						0						0	1
Penicillium sp. 11	EF694630								0				1[e]				1						0						0	1
Penicillium sp. 12	EU002902								0	1[h]							1						0						0	1
Penicillium sp. 13	DQ123664 (32575)								0					1[g]			1						0						0	1
Penicillium biverticillate	DQ123635 (35186)								0			1[g]					1						0						0	1
Penicillium brevicompactum	DQ682592	3							3	14[b,c,d,g,i,j,v]				6[b,e,j]			20						0						0	23
Penicillium brocae	EF634396 (35209)	1							1	1[g]						1[g]	2						0						0	3
Penicillium citrinum	EF634411 (35435)								0				3[g]				3						0						0	3
Penicillium coffeae	AY742705 (35366)								0				4[g,i]				4						0						0	4
Penicillium crustosum	DQ123647 (35178)		1	2					3								0					1[l]	1						0	4
Penicillium olsonii	DQ778918						1		1	20[a,b,d,e,f,g,h,j,q]	2[e]	1[h]	7[a,d,e,f,q]	2[e,g]			32						0		1	3			4	37
Penicillium oxalicum	DQ123663 (35183)	1							1								0						0						0	1
Penicillium sclerotiorum	EF634367 (32578)								0			2[a]	1[a]				3						0						0	3
Penicillium steckii	EF634408 (35368)								0		3[g]						3						0						0	3
Penicillium sumatraense	DQ861286								0								0				1[p]		1					1	1	2
Pestalotiopsis sp. 1	EU002905								0								0			1[m]			1						0	1
Pestalotiopsis sp. 2	EU002907								0	1[j]							1						0						0	1
Petriella sp.	EU002908							2	2								0						0						0	2
Pezizomycotina sp.	EF672296	1							1								0						0						0	1
Phlebiopsis sp. 1	EU002912								0	1[d]							1						0						0	1
Phlebiopsis sp. 2	EU002911								0				1[i]				1						0						0	1
Phomopsis sp. 1	EU002923	1							1								0						0						0	1
Phomopsis sp. 2	EU002919								0								0			1[p]			1						0	1
Phomopsis sp. 3	EU002920								0								0						0			1			1	1
Phomopsis sp. 4	EU002916								0		1[a]						1						0						0	1
Phomopsis sp. 5	EU002928	1		1					2								0						0						0	2
Phomopsis sp. 6	EU002926	1	1						2								0						0						0	2
Phomopsis sp. 7	EU002921			1					1								0						0						0	1
Phomopsis sp. 8	EU002917			1					1								0						0						0	1
Phomopsis sp. 9	EU002922			1					1								0						0						0	1
Phomopsis sp. 10	EU002915			1					1								0						0						0	1
Phomopsis sp. 11	EU002925	1							1								0						0						0	1
Phomopsis sp. 12	EU002927			1					1								0						0						0	1
Phomopsis sp. 13	EU002918			2					2								0						0						0	2
Phomopsis sp. 14	EF687935								0			1[d,q]					1						0						0	1
Phomopsis sp. 15	EF687933								0				1[f]				1						0						0	1
Phomopsis sp. 16	EU002930								0	1[i]		1[i,q]					2						0						0	2
Phomopsis sp. 17	EU002929								0	1[d,u]		1[d,u]					2	1[m]					1						0	3
Phomopsis sp. 18	EU002931								0			1[d,s]					1						0						0	1
Phomopsis sp. 19	EU002932								0			1[a]					1						0						0	1
Phomopsis sp. 20	EU002924								0			1[a]					1						0						0	1

Phomopsis sp. 21	EF687934								0						0		1[m]	1					0	1
Phomopsis sp. 22	EU002934								0						0		1[p]	1					0	1
Phomopsis sp. 23	EF687936								0	1[d]		1[d]			2			0					0	2
Phomopsis sp. 24	EU002933								0	1[d,t]			1[d]		2			0					0	2
Pleosporaceae *sp.*	EF687932			1	1				2						0			0					0	2
Pleosporales sp. 1	EF672298					1			1						0			0					0	1
Pleosporales sp. 2	EU002891								0				1[d,s]		1			0					0	1
Pleosporales sp. 3	EU002938								0			1[d]	1[d]		2			0					0	2
Pleosporales sp. 4	EF694662								0				1[d]		1			0					0	1
Pleosporales sp. 5	EU002937								0				1[j]		1			0					0	1
Pleosporales sp. 6	EU049289								0	1[j]					1			0					0	1
Pleosporales sp. 7	EU002939								0				1[d,q]		1			0					0	1
Pseudozyma sp.	EU002890			1					1						0			0					0	1
Schizophyllum sp. 1	EU002894								0						0			0			1		1	1
Schizophyllum sp. 2	EU002895			1					1						0			0					0	1
Sordariales sp.	EU002897							1	1						0			0					0	1
Sordariomycetes sp. 1	EF672297						1		1						0			0					0	1
Sordariomycetes sp. 2	EF694651				1				1						0			0					0	1
Sordariomycetes sp. 3	EU002935	1							1						0			0					0	1
Sordariomycetes sp. 4	EF687956				1				1						0			0					0	1
Sordariomycetes sp. 5	EU009995								0					1[j]	1			0					0	1
Sordariomycetes sp. 6	EU002898	1							1						0			0					0	1
Sporobolomyces sp. 1	EU009966								0						0			0			1		1	1
Sporobolomyces sp. 2	EU002893								0						0			0			1	2	3	3
Sporobolomyces sp. 3	EU002899								0						0			0		1	1	1	3	3
Sporobolomyces sp. 4	EU002892								0						0			0				1	1	1
Sporobolomyces sp. 5	EU009967						1		1						0			0					0	1
Stagonospora sp.	EU009968								0	1[g]					1			0					0	1
Stereum sp. 1	EU009970	1							1						0			0					0	1
Stereum sp. 2	EU009969					1			1						0			0					0	1
Tilletia sp. 1	EU009972		2						2						0			0					0	2
Tilletia sp. 2	EU009971			1					1						0			0					0	1
Tilletiopsis sp.	EU009973								0						0			0				1	1	1
Trametes sp. 1	EU009977	1		1	1				3		1[a]				1			0					0	4
Trametes sp. 2	EU009978								0				1[d]		1			0					0	1
Trichoderma sp. 1	EU009980						2		2						0			0					0	2
Trichoderma sp. 2	EU009981			1					1						0			0					0	1
Xylaria sp. 1	EU009989	1							1						0			0					0	1
Xylaria sp. 2	EU009958			1					1						0			0					0	1
Xylaria sp. 3	EU010000								0	1[c]					1			0					0	1
Xylaria sp. 4	EF634381 (35182)								0			1[h]			1			0					0	1
Xylariaceae sp. 1	EU009962								0						0			0	1				1	1
Xylariaceae sp. 2	EU009964								0						0			0	1				1	1
Xylariaceae sp. 3	EU009965								0						0	1[p]		1					0	1
Xylariaceae sp. 4	EU009987								0						0			0				1	1	1
Xylariaceae sp. 5	EU010005	1							1						0			0					0	1
Xylariaceae sp. 6	EU010004	1							1						0			0					0	1
Xylariaceae sp. 7	EU009959						1		1						0			0					0	1
Xylariaceae sp. 8	EU009998	2							2						0			0					0	2
Xylariaceae sp. 9	EF694657			2					2						0			0					0	2
Xylariaceae sp. 10	EU009997		1						1						0			0					0	1
Xylariaceae sp. 11	EU009999	1							1						0			0					0	1
Xylariaceae sp. 12	EU010001			1					1						0			0					0	1
Xylariaceae sp. 13	EU010003	1							1						0			0					0	1
Xylariaceae sp. 14	EU009992	1							1						0			0					0	1
Xylariaceae sp. 15	EU009985								0	27[d,i,q,v]					27			0					0	27
Xylariaceae sp. 16	EU009957								0	1[a]					1			0					0	1
Xylariaceae sp. 17	EU010002								0	1[i]					1			0					0	1
Xylariaceae sp. 18	EU009961								0	2[i,q]		1[b]			3			0					0	3
Xylariaceae sp. 19	EU009963								0	3[d]		1[d]			4			0					0	4
Xylariaceae sp. 20	EF694656								0	1[i]					1			0					0	1

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Table 1 – (continued)

Fungal ID	GenBank[1]	COLOMBIA[2]								HAWAI'I[3]								MEXICO[4]						PUERTO RICO[5]						
		Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	TOTAL	Leaf	Berry	Crown	Peduncle	Seed	Stem	Root	TOTAL	Leaf	Berry	Crown	Peduncle	Seed	TOTAL	Leaf	Berry	Crown	Peduncle	Seed	TOTAL	SUM
Xylariaceae sp. 21	EU010002								0			1[1]					1						0						0	1
Xylariaceae sp. 22	EU009993								0	2[j]		1[d]					3						0						0	3
Xylariaceae sp. 23	EU009991	1							1								0						0						0	1
Xylariaceae sp. 24	EU009990								0	1[g]							1						0						0	1
Xylariaceae sp. 25	EU009988								0			1[d]					1						0						0	1
Xylariaceae sp. 26	EU009986								0	1[cq]			1[cq]				2						0						0	2
Xylariales sp. 1	EU009994	1							1								0						0						0	1
Xylariales sp. 2	EU009996								0				1[d,l]				1						0						0	1
TOTAL		108	16	61	27	6	45	4	267	245	15	25	56	15	15	22	393	18	21	30	14	26	109	7	19	32	13	3	74	843

1. In most cases, only one sequence was deposited for all isolates that had identical sequences (genotypes). Number in parenthesis following GenBank is NRRL accession #
2. All Colombia isolates originate from Caldas, Chinchiná (N 5°00', W 75°36')
3. Hawaii locations: a. Kauai Coffee Co., Kauai, N 21°53'53", W 159°33'30"; b. Kona Coffee Co., Holualoa, Kona, Hawaii, N 19°32.020', W 155°55.746'; c. Howard Yamasaki Farm, Kona, Hawaii, N 19°25.976', W 155°52.902'; d. Kona Experiment Station, Kona, Hawaii, N 19°32.048', W 155°55.494'; e. Dragons Lair Coffee Farm, Kona, Hawaii, N 19°25.536', W 155°52.829'; f. Greenwell Coffee, Kona, Hawaii, N 19°30.673', W 155°55.308'; g. Kunia Field Station, Oahu, N 21°23.255', W 158°02.113'; h. Waimea Arboretum, Haleiwa, Oahu, N 21°37.834', W 158°02.877'; i. Lyon Arboretum, Manoa, Oahu, N 21°20.035', W 157°48.228'; j. Foster Arboretum, Honolulu, Oahu, N 21°18.959', W 157°51.444'
4. Mexico locations: k. Cacahuatan, Chiapas, N 15°00.330', W 092°09.994'; l. Rancho El Paraiso, Cacahoatan, Chiapas, N 15°00.276", W 92°09'51.2"; m. Finca Hamburgo, Chiapas, N 15°10.388', W 092°19.535'; n. Rosario Izapa, Chiapas, N 14°57.998', W 092°09.130'; o. Tapachula, Chiapas, N 14°57.697', W 092°15.227'; p. Unión Juárez, Chiapas, N 15°03.457', W 092°04.443'
5. All Puerto Rico isolates originate from the Estación Experimental Agrícola, Adjuntas (N 18°10.204', W 066°47.685')
6. Also isolated from: q. *Coffea liberica*; r. *C. macrocarpa*; s. *C. canephora*; t. *C. congensis*; u. *C. racemosa*; v. *C. stenophylla*

considered to belong to that family. Sequences that shared lower identity with members of several families within a single order were identified only at the ordinal level. A few sequences sharing less than 85 % identity with other sequences from multiple orders, or sharing higher identity to unidentified sequences in GenBank, were identified only to class or phylum.

Results and discussion

Despite the fact that the methodology used here is culture-dependent and slow growing and nonculturable taxa are unlikely to be isolated (Hyde & Soytong 2008), a high diversity of fungal endophytes was obtained. Various coffee plant tissues served as suitable substrata for a wide number of Ascomycota and a few Basidiomycota (Fig 1). A total of 843 fungal endophytes was isolated: 267 from Colombia (32 %); 393 from Hawai'i (46 %); 109 from Mexico (13 %); and 74 from Puerto Rico (9 %) (Table 1, Fig 1). These resulted in 257 unique ITS sequences, yielding the following number of genotypes for each region: Colombia, 113; Hawai'i, 126; Mexico, 32; and Puerto Rico, 40. The most common endophytes were species of *Colletotrichum* (251 isolates yielding 40 genotypes), *Fusarium* (177 isolates yielding 25 genotypes), *Penicillium* (128 isolates yielding 14 genotypes and 11 species) and Xylariaceae (62 isolates yielding 26 genotypes).

All endophytes recovered from *Coffea* spp. were members of the Dikarya. Eighty-seven percent of endophyte genotypes recovered here represented the Ascomycota, and were distributed throughout the Pezizomycotina. The majority represented the Sordariomycetes (Hypocreales, Phyllachorales, Diaporthales, Sordariales, and Xylariales), Eurotiomycetes (Eurotiales), and Dothideomycetes (Pleosporales) (Fig 2). The dominance of Sordariomycetes in these primarily tropical and near-tropical samples corroborates the findings of Arnold & Lutzoni (2007), who found that Sordariomycetes dominated the endophyte communities of tropical plants. However, the present study recovered a greater representation of Eurotiomycetes than other surveys of tropical plants (e.g., Lodge *et al.* 1996; Arnold *et al.* 2003), suggesting that *Coffea* spp. may be an important reservoir for the often ecologically and economically important Eurotiales. The most common endophyte was *Colletotrichum*; it has also been reported as the most common endophyte in tropical *Theobroma cacao* (Arnold *et al.* 2003), and is a commonly isolated endophytic genus in the tropics, having been reported in *Amomum siamense*, *Anacardium occidentale*, *Citrus* spp., *C. arabica*, *Euterpe oleracea*, *Glycine max*, *Guarea guidonia*, *Ilex paraguariensis*, *Malus domestica*, *Musa acuminata*, *Himatanthus sucuuba*, *Palicourea longiflora*, *Strychnos cogens*, *Spondias mombin*, *Zea mays*, and other species (Bussaban *et al.* 2001; Gamboa & Bayman 2001; Lumyong *et al.* 2002; Peixoto Neto *et al.* 2002; Arnold *et al.* 2003; Photita *et al.* 2004; Camatti-Sartori *et al.* 2005; Rubini *et al.* 2005; Arnold 2007; Huang *et al.* 2008).

Overall, 34 of the 257 genotypes recovered (13 %) represented Basidiomycota, but these were distributed across all three subphyla – Pucciniomycotina, Ustilaginiomycotina (Exobasidiomycetes: Exobasidiales and Tilletiales; and Ustilaginomycetes: Ustilaginales) and Agaricomycotina (Tremellomycetes: Tremellales; and Agaricomycetes: Agaricales and *incertae sedis*), with the majority belonging to the Agaricomycetes (Fig 2).

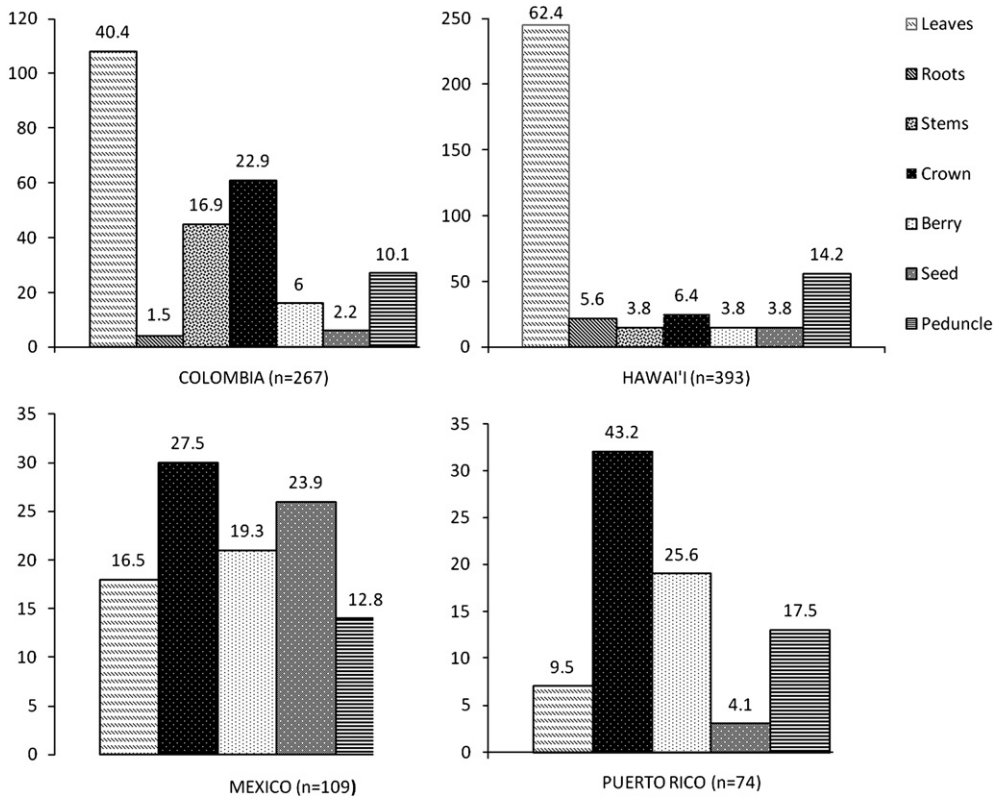

Fig 1 – Total number of fungal endophytes isolated in each country grouped by coffee plant tissues from which they were isolated. Numbers above bar represent percentages.

Although endophytic basidiomycetes are relatively rarely reported (Petrini 1986, Petrini *et al.* 1992), some have been recovered from temperate trees (e.g., *Marasmius*, *Rhizoctonia*, *Rhodotorula*; Petrini & Müller 1979; Widler & Müller 1984; Sieber-Canavesi & Sieber 1987, Arnold *et al.* 2007) and tropical trees (e.g., *Coprinellus*, *Fomitopsis*, *Phanerochaete*, *Pycnoporus*, *Schizophyllum*, *Sebacina*; Crozier *et al.* 2006; Rungjindamai *et al.* 2008; Tao *et al.* 2008).

Genotypic richness and diversity differed among countries, ranging from 32 genotypes among 109 isolates in Mexico

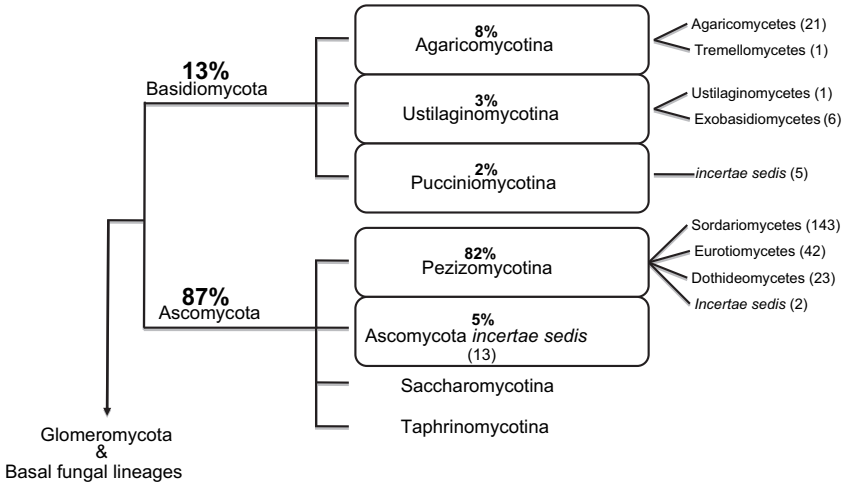

Fig 2 – Schematic representation of phylogenetic placement of 257 fungal genotypes derived from ITS sequences of endophytes isolated from coffee plants in Colombia, Hawai'i, Mexico, and Puerto Rico. Classification follows Hibbett *et al.* (2007). All 257 genotypes represent the crown fungal group Dikarya (Basidiomycota plus Ascomycota). Isolates were distributed among all three subphyla of Basidiomycota, but in only one of the three subphyla of Ascomycota (Pezizomycotina). Percentages indicate the total number of genotypes out of 257 for indicated group; total number of unique genotypes per taxonomic group is indicated in parentheses.

(Fisher's alpha = 14.9) to 113 genotypes among 267 isolates in Colombia (Fisher's alpha = 75.3) (Table 2). Mean diversity of fungi recovered from *Coffea* tissues in Puerto Rico, Hawai'i, and Colombia was significantly greater than in Mexico (Table 3). Inclusion of additional *Coffea* spp. in Hawai'i did not notably increase diversity of fungi recovered there, as all genotypes recovered from other *Coffea* species also were found in *C. arabica* (Table 1). Moreover, inclusion of multiple sites within a country (e.g., Mexico) did not strongly increase diversity relative to countries with only one thoroughly sampled site (e.g., Colombia) (Tables 1, 2). Although Fisher's alpha is robust to differences in sample size, more isolates from Mexico and Puerto Rico, sampling the same tissues in each country, and explicit evaluation of local microclimate conditions – which can strongly affect endophyte diversity (e.g., Hoffman & Arnold 2007) – would increase our confidence in the observed differences in diversity among countries.

Overall, only 107 genotypes (41.6 %) were recovered more than once. Of these, 70 genotypes (65.4 %) were found in only one country. Among the remaining 37 genotypes, 25 were found in two countries, 10 were found in three countries, and two were found in all four countries (Table 4). Genotypes found in three or more countries represented only three genera (*Colletotrichum*, seven genotypes; *Fusarium*, three genotypes; *Penicillium*, two genotypes). These commonly isolated genera also included genotypes found in two countries (*Colletotrichum*, five genotypes; *Fusarium*, three genotypes; *Penicillium*, six genotypes); genotypes found in only one country (*Colletotrichum*, 11 genotypes; *Fusarium*, 14 genotypes; and *Penicillium*, four genotypes). Genotypes that were found in at least two countries represented *Aspergillus*, *Beauveria*, *Botryosphaeria*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Mycosphaerella*, *Neosartorya*, *Paecilomyces*, *Penicillium*, *Phomopsis*, *Trametes* and an unidentified agaricomycete (Table 4). In contrast, none of the clavicipitaceous, pleosporaceous, xylariaceous genotypes was found in more than one country, and several genera were found only in one country's coffee plants (e.g., *Clonostachys*, *Petriella*, *Tilletia*, *Trichoderma*; Table 1).

Despite the occurrence of some genotypes in multiple sites, similarity indices based on presence/absence and abundance data showed low similarity among the fungal communities recovered in different countries. Hawai'i and Puerto Rico shared the fewest genotypes (JI = 0.092, SO = 0.169), whereas Colombia and Hawai'i (JI = 0.226, SO = 0.368) and Colombia and Mexico (JI = 0.210, SO = 0.347) shared the most genotypes (Table 5). These results were partially corroborated by abundance data, which showed the greatest similarity between fungal assemblages in Colombia and Hawai'i (MH = 0.302) and Colombia and Mexico (MH = 0.299; Table 5). Genotypes shared between Colombia and Hawai'i represented *Aspergillus*, *Beauveria*, *Botryosphaeria*, *Cladosporium*, *Neosartorya*, *Trametes*, and several genotypes of *Colletotrichum*, *Fusarium* and *Penicillium* (Tables 1, 4). Genotypes shared between Colombia and Mexico represented *Paecilomyces* and the most widely distributed genotypes of *Colletotrichum*, *Fusarium*, and *Penicillium* (Tables 1, 4).

The reasons underlying the especially wide distribution of some *Colletotrichum*, *Fusarium*, and *Penicillium* genotypes recovered here remain to be explored. It is possible that these genotypes are ubiquitous among coffee-growing regions because of intrinsic factors (i.e., global distribution of the fungi themselves) or because of the movement of *Coffea* plants and seeds, and their attendant endophytes, among coffee-growing regions. Notably, 55 % of genotypes recovered from seeds were found in multiple countries (Table 1). These data may hint that some genotypes were distributed with seeds to new locations, but this hypothesis needs further in-depth research. The occurrence of some genotypes in leaves and other tissues from multiple countries leaves open the possibility of global distributions of fungi. Future studies would benefit from a phylogenetic perspective that could explicitly trace the biogeographic or colonization patterns of particular endophytic lineages.

When Hawai'ian endophytes are divided by islands of origin (i.e., Oahu, Hawai'i, and Kauai) only 15 genotypes were shared by two islands. Of these, 14 were shared between Oahu and Hawai'i (Table 1). Only three endophytes (*Colletotrichum* sp. 24, *Colletotrichum* sp. 28, and *Penicillium olsonii*) were shared among the three islands. We found no evidence that endophyte communities differed markedly among *Coffea* species (Table 1), suggesting that endophyte communities are influenced more by site than by plant host species *per se*. This site-specific trend has been previously reported (Petrini 1985; Herre *et al.* 2005; Santamaría & Bayman 2005; see also Hoffman & Arnold 2007). In another perennial tropical crop, *T. cacao*, Arnold *et al.* (2003) reported a reduction in similarity among endophytes as distance between sampling sites increased >50 km. Santamaría & Bayman (2005) also reported significant differences in coffee fungal endophytes in Puerto Rico across different sites.

Table 2 – Sampling effort, genotypic richness, total diversity (Fisher's alpha), and dominant genera and genotypes of fungal endophytes recovered from surface-sterilized coffee tissues in Colombia, Hawai'i, Mexico, and Puerto Rico

Country	Isolates sequenced	Genotypes recovered	Diversity (Fisher's alpha)	Dominant genus (isolates, genotypes)	Dominant genotype (isolates, tissue type)[a]
Colombia	267	113	75.3	*Colletotrichum* (106, 25)	*Colletotrichum* sp. 2 (25; L,C,P,St)
Hawai'i	393	126	64.0	*Colletotrichum* (121, 20)	*Penicillium olsonii* (32; L,B,C,P,Se)
Mexico	109	32	14.9	*Fusarium* (68, 8)	*Fusarium* sp. 16 (48; L,B,C,P,Se)
Puerto Rico	74	40	35.6	*Fusarium* (24, 8)	*Fusarium* sp. 18 (8; B,C); *Fusarium* sp. 2 (8; B,C,P,Se)
Total	843	257	125.9	*Colletotrichum* (251, 40)	*Fusarium* sp. 16 (64; L,B,C,P,Se,St,R)

a Tissue types: L, leaf; B, berry; C, crown; P, peduncle; Se, seed; St, stem; R, root.

Table 3 – Mean and standard error of the mean for diversity (Fisher's alpha) of fungal endophytes as a function of tissue type (panel A) and country (panel B). Within each panel, means with the same numerical subscripts do not differ significantly

Tissue type	Countries[a]	Fisher's alpha[b]	Standard error of mean
A			
Berry	4	23.5	9.6
Crown	3	16.5	6.7
Leaf	3	31.1	8.6
Peduncle	4	27.6	10.1
Root	1	13.5	NA
Seed	2	4.6	2.2
Stem	2	17.0	3.2
B			
Country	Tissues[a]	Fisher's alpha[c]	Standard error of mean
Colombia	5	27.7	4.8
Hawai'i	6	22.6	6.3
Mexico	5	5.2	2.9
Puerto Rico	3	34.3	8.5

a Data are included if ≥10 isolates were recovered and sequenced from a given tissue in a given country. Data from crown samples in Hawai'i were excluded because the diversity value, reflecting the recovery of 24 genotypes from 25 crown samples, was more than three standard deviations greater than the mean.
b Statistical analyses reflect ANOVA with alpha = 0.05, followed by post-hoc comparisons.
c Statistical analyses reflect ANOVA with alpha = 0.05, followed by post-hoc comparisons; $F_{3,15} = 4.4682$, $P = 0.0197$.

Even though it has been suggested that endophytes in the leaves of woody plants are acquired from air spora in the environment (Petrini 1991; Arnold & Herre 2003), our results indicate that vertical transmission might also be possible based on the isolation of several endophytic fungi from coffee seeds in all the countries sampled (Table 1) as well as from green coffee seeds examined in a previous study (Vega *et al.* 2008a). Endophytic fungi have been reported in seeds of *Pseudotsuga menziesii* (Bloomberg 1966), *Casuarina equisetifolia* (Bose 1947), and *Cecropia* spp. (Gallery *et al.* 2007). All genotypes recovered from seeds in our study were also found as endophytes in other tissues (Table 1).

In the present study diversity of fungi associated with different tissue types did not differ significantly (ANOVA; $F_{6,12} = 0.7746$, $P = 0.6048$), although diversity was nearly sevenfold higher in leaves (Fisher's alpha mean ± SEM = 31.1 ± 8.6) than in seeds (Fisher's alpha = 4.6 ± 2.2) (Table 3). The vast majority of nonsingleton genotypes (75 genotypes, or 70 % of those found more than once) were recovered from more than one tissue type (e.g., leaves, stems, roots); the remaining 32 nonsingleton genotypes were found in only one tissue type. Among the genotypes found only in one tissue type, 15 were found only in leaves, three only from berries, four only from crowns, five only from peduncles, two only from roots, and three only from stems (Table 6).

Most of those single-tissue genotypes (28 of 32) were isolated from a given tissue type in only one country. However, four genotypes found only in leaves were recovered from foliage in multiple countries (Table 6). These fungi may be especially interesting for further study: on the one hand, they may have global distributions and simply represent opportunistic infections by generalist fungi. Alternatively, they may represent closely co-evolved endophytes of *Coffea* that have moved with plants across the coffee-growing regions of the world.

In several cases, endophytes associated with only one tissue type in one locality were subsequently found in additional tissue types in other countries. For example, *Colletotrichum* sp. 2 was recovered from leaves in Mexico, but from crown, peduncle, stem, and leaf tissue in Colombia (Table 1). In several cases, genotypes that would have been assigned to singleton genotypes in survey of only one site or tissue type were shown to be quite common in other tissues or sites, underscoring the need to broadly sample different tissues and sites to adequately address the frequency and tissue specificity of endophytic fungi (Table 1).

Overall, 63 of the 257 unique genotypes (including singletons) were isolated only from leaves. It is likely that these fungi, as has been reported for woody endophytes in general, do not have the capacity to move through the plant and only occur locally relative to the point of entrance (see Saikkonen *et al.* 1998, Herre *et al.* 2007). The fungal species found in leaf tissue, like most tissues, could reflect the prevalence of aerial spores at a particular site, the ability of spores of individual species to grow into the leaf, and presence of favorable conditions that allow ambient microfungi to enter internal plant tissues.

Widler & Müller (1984), in what to our knowledge is the most comprehensive analysis of fungal endophytes in one plant species, reported more than 190 different fungal endophytes in the leaves, roots, and branches of *Arctostaphylos uva-ursi* in two locations in Switzerland. Our coffee survey surpasses this figure, with a total of 257 genotypes recovered. While these genotypes do not correspond to species – indeed, they overestimated species boundaries for *Penicillium* – our data provide a fine scale of resolution for determining the occurrence of endophytes among different tissue types, *Coffea* spp., and geographic regions.

The high biodiversity of fungal endophytes in coffee plants may indicate that most of these are ''accidental

Table 4 – Number of isolates of fungal endophyte genotypes that were recovered from at least two countries among the four sampled (Colombia, Hawai'i, Mexico, and Puerto Rico)

Genotype	Colombia	Hawai'i	Mexico	Puerto Rico
Agaricomycetes sp. 1		1		1
Aspergillus sp. 3		1	1	
Aspergillus pseudodeflectus	1	3		
Beauveria bassiana	12	1		
Botryosphaeria sp.	2	2		
Cladosporium sp. 3	4	1		
Colletotrichum sp. 2	25	6	7	
Colletotrichum sp. 4		3		1
Colletotrichum sp. 5	1	1		
Colletotrichum sp. 7	1			2
Colletotrichum sp. 11	2			1
Colletotrichum sp. 20	1	1	1	
Colletotrichum sp. 21	9	3		4
Colletotrichum sp. 23	2	3	1	
Colletotrichum sp. 24	12	30	1	
Colletotrichum sp. 25	10	3	1	
Colletotrichum sp. 28		31	1	
Colletotrichum sp. 29	4	7	1	
Fusarium sp. 2	1			8
Fusarium sp. 4			1	1
Fusarium sp. 6			1	1
Fusarium sp. 13	2	5	2	
Fusarium sp. 16	13	3	48	
Fusarium sp. 18	13	7	5	8
Mycosphaerella sp.		4		1
Neosartorya sp.	1	1		
Paecilomyces sp. 2	1		1	
Penicillium sp. 1	1		8	
Penicillium sp. 2	1			1
Penicillium sp. 9	12	7	1	3
P. brevicompactum	3	20		
P. brocae	1	2		
P. crustosum	3		1	
P. olsonii	1	32		4
P. sumaetrense			1	1
Phomopsis sp. 17		2	1	
Trametes sp.	3	1		

A total of 843 endophytes was isolated, from which 257 unique ITS sequence genotypes were identified; of these, 220 genotypes were only found in one country; 25 genotypes were shared by two countries; 10 genotypes were shared by three countries; and two genotypes were shared by four countries.

Table 5 – Number of nonsingleton genotypes (genotypes that occurred more than once), percent of all genotypes occurring more than once, and similarity of fungal communities recovered from coffee tissues in Colombia, Hawai'i, Mexico, and Puerto Rico, considering presence/absence data (Jaccard's index, JI; Sørensen's index, SO) and abundance data (Morisita–Horn index, MH)

Country 1	Country 2	Nonsingleton genotypes country 1 (%)	Nonsingleton genotypes country 2 (%)	JI	SO	MH
Colombia	Hawai'i	52 (46.0 %)	62 (49.2 %)	0.226	0.368	0.302
Colombia	Mexico	52 (46.0 %)	23 (71.8 %)	0.210	0.347	0.299
Colombia	Puerto Rico	52 (46.0 %)	21 (52.5 %)	0.123	0.219	0.277
Hawai'i	Mexico	62 (49.2 %)	23 (71.8 %)	0.181	0.306	0.064
Hawai'i	Puerto Rico	62 (49.2 %)	21 (52.5 %)	0.092	0.169	0.202
Mexico	Puerto Rico	23 (71.8 %)	21 (52.5 %)	0.128	0.227	0.050

Approximately half of all genotypes found in Colombia, Hawai'i, and Puerto Rico were found only once; in contrast, the majority of genotypes recovered in Mexico were found more than once.

Table 6 – Fungal endophytes of *Coffea* spp. recovered from only one tissue type: genotype identification, tissue, and country of origin

Genotype	Tissue	Countries of origin
Aspergillus fumigatus	Leaf	Colombia
Aspergillus sp. 4	Stem	Colombia
Aspergillus westerdijkiae	Peduncle	Hawai'i
Beauveria sp.	Leaf	Colombia
Cladosporium sp. 5	Leaf	Hawai'i
Clavicipitaceae sp.	Berry	Puerto Rico
Clonostachys cf. *rosea*	Leaf	Colombia
Colletotrichum sp. 5	Leaf	Colombia, Hawai'i
Colletotrichum sp. 12	Crown	Colombia
Colletotrichum sp. 16	Leaf	Colombia
Colletotrichum sp. 20	Leaf	Colombia, Hawai'i, Mexico
Colletotrichum sp. 22	Stem	Colombia
Colletotrichum sp. 29	Leaf	Colombia, Hawai'i, Mexico
Colletotrichum sp. 30	Leaf	Hawai'i
Colletotrichum sp. 32	Leaf	Hawai'i
Colletotrichum sp. 36	Leaf	Hawai'i
Colletotrichum sp. 40	Leaf	Hawai'i
Fusarium sp. 19	Root	Hawai'i
Fusarium sp. 5	Peduncle	Mexico
Fusarium sp. 7	Crown	Puerto Rico
Fusarium sp. 9	Peduncle	Mexico
Neosartorya sp.	Leaf	Colombia, Hawai'i
Penicillium citrinum	Peduncle	Hawai'i
Penicillium coffeae	Peduncle	Hawai'i
Penicillium steckii	Berry	Hawai'i
Petriella sp.	Root	Colombia
Phomopsis sp. 13	Crown	Colombia
Tilletia sp. 1	Berry	Colombia
Trichoderma sp. 1	Stem	Colombia
Xylariaceae sp. 15	Leaf	Hawai'i

tourists'' with no role in the plant, in contrast to endophytes that could be defined as ''influential passengers'' and whose role on the plant has been elucidated. Using ribosomal DNA sequence comparisons, Promputtha *et al.* (2007) suggested that endophytic *Colletotrichum*, *Fusarium*, and other taxa in *Magnolia liliifera* can change their lifestyle and become saprotrophic after host senescence. Such ''lifestyle switching'' (Rodriguez & Redman 2005) might help explain the possible roles of some fungal endophytes. Similarly, most members of the Xylariaceae (Ascomycota: Sodariomycetes) are considered to be saprotrophs (Petrini & Petrini 1985; Weber & Anke 2006), however, they are especially common as endophytes of tropical hosts (Gamboa & Bayman 2001; Peixoto Neto *et al.* 2002; Takeda *et al.* 2003; Tomita 2003; Crozier *et al.* 2006) and it is possible that these play a saprotrophic role in coffee and other plants after host senescence. The ecological roles of *Penicillium* species remain to be explored and represent an area of special interest to us for future studies. However, experimental trials are needed to confirm the ecological roles in living plants, or lack thereof, of the many fungi recovered here (see Saikkonen *et al.* 2006 for possible roles). Notably, Arnold *et al.* (2003) showed that resistance of *T. cacao* seedlings to invasion by a virulent pathogen (*Phytophthora* sp.) occurred in the presence of multiple endophyte species in the same leaf tissues. Similarly, Arnold & Lewis (2005) reviewed several cases in which the entomopathogen *B. bassiana* was able to protect host plants against a significant herbivore even in the context of additional fungal inhabitants of the same plants.

The large number of singletons recovered here suggests that we have barely scratched the surface of the diversity of endophytes associated with *Coffea*. In particular, sampling these plants in their biogeographic regions of origin would elucidate the ways in which introduction to novel environments change the fungal communities with which economically important plants associate. Hoffman & Arnold (2007) showed that trees in the Cupressaceae, when cultivated in non-native environments, maintained a lower diversity of fungi than did closely related, native species. Moreover, the introduced species examined in that study consistently harbored more cosmopolitan, less-specific endophytes than did their native relatives in the same environments. The movement of *Coffea* throughout the coffee-growing regions of the world provides a useful framework for addressing similar questions in an economically important plant in the fungus-rich tropics. Furthermore, if other molecular techniques (e.g., DNA cloning: Guo *et al.* 2000, 2001; Seena *et al.* 2008; denaturing gradient gel electrophoresis (DGGE): Nikolcheva *et al.* 2003; Nikolcheva and Bärlocher 2004, 2005; Duong *et al.* 2006; Tao *et al.* 2008; or terminal-restriction fragment length polymorphism (T-RFLP): Nikolcheva *et al.* 2003; Nikolcheva & Bärlocher 2005) were applied to identify fungal DNA from leaves or other parts of the coffee plant, many slow growing or unculturable fungi could be identified. A microarray

hybridization technique known as PhyloChip, currently used for the identification of archaeal and bacterial organisms (Brodie *et al.* 2007; DeSantis *et al.* 2007) is currently being developed for the identification of fungal diversity (M. Blackwell, pers. comm.). This technique will greatly enhance our understanding of fungal endophyte communities. We expect that many more fungal endophytes in coffee remain to be identified. Future research will focus on fungal endophyte biodiversity in Africa, and on the potential applications of these phylogenetically diverse and species-rich fungal associates of *Coffea* plants.

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REFERENCES

Arnold AE, 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biology Reviews* **21**: 51–66.

Arnold AE, Henk DA, Eells RL, 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.

Arnold AE, 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.

Arnold AE, Lewis LE, 2005. Ecology and evolution of fungal endophytes and their roles against insects. In: Vega FE, Blackwell M (eds), *Insect-Fungal Associations: Ecology and Evolution*. Oxford University Press, New York, pp. 74–96.

Arnold AE, Lutzoni F, 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* **88**: 541–549.

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA, 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 15649–15654.

Bloomberg WJ, 1966. The occurrence of endophytic fungi in Douglas fir seedlings and seeds. *Canadian Journal of Botany* **44**: 413–420.

Bose SR, 1947. Hereditary (seed-borne) symbiosis in *Casuarina equisetifolia*. *Nature* **159**: 512–514.

Brodie EL, DeSantis TZ, Moberg Parker JP, Zubietta IX, Piceno YM, Andersen GL, 2007. Urban aerosols harbor diverse and dynamic bacterial populations. *Proceedings of the National Academy of Sciences of the Unites States of America* **104**: 299–304.

Bussaban B, Lumyong S, Lumyong P, McKenzie EHC, Hyde KD, 2001. Endophytic fungi from *Amomum siamense*. *Canadian Journal of Microbiology* **47**: 943–948.

Camatti-Sartori V, da Silva-Ribeiro RT, Valdebenito-Sanhueza RM, Pagnocca FC, Echeverrigaray S, Azevedo JL, 2005. Endophytic yeasts and filamentous fungi associated with southern Brazilian apple (*Malus domestica*) orchards subjected to conventional, integrated or organic cultivation. *Journal of Basic Microbiology* **45**: 397–402.

Crozier J, Thomas SE, Aime MC, Evans HC, Holmes KA, 2006. Molecular characterization of fungal endophytic morphospecies isolated from stems and pods of *Theobroma cacao*. *Plant Pathology* **55**: 783–791.

Davis AP, Govaerts R, Bridson DM, Stoffelen P, 2006. An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). *Botanical Journal of the Linnean Society* **152**: 465–512.

DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL, 2007. High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. *Microbial Ecology* **53**: 371–383.

Duong LM, Jeewon R, Lumyong S, Hyde KD, 2006. DGGE coupled with ribosomal DNA phylogenies reveal uncharacterized fungal phylotypes. *Fungal Diversity* **23**: 121–138.

Freeman S, Rodriguez RJ, 1993. Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. *Science* **260**: 75–78.

Gallery RE, Dalling JW, Wolfe B, Arnold AE, 2007. Role of seed-infecting fungi in the recruitment limitation of neotropical pioneer species. In: Dennis A, Green R, Schupp E, Westcott D (eds), *Seed Dispersal: Theory and its Application in a Changing World*. CABI Press, Wallingford, pp. 479–498.

Gamboa MA, Bayman P, 2001. Communities of endophytic fungi in leaves of a tropical timber tree (*Guarea guidonia*: Meliacea). *Biotropica* **33**: 352–360.

Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Guo LD, Hyde KD, Liew ECY, 2000. Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. *New Phytologist* **147**: 617–630.

Guo LD, Hyde KD, Liew ECY, 2001. Detection and taxonomic placement of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequences. *Molecular Phylogenetics and Evolution* **20**: 1–13.

Herre EA, Mejía LC, Kyllo DA, Rojas E, Maynard Z, Butler A, Van Bael SA, 2007. Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. *Ecology* **88**: 550–558.

Herre EA, Van Bael SA, Maynard Z, Robbins N, Bischoff J, Arnold AE, Rojas E, Mejia LC, Cordero RA, Woodward C, Kyllo DA, 2005. Tropical plants as chimera: some implications of foliar endophytic fungi for the study of host-plant defence, physiology, and genetics. In: Burslem DFRP, Pinard MA, Hartley SE (eds), *Biotic Interactions in the Tropics: Their Role in the Maintenance of Species Diversity*. Cambridge University Press, Cambridge, pp. 226–237.

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Canon PF, Eriksson OE, *et al.*, 2007. A higher-level phylogenetic classification of the fungi. *Mycological Research* **111**: 509–547.

Hoffman M, Arnold AE, 2007. Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. *Mycological Research* **112**: 331–344.

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M, 2008. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity* **33**: 61–75.

Hyde KD, Soytong K, 2008. The fungal endophyte dilemma. *Fungal Diversity* **33**: 163–173.

Lewin B, Giovannucci D, Varangis P, 2004. *Coffee Markets: New Paradigms in Global Supply and Demand*. Agricultural and Rural Development Discussion Paper No. 3. International Bank for Reconstruction and Development, Washington D.C., USA.

Lodge DJ, Fisher PJ, Sutton BC, 1996. Endophytic fungi of *Manilkara bidentata* leaves in Puerto Rico. *Mycologia* **88**: 733–738.

Lumyong S, Lumyong P, McKenzie EHC, Hyde KD, 2002. Enzymatic activity of endophytic fungi of six native species from Doi Suthep-Pui National Park, Thailand. *Canadian Journal of Microbiology* **48**: 1109–1112.

Muller RA, Berry D, Avelino J, Bieysse D, 2004. Coffee diseases. In: Wintgens JN (ed), *Coffee: Growing, Processing, Sustainable Production*. Wiley-VCH, Weinheim, pp. 491–545.

Nikolcheva LG, Bärlocher F, 2004. Taxon-specific fungal primers reveal unexpectedly high diversity during leaf decomposition in a stream. *Mycological Progress* **3**: 41–49.

Nikolcheva LG, Bärlocher F, 2005. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. *Environmental Microbiology* **7**: 270–280.

Nikolcheva LG, Cockshutt AM, Bärlocher F, 2003. Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. *Applied and Environmental Microbiology* **69**: 2548–2554.

Osorio N, 2002. *The Global Coffee Crisis: a Threat to Sustainable Development*. International Coffee Organization, London.

Peixoto Neto PA de S, Azevedo JL, Araújo WL, 2002. Microrganismos endofíticos. *Biotecnologia Ciência & Desenvolvimiento* **29**: 62–77.

Perfecto I, Armbrecht I, Philpott S, Soto Pinto L, Dietsch TV, 2007. Shade coffee and the stability of forest margins in Northern Latin America. In: Tscharntke T, Zeller M, Leuschner C (eds), *The Stability of Tropical Rainforest Margins: Linking Ecological, Economic and Social Constraints*. Springer-Verlag, Berlin, pp. 225–261.

Peterson SW, Vega FE, Posada F, Nagai C, 2005. *Penicillium coffeae*, a new endophytic species isolated from a coffee plant and its phylogenetic relationship to *P. fellutanum*, *P. thiersii* and *P. brocae* based on parsimony analysis of multilocus DNA sequences. *Mycologia* **97**: 659–666.

Petrini O, 1985. Wirtsspezifität endophytischer Pilze bei einheimischen Ericaceae. *Botanica Helvetiva* **95**: 213–238.

Petrini O, 1986. Taxonomy of endophytic fungi in aerial plant tissues. In: Fokkema NJ, van den Heuvel J (eds), *Microbiology of the Phyllosphere*. Cambridge University Press, Cambridge, pp. 175–187.

Petrini O, 1991. Fungal endophytes in tree leaves. In: Andrews JH, Hirano SS (eds), *Microbial Ecology of Leaves*. Springer-Verlag, New York, pp. 179–197.

Petrini O, Müller E, 1979. Pilzliche Endophyten, am Beispiel von *Juniperus communis* L. *Sydowia* **32**: 224–251.

Petrini L, Petrini O, 1985. Xylariaceous fungi as endophytes. *Sydowia* **38**: 216–234.

Petrini O, Sieber TN, Toti L, Viret O, 1992. Ecology, metabolite production, and substrate utilization in endophytic fungi. *Natural Toxins* **1**: 185–196.

Photita W, Lumyong S, Lumyong P, McKenzie EHC, Hyde KD, 2004. Are some endophytes of *Musa acuminata* latent pathogens? *Fungal Diversity* **16**: 131–140.

Posada F, Aime MC, Peterson SW, Rehner SA, Vega FE, 2007. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales). *Mycological Research* **111**: 749–758.

Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R, 2007. A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microbial Ecology* **53**: 579–590.

Rayner RW, 1948. Latent infection in *Coffea arabica* L. *Nature* **161**: 245–246.

Rehner SA, Buckley EP, 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**: 84–98.

Rodriguez RJ, Redman RS, 2005. Symbiotic lifestyle expression by fungal endophytes and the adaptation of plants to stress: unraveling the complexities of intimacy. In: Dighton J, Oudemans P, White J (eds), *The Fungal Community: Its Organization and Role in the Ecosystem*. CRC Press, Boca Raton, pp. 683–695.

Rubini MR, Silva-Ribeiro RT, Pomella AWV, Maki CS, Araujo WL, dos Santos DR, Azevedo JL, 2005. Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' broom disease. *International Journal of Biological Sciences* **1**: 24–33.

Rungjindamai N, Pinruan U, Choeyklin R, Hattori T, Jones EBG, 2008. Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*, in Thailand. *Fungal Diversity* **33**: 139–161.

Saikkonen K, Faeth SH, Helander ML, Sullivan TJ, 1998. Fungal endophytes: a continuum of interactions with host plants. *Annual Review of Ecology and Systematics* **29**: 319–343.

Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth SH, 2006. Model systems in ecology: dissecting the endophyte-grass literature. *Trends in Plant Sciences* **11**: 428–433.

Santamaría J, Bayman P, 2005. Fungal epiphytes and endophytes of coffee leaves (*Coffea arabica*). *Microbial Ecology* **50**: 1–8.

Schulz B, Boyle C, 2005. The endophytic continuum. *Mycological Research* **109**: 661–686.

Schulz B, Boyle C, 2006. What are endophytes? In: Schulz B, Boyle C, Sieber TN (eds), *Microbial Root Endophytes*. Springer, Berlin, pp. 1–13.

Seena S, Wynberg N, Bärlocher F, 2008. Fungal diversity during leaf decomposition in a stream assessed through clone libraries. *Fungal Diversity* **30**: 1–14.

Sieber-Canavesi F, Sieber TN, 1987. Endophytische Pilze in Tanne (*Abies alba* Mill.). Vergleich zweier Standorte im Schweizer Mittelland (Naturwald-Aufforstung). *Sydowia* **40**: 250–273.

Takeda I, Guerrero R, Bettucci L, 2003. Endophytic fungi of twigs and leaves from *Ilex paraguariensis* in Brazil. *Sydowia* **55**: 372–380.

Tao G, Liu ZY, Hyde KD, Liu XZ, Yu ZN, 2008. Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (Orchidaceae). *Fungal Diversity* **33**: 101–122.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC, 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* **31**: 21–32.

Tomita F, 2003. Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. *Fungal Diversity* **14**: 187–204.

Vega FE, Pava-Ripoll M, Posada F, Buyer JS, 2005. Endophytic bacteria in *Coffea arabica* L. *Journal of Basic Microbiology* **45**: 371–380.

Vega FE, Posada F, Aime MC, Peterson SW, Rehner SA, 2008a. Fungal endophytes in green coffee seeds. *Mycosystema* **27**: 74–83.

Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA, 2008b. Entomopathogenic fungal endophytes. *Biological Control* **46**: 72–82.

Vega FE, Posada F, Peterson SW, Gianfagna T, Chaves F, 2006. *Penicillium* species endophytic in coffee plants and ochratoxin A production. *Mycologia* **98**: 31–42.

Vilgalys R, 2003. Taxonomic misidentification in public databases. *New Phytologist* **160**: 4–5.

Weber RWS, Anke H, 2006. Effects of endophytes on colonization by leaf surface microbiota. In: Bailey MJ,

Lilley AK, Timms-Wilson TM, Spencer-Phillips PTM (eds), *Microbial Ecology of Aerial Plant Surfaces*. CABI, Wallingford, pp. 209–222.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds), *PCR Protocols: a Guide to Methods and Applications*. Academic, San Diego, pp. 315–322.

Widler B, Müller E, 1984. Untursuchungen über endophytische Pilze von *Arctostaphylos uva-ursi* (L.) Sprengel (Ericaceae). *Botanica Helvetica* **94**: 307–337.

Wilson D, 1995. Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos* **73**: 274–276.

Wintgens JN (ed), 2004. *Coffee: Growing, Processing, Sustainable Production*. Wiley-VCH, Weinheim.