Linking ex planta fungi with their endophytic stages: Perisporiopsis, a common leaf litter and soil fungus, is a frequent endophyte of Hevea spp. and other plants

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Introduction

Endophytes are microorganisms (e.g., fungi and bacteria) that live for all or part of their life cycle inside the tissues of aboveground plant parts without causing any visible sign of infection (Hyde & Soytong 2008). Of all the plants (and their organs) sampled until now, most have a high diversity of culturable and non-culturale endophytic fungi (Carroll 1988; Evans et al. 2003; Berg et al. 2005; Mahesh et al. 2005; Summerbell 2005a; Crozier et al. 2006; Samuels et al. 2006; Arnold et al. 2007; Baird et al. 2007; Gond et al. 2007; Verma et al. 2007). Studies have shown that individual plants may harbor several dozens of endophytic fungal species (Lodge et al. 1996; Arnold et al. 2000; Frohlich et al. 2000; Gazis & Chaverri 2010). This high diversity of endophytic fungi, in addition to the non-sporulating characteristic of many of them, poses a challenge for their identification.

It has been hypothesized that endophytic fungi originate from local environmental sources, and endophyte identity and composition can be predicted based on surrounding ex planta fungi. As part of a pilot study to characterize endophytes and ex planta fungi (e.g., saprotrophs) of wild Hevea trees, endophytes were isolated with almost identical ITS sequences, but these were unidentifiable using GenBank database. Along with the endophytes, ex planta fungi on Hevea leaves were also collected at the sampling sites. The objective of this study was to determine if selected unknown Hevea endophytes could be linked with the ex planta fungi using DNA sequences. Methods included BLAST searches, phylogenetic analysis, and morphological characterizations of the endophytes and saprotrophs. Results show that the BLAST-unidentifiable Hevea endophytes belong in the fungal genus Perisporiopsis (Parodiopsidaceae, Dothideomycetes, Ascomycota). In addition, this Hevea endophyte is at least 98% identical to >20 other sequences from GenBank, demonstrating that Perisporiopsis is a common endophyte from various plants, and a leaf litter and soil fungus. Connecting a DNA sequence with a name that has biological meaning enables microbial (i.e., fungal) biologists to make more accurate inferences about the functional ecology of a particular species in a community or ecosystem.

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such as ascospores or conidia) (Clay 1993; Saikkonen et al. 1998; Schulz & Boyle 2005). Endophytes of woody plants are thought to be transmitted horizontally due to their generalized absence from tree seeds (Espinosa-Garcia & Langenheim 1990; Faeth & Hammon 1997; Arnold et al. 2000; Boyle et al. 2001). Thus, infection by horizontally transmitted endophytes depends on the availability and viability of fungal propagules in the surrounding environment (Schulz & Boyle 2005). In addition, the life cycle of some fungi may include an endophytic stage, and thus, for example, some endophytes may become saprotrophs at host senescence or pathogens when the host becomes susceptible (Petri 1986; Saikkonen et al. 1998; Schulz & Boyle 2005; Hyde et al. 2007; Promputtha et al. 2007; Duong et al. 2008; Oses et al. 2008; Parfitt et al. 2010). Studies suggest that saprotrophic fungi having an endophytic stage may have an advantage over those that are exclusively saprotrophic because when the plant dies they are the first or “pioneer” colonizers of the decaying plant material (Müller et al. 2001; Oses et al. 2008; Parfitt et al. 2010).

As part of a pilot study to characterize endophytes and ex planta fungi (e.g. saprotrophs) of wild Hevea spp. trees (including Hevea brasiliensis, one of the source of natural rubber), several endophyte samples with almost identical ITS sequences were isolated. A BLAST search showed the Hevea endophyte sequences had 98% identity with 26 sequences in GenBank, most of them unidentified endophytes, soil fungi, and leaf litter fungi. When sampling for endophytes, some saprotrophs were also collected on decaying Hevea leaves. The objective of this study was to determine if it was possible to link the unknown Hevea endophytes with the ex planta fungi using DNA sequences. By identifying and naming the ex planta stage of the fungal endophyte, it will be possible to identify and name the unknown Hevea endophytes.

Materials and methods

Endophyte isolation

Living leaves and sapwood (cambium and vascular phloem) of ca. 50 wild H. brasiliensis and Hevea guianensis (Euphorbiaceae) trees were sampled in various sites in the Peruvian Amazon, i.e. Iquitos (Dpto. Loreto, Prov. Maynas), Tambopata (Dpto. Madre de Dios, Prov. Tambopata), Los Amigos (Dpto. Madre de Dios, Prov. Manú), and Iberia (Dpto. Madre de Dios, Prov. Tahuamanu). To isolate endophytes from leaves, three healthy leaflets from different parts of each tree were collected. In the field station, five segments from each leaflet were surface-sterilized through sequential immersion in 2% sodium hypochlorite (bleach), 70% ethanol, and sterilized water (Arnold et al. 2001, 2003). Each segment was placed in a 3 cm Petri plate containing BBL™ cornmeal-dextrose-agar plus 2% dextrose (CMD) supplemented with a 0.2% antibiotic solution (Sigma–Aldrich neomycin–streptomycin–penicillin). Endophytes from the living sapwood tissue were isolated using methods modified from Evans et al. (2003) and Kowalski & Kehr (1996). Three slivers of ca. 3 x 6 cm of dead bark were shaved from each tree in the field. Five pieces of ca. 5 mm of sapwood were cut from the exposed sapwood and transferred to 3 cm Petri plates containing CMD plus antibiotics. Petri plates were incubated for several days and then transported to the U.S. for analyses and purification. Once cultures were purified and transferred to clean plates, they were incubated at 25°C with alternating 12 hr darkness/12 hr fluorescent light. The pure cultures were then preserved in cryovials with 20% glycerol at −80°C at University of Maryland. The unknown endophytes with almost identical ITS sequences were isolated from the sapwood in Tambopata (PP 75, PP 114), and from leaves in Iquitos (IQ 232) and Iberia (IB 145b).

Saprotroph collection

Selected sexual fruit bodies (ascomata) on leaves were collected from two sites, i.e. Los Amigos and Tambopata, with specimen numbers P.C. 987 and P.C. 811, respectively. (Not all ascomata found were collected or processed; future studies will include an extensive collection of saprotrophs.) Fungal fruit bodies on decaying Hevea spp. leaves were collected near the base of Hevea trees and placed in brown paper bags. Specimens were air dried and placed in glassine bags within plastic Ziploc™ bags with silica gel for culturing in the United States. Although sexual spore (ascospore) germination was attempted by isolating them onto CMD supplemented with antibiotics (Sigma–Aldrich; streptomycin–neomycin–penicillin) (Chaverri & Samuels 2003), these did not germinate after 2 weeks. Specimens were preserved and labelled for accession in the U.S. National Fungus Collection (P.C. 987 = BPI 880186; P.C. 811 = BPI 880185).

Morphological characterization of the saprotrophs and endophytes

For morphological characterization of the sexual state (teleomorph), the macro- and micro-morphology of the sexual fruit bodies (ascomata) and spores (ascospores) were observed and described. To observe internal and microscopic characteristics, the ascomata were rehydrated briefly in KOH, then supported by Tissue-Tek O.C.T. Compound 4583 (Miles Inc., Elkhart, Indiana, U.S.A.), and sectioned at a thickness of ca. 15 µm with a freezing microtome. Characteristics of the ascospores were observed by rehydrating the ascomata in 3% potassium hydroxide (KOH), removing part of the center with a fine glass needle, and placing it on a glass slide. Measurements of continuous characters such as length and width were made using Scion Image software beta version 4.0.2 (Scion Corporation, Frederick, Maryland, U.S.A.). Continuous measurements were based on at least 30 measured units. Morphological observations of the colonies and asexual state (anamorph) of the endophytes in culture were based on isolates grown on Difco™ potato-dextrose-agar (PDA) for 2 weeks at 25°C with alternating 12 hr/12 hr fluorescent light/darkness. Color terminology is from Rayner (1970).

DNA extraction, PCR and sequencing

Cultures of the endophytes (PP 75, PP 114, IQ 232, IB 145b) were grown in 6 cm diam Petri dishes containing Difco™ potato-dextrose broth. Plates were incubated at 25°C for ca. 1 week. DNA was extracted from the mycelial mat harvested from the surface of the broth using the PowerPlant™ DNA Isolation Kit.
Partial large subunit (LSU) and complete internal transcribed spacers 1 and 2 sequences including 5.8S (ITS) of the nuclear ribosomal DNA were used in the analyses. The primers used for LSU were LROR and LRS (Vilgalys & Hester 1990); and for ITS were ITS 5 and ITS 4 (White et al. 1990). Each 50 μl PCR reaction consisted of 25 μl GoTaq® Green Master Mix (Promega Corporation, Madison, Wisconsin, U.S.A.), 2.5 μl 10 mM ITS 5, 2.5 μl 10 mM LRS, 1 μl of the DNA template, and 19 μl of sterile RNAase-free water. PCR reactions were run in an Eppendorf Mastercycler ep using the following parameters: (1) 94 °C for 5 min, (2) 35 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min, and (3) 72 °C for 5 min. PCR products were cleaned using ExoSAP-IT® (USB Corporation, Cleveland, Ohio, U.S.A.) following the manufacturers instructions. Clean PCR products were sequenced using ITS 5, ITS 4, LROR, and LRS primers at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, U.S.A.). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin, U.S.A.). Sequences have been deposited in GenBank (Table 1).

**Phylogenetic analysis**

Ninety-six ITS and 18 LSU sequences were analyzed, including Leptosphaeriaceae and Phaeosphaeriaceae sequences from GenBank (accession numbers are indicated in Figs 1 and 2). Several taxa in the Pleosporaceae and Botryosphaeriaceae were used as outgroups for the ITS and LSU trees, respectively. Sequences were aligned with MAFFT 5 (Katoh et al. 2005) using the E-INS-i strategy. The alignment was improved by hand with Seaview 2.4 (Galtier et al. 1996) and MESQUITE 2.5 (Maddison & Maddison 2009). Gaps (insertions/deletions) were treated as missing data. Neighbor-Joining (NJ) and Maximum Likelihood (ML) were performed with all sequences. jMODELTEST (Rannala & Yang 1996; Posada & Buckley 2004; Posada 2008) was used to select the models of nucleotide substitution for the ML analysis. Once the likelihood scores were calculated, the models were selected according to the Akaiae Information Criterion (AIC), and corrected for smaller samples (AICc). According to jMODELTEST, the general time reversible model with gamma distribution (GTR+G) best fit the sequence data for both LSU and ITS regions.

GARLI version 0.96 (Zwickl 2006) was used for the ML and bootstrap analyses. To search for the best tree in GARLI, the starting tree was obtained by random stepwise-addition, the number of independent searches was set to 10, and the number of generations was set to 20 000. For the bootstrap analysis (BP), the starting tree was obtained randomly, was replicated 1 000 times, and the consensus of the resulting bootstrap trees was calculated in PAUP® version 4.0a109 (Swoford 2002). PAUP® was used for the NJ analysis and the tree search was done with the LogDet/Paralinear distance option. NJ bootstrap analysis was also done in PAUP® (1 000 replicates).

A BLAST search or query in the nucleotide database using the “megablast” option was done with the unknown endophytic isolates. To define if the unknown endophyte belonged to the same “phylogenetic species” or Operational Taxonomic Unit (OTU) as sequences in GenBank, a 98 % similarity threshold was used (Nilsson et al. 2008). The resulting sequences from the BLAST search were downloaded and compared to those produced in the present study. Only sequences with ca. ≥95 % query coverage were used in the analyses.

**Results**

**Saprotroph and endophyte collections**

Approximately 248, 190, 246 and 175 endophytic strains were collected from Iberia, Iquitos, Los Amigos and Tambopata, respectively. These endophytic strains represent an estimated 76, 50, 39 and 58 OTUs (or putative species), respectively, based on a 98 % ITS-sequence similarity threshold (Nilsson et al. 2008). The identification of these isolates is still in progress. The most abundant genera were Clonostachys, Colletotrichum, Fusarium, Lasiodiplodia, Pestalotiopsis, Phomopsis, Trichoderma and Xylaria (results not shown, but see Gazis & Chaverri 2010 for results from Tambopata). The endophytes selected for this study were not abundant, but were found at three sites: Tambopata (PP 75, PP 114), Iquitos (IQ 232) and Iberia (IB 145b). Saprotrophs mainly from Dothideomycetes and Hypocreales (Sordariomycetes) were collected only in Tambopata and Los Amigos; their identification is still in progress. The leaf saporoph used in the present study (P.C. 811 and P.C. 987) was selected because its sequences matched those of the above-mentioned endophytes (see following sections).

**Identification of the unknown endophyte using a BLAST search**

One of the sequences of the unknown endophytes (IB 145b) was used in the BLAST search. The query resulted in ca. 25 sequences with ≥98 % similarity or “maximum identity” (Table 1), indicating that all of these sequences may represent one OTU. Out of these resulting sequences, the majority were unidentified soil, leaf litter or endophytic fungi. Isolate IB 145b was 99.5 % identical to a Perisporiopsis (P.C. 811, accession number FJ884129), which was collected from decaying Hevea leaves as part of this study. One of the sequences was labelled as Doakia monthandangii (98.3 % identical) and others were identified as “Leptosphaeria” sp. In the ITS tree, all of the sequences with ≥98 % similarity clustered with the unknown endophytes in Clade A (Fig 1). Other close BLAST matches identified to species were Phoma leveillei FJS51477 (97.8 % identical), Pyrenochaeta nobilis EU930011 (86.4 % identity) and Orochladosporium elatum GU248334 (85.4 % identity), all members of the Dothideomycetes.

**Phylogenetic analysis**

Alignment of ITS and LSU sequences resulted in 680 and 905 base pairs (bp), respectively, including gaps. An ambiguously aligned region of 125 characters in the ITS alignment was
Table 1 – Results of BLAST search, using ITS sequence of IB 145b as the query, and sorted by % identity. Only % identities > 95 % are included. GenBank accession numbers for the unknown endophytes are included.

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<tr>
<th>Accession number</th>
<th>Description</th>
<th>Substrate</th>
<th>% identity</th>
</tr>
</thead>
<tbody>
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<td>Perisporiopsis lateritii IB 145b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Leaf endophyte, Hevea brasiliensis</td>
<td></td>
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<tr>
<td>JF884129</td>
<td>Perisporiopsis lateritii P.C. 811&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Decaying leaf of Hevea brasiliensis</td>
<td>99.52</td>
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<tr>
<td>JF884130</td>
<td>Perisporiopsis lateritii PP 114&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&gt;99</td>
</tr>
<tr>
<td>JF890410</td>
<td>Perisporiopsis lateritii PP 75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Sapwood endophyte, Hevea brasiliensis</td>
<td>&gt;99</td>
</tr>
<tr>
<td>HM031458</td>
<td>Perisporiopsis lateritii IQ 232&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&gt;99</td>
</tr>
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<td>FJ450030</td>
<td>Fungal endophyte strain 1168</td>
<td>Endophyte, Dendrobiurn sp.</td>
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<td>AF032815</td>
<td>Leaf litter ascomycete strain its301</td>
<td>Leaf litter</td>
<td>99.17</td>
</tr>
<tr>
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<td>Ceratonia insignis</td>
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<td>Soil?</td>
<td>98.96</td>
</tr>
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<td>Soil fungal sp. 4-M-1</td>
<td>Soil</td>
<td>98.96</td>
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<td>Spore trap</td>
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<td>Soil</td>
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<td>Soil</td>
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<td>Soil</td>
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<td>Fungal sp. GMG_ppb12</td>
<td>Soil</td>
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<td>Uncultured fungus isolate F-RISA</td>
<td>Soil</td>
<td>98.34</td>
</tr>
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<td>Dokmaia monlandangui</td>
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</tr>
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<td>Marine sponge</td>
<td>97.77</td>
</tr>
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<td>Leptosphaeriaceae HKC16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Marine sponge</td>
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<td>Soil</td>
<td>95.26</td>
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</table>

<sup>a</sup> Sequences produced for the present study. All other sequences were obtained from GenBank.

<sup>b</sup> Not included in Fig 1.

excluded from the analysis. Maximum Likelihood and Neighbor-Joining analyses showed that the unknown endophytes (isolates IB 145b, IQ 232, PP 75, PP 114) clustered with a specimen found in surrounding decaying Hevea leaves (P.C. 811 from Tambopata) identified as Perisporiopsis (Fig 1: ITS tree, clade A). These specimens also cluster with the additional sequences obtained from GenBank that had >98 % similarity in the BLAST search (Fig 1, Clade A). Phylogenetic analyses of the ITS and LSU regions also showed that the unidentified endophytes and saprotrophs of Hevea are taxonomically related to species in the fungal families Leptosphaeriaceae and Phaeosphaeriaceae (Dothideomycetes, Ascomycota) (Figs 1 and 2). However, the endophytes and saprotrophs did not seem to cluster within any of those families.

**Identification using morphology**

The specimens of saprotrophs on decaying leaves of *H. brasiliensis* and *H. guianensis*, P.C. 811 and P.C. 987, respectively, were identified as Perisporiopsis (Ascomycota, Dothideomycetes, Parodiopsidaceae) by examination and characterization of sexual and asexual stages on the natural substratum, and by the use of taxonomic literature. Specimens P.C. 811 and P.C. 987 had abundant sexual and asexual fruiting structures on the underside of the decaying leaves. The characteristics of the sexual and asexual stages did not match any of the previously described species of Perisporiopsis (Sivanesan 1984). Therefore, this species is considered as new and is being formally described as Perisporiopsis lateritii (Chaverri & Gazis 2010).

The colony appearance matched that of other members in the Dothideomycetes and the microconidial anamorph that of Perisporiopsis (Sivanesan 1984). The cultures of the unknown endophytes on PDA were discrete, somewhat cotty or wooly, slow growing, reaching ca. 30 mm after 2 weeks, the mycelium at first colourless with tinges of pale grayish-brown, and with a grayish sepal pigmentation of the reverse of the colony. The cultures sporulated after 2 weeks, forming few
Fig 1 – ITS nrDNA Neighbor-Joining tree. Bootstrap support ≥70% in NJ and ML analyses indicated by the thicker branches. Log likelihood (Ln) of the ML tree = −11194.0284. Clade A includes resulting sequences from a BLAST search. Outgroup taxa: Pleosporales and Alternaria sp.

Perisporiopsis aff. lateritia

98% identity from a BLAST search

Substrate/source:
Endophyte (bold)
Leaf litter
Soil
Substrate unknown?

LEPTOSPHAERIACEAE

PHAEOSPHERIACEAE
brown conidiophores and colourless unicellular spores near the source of inoculum.

The original description (Promputtha et al. 2002) and holotype (PDD 74980) of D. monthadangii, a leaf litter fungus that had 98% sequence identity with the Hevea endophytes, did not match the morphology of either the Hevea endophytes or saprotrophs. The morphology of the asexual fruit bodies on PDA described for D. monthadangii also did not match that of the Hevea endophytes on PDA. In addition, none of the asexual stages of Leptosphaeriaceae, Parodiopsidaceae, Phaeosphaeriaceae, or other related families in the Dothideomycetes reported in the literature have morphology similar to Dokmaia.

**Discussion**

Based on the results of the DNA sequence analyses (BLAST and phylogenies) and morphological comparisons of endophytes and saprotrophs of Hevea, it is concluded that the BLAST-unidentifiable endophytes (IB 145b, IQ 232, PP 75, PP 114) are P. lateritia (P.C. 811). All other species of Perisporiopsis are also described as being hypophyllous (growing on the underside of decaying leaves) in tropical regions (Sivanesan 1984). According to Sivanesan (1984) Perisporiopsis is taxonomically related to the Leptosphaeriaceae in the Dothideomycetes and our phylogenetic analyses confirm this relationship (Figs 1 and 2). The identification of the sequence with GenBank accession number DQ780454 as D. monthadangii is doubtful. First, the authors who described D. monthadangii mention its taxonomic relationship to Phaeoisaria (Promputtha et al. 2002), a genus that belongs in the Sordariomycetes, not in the Dothideomycetes. Second, Promputtha et al. (2002) reported the formation of asexual fruiting structures (synnemata) and spores in culture; none of the endophytic cultures studied in the present paper formed synnemata in culture. Therefore, the more likely name for these Hevea endophytes is Perisporiopsis.

Little is known about the ecology of Perisporiopsis. This genus includes 19 hypophyllous species, all of them from tropical

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Fig 2 – LSU (28S) nrDNA Neighbor-Joining tree. Bootstrap support ≥70% in NJ and ML analyses indicated by the thicker branches. Log likelihood (Ln) of the ML tree = –2540.7520. Endophytes of Hevea spp. are in bold. Outgroup taxa: Botryosphaeria spp.
regions (Sivanesan 1984). In the present study, two endophytic isolates originated from the sapwood of Hevea, yet most other endophytic isolates, including those sequences from GenBank (Table 1), show a substratum preference for leaves (Schulz & Boyle 2005; Arnold 2007; Hyde & Soytong 2008; Tao et al. 2008; Gazis & Chaverri 2010). The results of the present study also suggest that for part of its life cycle this fungus lives in the soil, possibly “waiting” for the preferred host or substratum (Arnold 2007; Hyde & Soytong 2008; Tao et al. 2008; De Errasti et al. 2010). The darkly pigmented mycelium and asexual spores may allow these fungi to lay dormant in the soil (Rotem & Aust 1991; Robinson 2001).

Species in the genus Perisporiopsis appear to be plant host specific, at least at the genus or higher level (Sivanesan 1984). For example, Perisporiopsis brachystegiae and Perisporiopsis fusiclora are known only from legumes in Africa and Tropical America, respectively. Perisporiopsis megalospora is known from various genera in the Malpighiales, and Perisporiopsis melioloides from Myrtaceae. There is only one other species that has been found on Euphorbiaceae, Perisporiopsis kwangensis. In our phylogenetic analyses of ITS (Fig 1), Clade A contained sequences of endophytic fungi from different plants, e.g. Cecropia, Dactylis, Dendrobium and Holcus. Thus, it is not clear if Clade A represents one species, based on the definition of “phylogenetic species” or Operational Taxonomic Unit (OTU) using a 98% sequence identity in ITS (Nilsson et al. 2008). A number of studies have demonstrated that ITS is not sufficient for species identification, especially in rapidly evolving or highly diverse genera or species complexes (Chaverri et al. 2003; Chaverri & Samuels 2003; Cai et al. 2009; Pavlic et al. 2009). Therefore, it is possible that there is more than one species of Perisporiopsis in Clade A; taxonomic studies are needed.

Due to the high diversity and abundance of endophytes, most studies characterize endophytic fungi using DNA sequences. Until now, the preferred molecular marker has been ITS because of its extensive database in GenBank (ca. 600,000 sequences). In addition, the ITS region is easy to amplify and, in many cases, provides enough variation to identify species (Nilsson et al. 2008; Seifert 2009). Unfortunately, the rate of submission of correctly identified, named and vouchered sequences in GenBank has not kept pace with the number of easily obtained, unidentified sequences from environmental samples (Nilsson et al. 2006). Consequently, many, even most, endophyte sequences in GenBank remain unidentified. For example, when querying the GenBank nucleotide database using the keyword “endophyte”, almost 50% were unidentified, labelled as “fungai endophyte”, “uncultured endophytic fungus”, or similar. Many others were identified to family or to genus, but few were identified to species.

One of the goals of the Barcode of Life Initiative (BOLI) is to provide diagnostic reference DNA sequences for accurately named species (www.dnabarcodes.org). This Initiative will enable biologists to rapidly and correctly identify unknown specimens/isolates/sequences. Especially, it will contribute to studies involving highly diverse environmental samples, such as soil fungi, endophytes and airborne fungi. Thus, achieving the goals of BOLI will provide an essential identification tool for those conducting environmental sampling. The taxonomic approach taken in the present study to identify and name BLAST-identifiable endophytes by sampling and accurately identifying the ex planta fungi suggests a method to be used for future fungal diversity studies. This approach includes discovering the substrata or niches a fungus can exploit, and the stages in the life cycle of fungi, including being an endophyte for part of their lives and a saprotroph when its plant host dies. Connecting a DNA sequence with a name that has biological meaning enables microbial (i.e. fungal) biologists to more accurately infer the functional ecology of a particular species in a community or ecosystem.

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