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Molecular characterisation of fungal endophytic morphospecies associated with the indigenous forest tree, *Theobroma gileri*, in Ecuador☆

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ABSTRACT

Fungal endophytes were isolated from healthy stems and pods of *Theobroma gileri*, an alternative host of the frosty pod rot pathogen of cacao. Non-sporulating isolates were grouped into 46 different morphological species according to their colony morphology. Many of these morphospecies were assumed to be basidiomycetes and, therefore, were of particular interest. Basidiomycetous endophytes have received far less attention than ascomycetes and also have potential as biological control agents of the basidiomycetous pathogens of *T. cacao: Moniliophthora roreri* (frosty pod rot pathogen) and *M. perniciosa* (witches' broom disease). The morphospecies were further characterised by molecular analyses. Amplification of the nuLSU was undertaken for phylogenetic placement of these non-sporulating cultures and revealed a total of 31 different taxa of which 15 were basidiomycetes belonging to the class *Agaricomycetes*, and 16 ascomycetes primarily belonging to the *Sordariomycetes*.

Introduction

Theobroma gileri (*Malvaceae*) is an understorey tree in submontane forests of the Chocó phytogeographic region of northwest Ecuador and Colombia, an area of high plant endemism (Dodson & Gentry 1991). *T. gileri* appears confined between 550–700 m a.s.l. and can be abundant in ravines and rocky slopes, often reaching a height of between 15–20 m (Evans

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et al. 2003). Interest in this species of *Theobroma* and its associated mycoflora originated when it was identified as a forest host of one of the major basidiomycete pathogens of the commercially cultivated *T. cacao* (Baker *et al.* 1954).

T. cacao is the source of the internationally traded commodity, cocoa. In Latin America production of cocoa is currently affected by the basidiomycetous pathogens *Moniliophthora perniciosa* (causal agent of witches' broom disease) and *M. roreri* (causal agent of frosty pod rot). Conventional control approaches have proved unable to halt the advance of these diseases, especially frosty pod rot, which was identified in 2005 as having reached as far north as Mexico (Phillips-Mora *et al.* 2006). *M. roreri* is also posing a direct threat to Bolivia and Brazil from its base in Peru (Evans 2002a). Biological control, in particular classical biological control, is a management option being pursued (Holmes *et al.* 2004). Classical biocontrol aims to redress the ecological imbalance by introducing coevolved natural enemies, selected for specificity and biocontrol activity, from the evolutionary centre of origin of the invasive, alien pest or pathogen. This strategy has traditionally been exploited for the control of invasive alien weeds, using both insect and fungal agents (Evans 2002b; McFadyen 1998) as well as against exotic arthropods (Greathead 1995).

T. gileri was first described from north-west Ecuador in the early 1950s (Cuatrecasas 1953, 1964) and was subsequently reported to occur along the Pacific slopes of the Andes up to northern Colombia (Baker *et al.* 1954). This means that *T. gileri*, which grows in the Chocó phytogeographic region that is a recognised biodiversity 'hotspot' (Myers *et al.* 2000), has been evolutionarily isolated from *T. cacao*, which appears to have originated east of the Andes (Motomayor *et al.* 2002). This isolation supports the hypothesis that this *Theobroma* species may be a source of novel fungal endophytes, with potential as biocontrol agents. Its isolated forest habitat and non-cultivated status may have enabled *T. gileri* to retain its rich mycoflora, which has been lost by cultivated *T. cacao*; this is supported by the previous study on *T. gileri* endophytes (Evans *et al.* 2003).

In a survey undertaken in Ecuador in 1999, Evans *et al.* (2003) located the type locality of *T. gileri* in order to isolate potential classical biological control agents for frosty pod rot. Fungal endophytes were isolated from the stems and pods of *T. gileri*. A rich endophytic assemblage was obtained that consisted of 373 isolates (Evans *et al.* 2003). With the aid of morphological keys, 258 of these were identified at least to genus level. The taxonomic range of endophytic fungi isolated was different from previously documented studies of tree endophytes with the majority of isolates belonging to anamorphs of the *Hypocreales*, as well as to basidiomycete orders (Evans *et al.* 2003). However, it was not possible to identify 115 (31 %) of the isolates collected as they did not sporulate on artificial culture media. These were grouped into 46 'morphological species' according to their colony characteristics (Fig 1).

This paper reports on the subsequent molecular characterisation undertaken to identify these 46 morphospecies isolated from healthy stems and pods of *T. gileri* in native forest. This paper has given special consideration to the

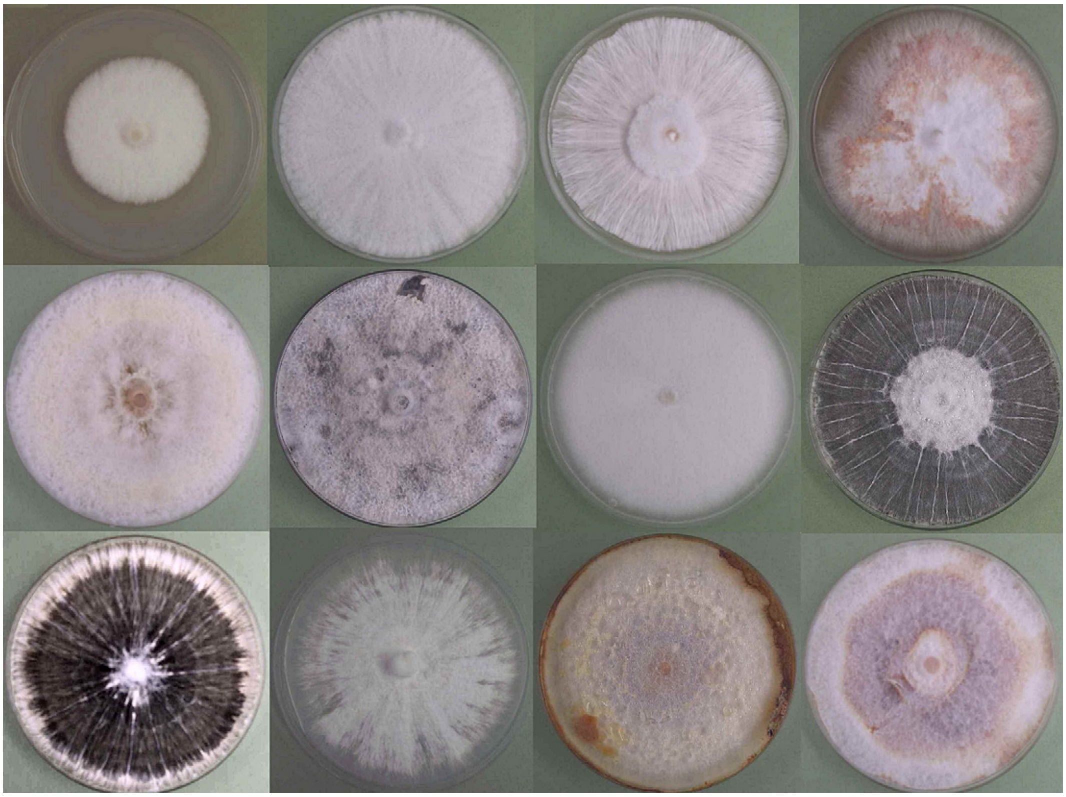

Fig 1 – Morphospecies isolated from stems of *Theobroma gileri*.

phylogenetic breadth of the basidiomycete endophytes identified as these have been less well studied in comparison with ascomycetes and may be useful as potential novel biocontrol agents for the basidiomycetous pathogens of *T. cacao*.

Methods

Selection of endophytic fungi

Details on the endophyte isolation techniques and the two study sites, one on the northwestern slopes of the Andes in the Ecuadorian Province Esmeraldas and the other in the neighbouring Pichincha Province, can be found in Evans *et al.* (2003). This subsequent study focuses on the 115 isolates that failed to form fruiting structures in culture, or only produced arthrosporic stages. These isolates were grouped into 46 morphospecies according to their colony characteristics and a representative isolate of each was selected for molecular analysis. All isolates are stored under oil or at 10 °C as DIS codes at CABI, UK (Tables 1 and 2).

DNA extraction, PCR amplification, and rDNA sequencing of endophytic morphospecies

DNA was extracted from the fungal isolates using a MoBio UltraClean Plant DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). An agar block (*ca* 5 mm × 5 mm × 2 mm)

Table 1 – Identification of *T. gileri* basidiomycetes isolates based on LSU rDNA sequence data

Isolate code	Genbank no.	Tentative identification	Nearest match	Query coverage (%)	Max identity (%)	Site/Tissue			
						Esmeraldas		Pichincha	
						Stem	*Pod*	*Stem*	*Pod*
DIS 360d	DQ674802	*Coprinellus* sp.	AY207180.1 *Coprinellus disseminatus*	99	99	+	–	–	–
DIS 274g	–	*Coprinellus* sp.				+	–	–	–
DIS 320g	–	*Coprinellus* sp.				–	–	+	–
DIS 337bii	–	*Coprinellus* sp.				–	–	+	–
DIS 355e	–	*Coprinellus* sp.				–	–	+	–
DIS 357aii	–	*Coprinellus* sp.				–	–	+	–
DIS 276d	DQ674804	*Ganoderma* sp.	DQ208418.1 *Fomes fomentarius*	100	99	–	+	–	–
DIS 276b	–	*Ganoderma* sp.				–	+	–	–
DIS 276f	–	*Ganoderma* sp.				–	+	–	–
DIS 358a	DQ674805	*Lachnocladiaceae* sp.	DQ094786.1 *Peniophora cinerea*	100	97	+	–	–	–
DIS 360e	DQ674807	*Lentinus* sp. 1	AF261563.1 *Lentinus squarrosulus*	100	98	+	–	–	–
DIS 326i	DQ674806	*Lentinus* sp. 2	AF261563.1 *Lentinus squarrosulus*	100	98	+	–	–	–
DIS 274b	DQ674808	*Melanotus subcuneiformis*	AF261511.1 *Melanotus subcuneiformis*	93	100	+	–	–	–
DIS 330i	DQ674803	*Meripilus* sp.	AY684166.1 *Albatrellus higanensis*	100	95	–	+	–	–
DIS 276i	DQ674809	*Phlebioid* sp.	EU118665.1 *Scopuloides hydnoides*	100	99	–	+	–	–
DIS 330fi	DQ674810	*Piptoporus* sp.	AY684164.1 *Fomitopsis pinicola*	100	96	–	+	–	–
DIS 360aii	DQ674813	*Polyporaceae* sp. 1	DQ208421.1 *Fomes fomentarius*	100	97	+	–	–	–
DIS 357f	–	*Polyporaceae* sp. 1				–	–	+	–
DIS 328b	DQ674812	*Polyporaceae* sp. 2	DQ208421.1 *Fomes fomentarius*	100	97	+	–	–	–
DIS 227b	–	*Polyporaceae* sp. 2				–	–	+	–
DIS 274c	–	*Polyporaceae* sp. 2				+	–	–	–
DIS 356a	–	*Polyporaceae* sp. 2				–	–	+	–
DIS 357b	–	*Polyporaceae* sp. 2				–	–	+	–
DIS 229c	DQ674811	*Polyporaceae* sp. 3	AY684163.1 *Ganoderma tsugae*	100	97	–	–	+	–
DIS 359c	DQ674814	cf. *Pycnoporus* sp.	AY586703.1 *Pycnoporus cinnabarinus*	100	98	+	–	–	–
DIS 372b	DQ674815	*Schizophyllum* sp.	DQ071725.2 *Schizophyllum commune*	99	99	–	+	–	–
DIS 229e	DQ674816	*Wrightoporia* sp.	AF506490.1 *Wrightoporia tropicalis*	100	99	–	–	+	–

Table 2 – Identification of *Ascomycetes* isolates from the stem tissue of *T. gileri*, based on LSU rDNA sequence data

Isolate code	Genbank no.	Tentative identification	Nearest match	Query coverage (%)	Max identity (%)	Site	
						Esmeraldas	Pichincha
DIS 325h	DQ674824	*Bionectriaceae* sp.	AY686634.1 *Bionectria ochroleuca*	100	99	+	–
DIS 341i(ii)	DQ674828	*Clavicipitaceae* sp. 1	AF373284.1 *Chaunopycnis alba*	100	99	–	+
DIS 217j	DQ674829	*Clavicipitaceae* sp. 2	AF373284.1 *Chaunopycnis alba*	100	99	+	–
DIS 216a	DQ674830	*Clavicipitaceae* sp. 3	AF373282.1 *Chaunopycnis pustulata*	100	99	+	–
DIS 274a	–	*Clavicipitaceae* sp. 3				+	–
DIS 226a	DQ674831	*Clavicipitaceae* sp. 4	AF389190.1 *Chaunopycnis pustulata*	100	99	–	+
DIS 358f	DQ674821	*Hypocreales* sp. 1	AB067709.1 *Cordyceps sinensis*	100	96	+	–
DIS 106g	DQ674822	*Hypocreales* sp. 2	AY097325.1 *Fusarium lichenicola*	100	99	+	–
DIS 355a	DQ674825	*Pleosporaceae* sp.	DQ678044.1 *Lewia eureka*	100	98	–	+
DIS 341k	DQ674820	*Sordariales* sp.	AY780067.1 *Cercophora terricola*	100	99	–	+
DIS 360g	DQ674817	*Xylariaceae* sp. 1	AY544676.1 *Xylaria acuta*	100	98	+	–
DIS 354b	DQ674818	*Xylariaceae* sp. 2	AY544676.1 *Xylaria acuta*	100	98	–	+
DIS 327e	DQ674819	*Xylariaceae* sp. 3	AY544676.1 *Xylaria acuta*	100	96	+	–
DIS 341eii	DQ674823	*Xylariaceae* sp. 4	DQ923534.1 *Phlogicylindrium eucalypti*	100	96	–	+
DIS 327b	DQ674826	*Xylariaceae* sp. 5	AY544676.1 *Xylaria acuta*	100	97	+	–
DIS 216i	DQ674827	*Xylariaceae* sp. 6	AY544676.1 *Xylaria acuta*	99	97	+	–
DIS 227k	DQ674832	*Xylariaceae* sp. 7	EF420088.1 Fungal endophyte	96	99	–	+
DIS 274j	–	*Xylariaceae* sp. 7				+	–
DIS 322b	–	*Xylariaceae* sp. 7				–	+

containing the mycelium of each isolate was cut from the growing edge of a potato–dextrose agar (PDA) plate and transferred to a 2 ml bead solution (supplied in the kit) using a sterile scalpel. DNA was extracted following the instructions supplied with the kit.

Subsequent analysis followed a similar protocol to that in Crozier *et al.* (2006). The first 1 kb of the nuLSU DNA was chosen for initial amplification (with primers LROR and LR6) and sequencing (with primers LROR, LR3R, LR5 and LR6), because this region has been used most frequently in basidiomycete systematics and comprehensive LSU datasets were already available for sequence comparisons and analyses (e.g. Moncalvo *et al.* 2002). Methods for PCR amplification and sequencing follow Aime & Phillips-Mora (2005). Sequences have been deposited in GenBank (Tables 1 and 2).

Sequences obtained were initially blasted in GenBank (http://www.ncbi.nlm.nih.gov) to predict the family and/or order for each isolate. For closer phylogenetic placement, a data matrix of LSU sequences was then constructed in the following manner: (1) a skeletal LSU dataset was constructed by pruning that of Moncalvo *et al.* (2002) to exclude redundant taxa from lineages not related to any of the fungal endophytes as indicated by BLAST analyses; (2) additional LSU sequences were then added to this dataset by including all close (>94 % similarity) BLAST results for the isolates; (3) additional exemplar sequences were included from families and orders of homobasidiomycetes to which BLAST analyses indicated the majority of endophytes had taxonomic affinities; and (4) several heterobasidiomycete (*Dacrymycetes* and *Auriculariales*) sequences were included as outgroups. GenBank accession numbers for additional sequences used in these analyses are shown in Fig 2. Sequences were manually aligned in Se-Al: Sequence Alignment Editor (Andrew Rambaut, Dept. Zoology, University of Oxford, U.K.; http://evolve.zoo.ox.ac.uk). Gaps were introduced to maintain alignment through regions where indels occurred in one or more sequences. Regions with ambiguous alignment were excluded from analyses. The assembled dataset contained 171 taxa. MP analyses were conducted in PAUP 4.0b10 (Swofford 2002) as heuristic searches with ten random addition replicates and tree bisection–reconnection (TBR) branch swapping; gaps were coded as missing data. Support for the branching topologies was evaluated by BS analysis derived from 1K replicates with ten random addition replicates each. Formal names for the resulting fungal clades follow Hibbett *et al.* (2007).

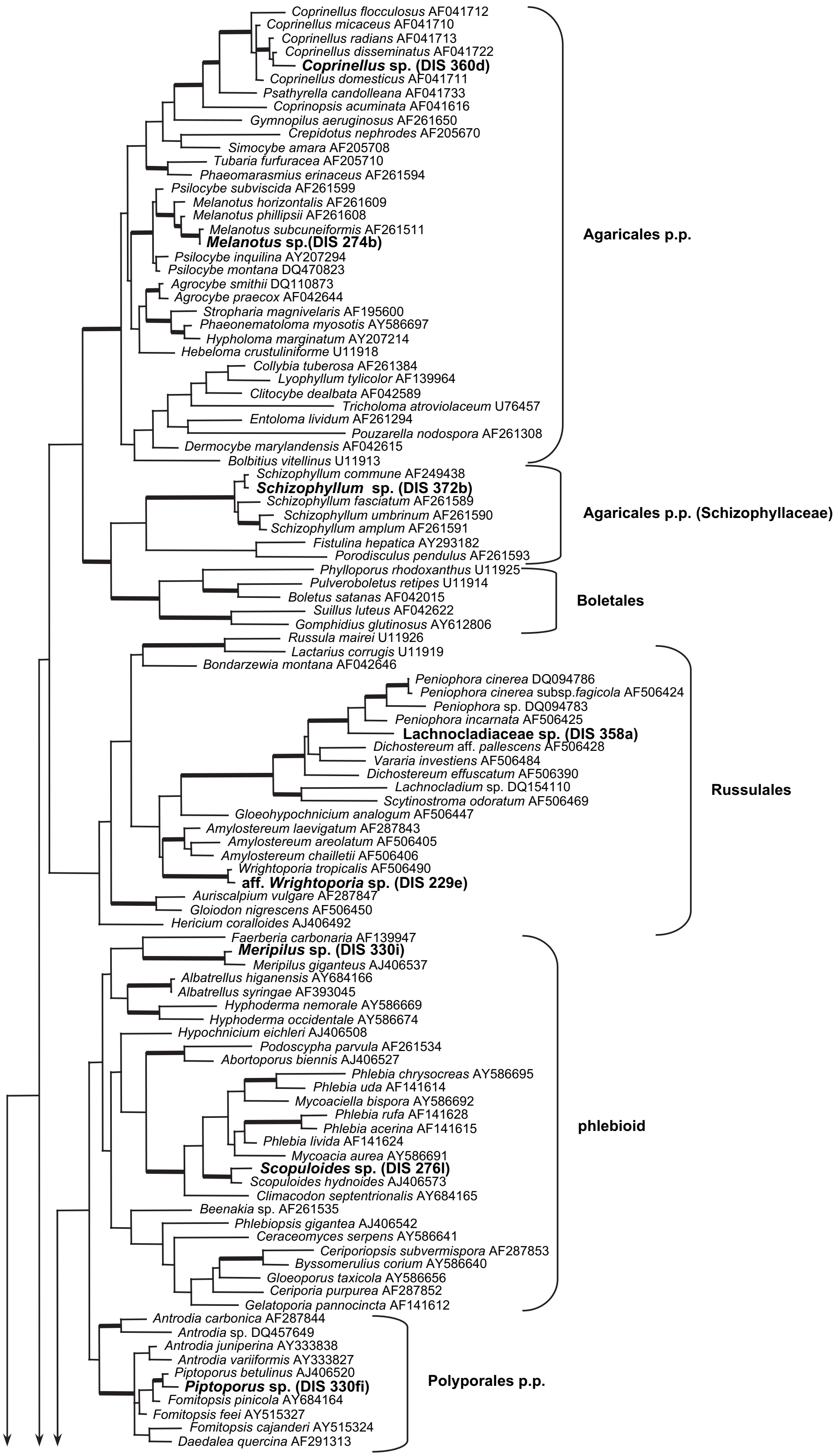

Fig 2 – Parsimony analysis of LSU rDNA sequences showing phylogenetic position of endophytic basidiomycetes within the major lineages of *Agaricomycotina*. Thickened branches in this tree indicate BS support of >60 %. Endophytic isolates are indicated by bold type. Clade names are from Hibbett *et al.* (2007), Larsson *et al.* (2004), and Matheny *et al.* (2006).

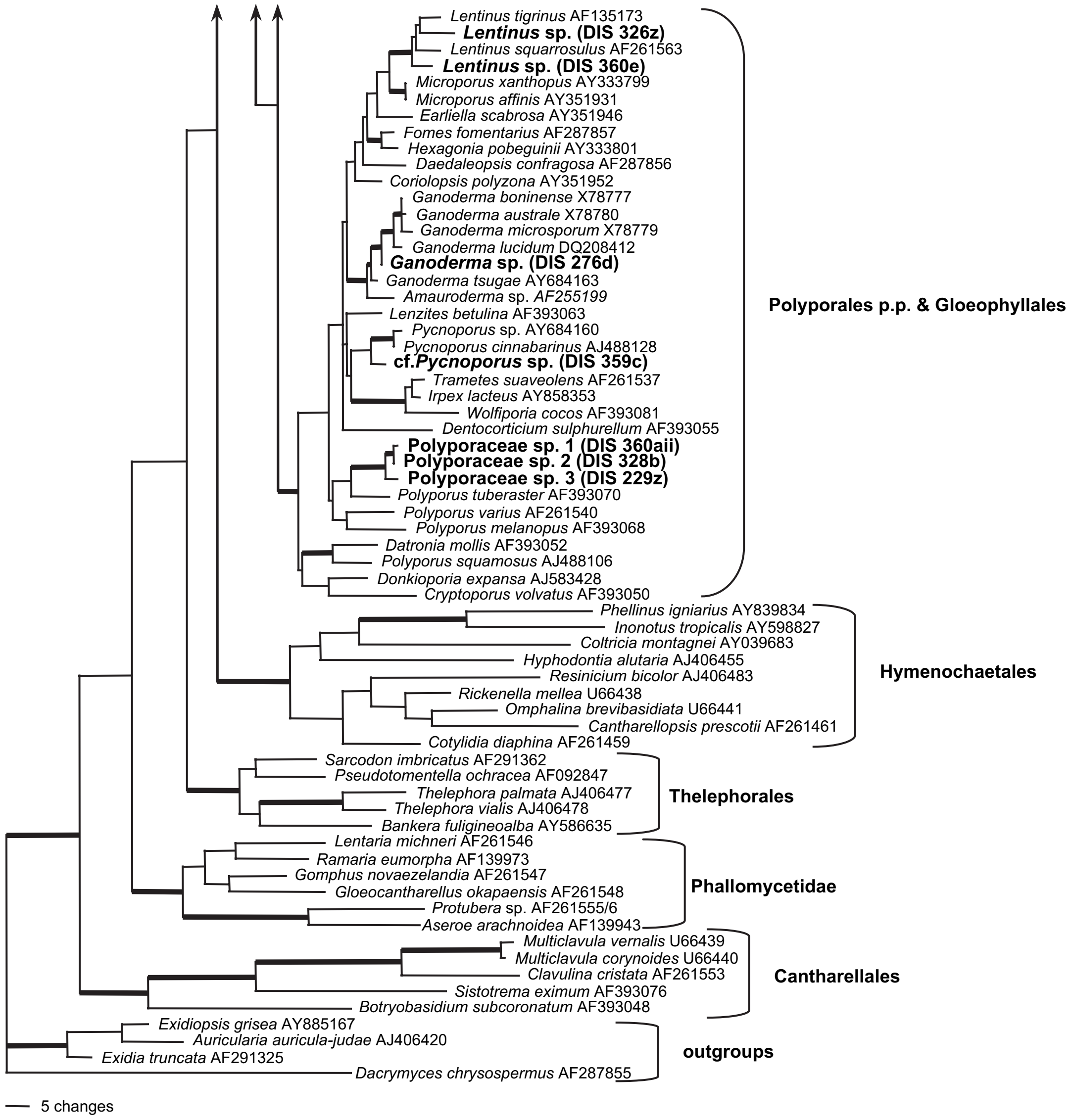

Fig 2 – (*continued*)

Results

Molecular analysis indicates that the 46 fungal endophyte morphospecies included in the study consisted of 31 different taxa, based on sequence data for the first 1 kb of the 5′ end of the 28 nuLSU gene. BLAST analysis revealed that 15 of the taxa were basidiomycetes (Table 1) and 16 of the taxa were ascomycetes (Table 2). *Basidiomycota* were identified by additional parsimony analyses (Fig 2). Of the 1130 included characters, 84 were variable but parsimony-uninformative, and 352 were parsimony-informative. Analyses yielded a single most parsimonious tree (length = 3141, CI = 0.228, RI = 0.666; Fig 2). Ascomycetes were not further analysed in this study.

All of the endophytic basidiomycetes were members of the *Agaricomycetes*. Eight of the basidiomycete isolates belonged to polyporoid lineages (*Polyporales, Corticiales*, and *Gloeophyllales sensu* Hibbett *et al.* 2007, and the phlebioid clade of Larsson *et al.* 2004). The remainder belonged to the *Agaricales* (including the *Schizophyllaceae sensu* Matheny *et al.* 2006), and *Russulales*. Those isolates allied within the *Russulales* belong to clades of non-ectomycorrhizal polyporoid taxa.

Discussion

A total of 373 fungal endophytes were isolated from stems and pods of *Theobroma gileri* (Evans *et al.* 2003). One hundred and fifteen (31 %) of these could not be identified by traditional morphological means and were grouped into 46 morphospecies. Morphospecies are artificial groupings that may not necessarily reflect taxonomic relationships (Guo *et al.* 2003). Artificial grouping of fungal isolates can be useful, but this study highlights morphological variability in colony characteristics on artificial fungal media and the limited use of morphospecies for inferring taxa. What were believed to be 46 different morphospecies belonged to only 31 taxa. Conversely, if all 115 unidentified isolates had been analysed molecularly, it is possible that additional fungal taxa would have been identified.

BLAST analysis revealed that 27 of the morphospecies were basidiomycetes and 19 were ascomycetes. Further molecular analysis of the 27 basidiomycete isolates led to the identification of 15 different taxa. Within the *Basidiomycota*, all of the isolates were members of the *Agaricomycotina*, with the majority of these isolates occurring within the *Polyporales sensu* Hibbett *et al.* (2007). Nineteen ascomycetous morphospecies were identified (corresponding to 16 taxa) with the most commonly isolated endophytes belonging to the *Xylariaceae*. Xylariaceous fungi are widespread wood decomposers and are particularly common as plant endophytes in the tropics (Petrini *et al.* 1995; Rodrigues & Petrini 1997). Santamaria & Bayman (2005) found *Xylaria* to be one of the most common endophytic genera isolated from coffee leaves (*Coffea arabica*) after molecular analysis of unidentified morphospecies. A previous study on another *Theobroma* species, *T. cacao* produced a similar range of ascomycetes, although these were from a range of ecological environments including natural forest and agroforestry (Crozier *et al.* 2006), as shown in Table 3.

The basidiomycetes were the main focus of this study. As noted previously (Guo *et al.* 2003; Crozier *et al.* 2006) there are limitations to the identification of basidiomycetes with molecular data only. The current sequence data for homobasidiomycetes available in public databases constitute less than 10 % of the known species and without the production of representative fruiting structures systematic placement is problematic (Crozier *et al.* 2006). As a result, until additional gene sequences are available definitive identification will be tentative at best.

Many previous studies have revealed a range of sterile and otherwise unidentifiable fungi, which have often been disregarded. Many have been grouped into morphospecies (Arnold *et al.* 2000; Fröhlich *et al.* 2000; Guo *et al.* 2003), but few studies have used molecular techniques to further identify these morphospecies and only one has concerned a species of *Theobroma* (Crozier *et al.* 2006). In general, basidiomycetes have only been identified in limited numbers in previous endophyte studies (Bills 1996; Carroll 1988; Oses *et al.* 2006; Sridhar & Raviraja 1995) and only a few have sampled from similar woody stem tissues (Evans *et al.* 2003; Rubini *et al.* 2005; Simeto *et al.* 2005). The assemblage presented here differs from the previous basidiomycete profiles from *T. cacao* (Crozier *et al.* 2006), which were more diverse, likely from being from a range of geographical and ecological zones (see Table 4). The basidiomycetes isolated from *T. gileri* were predominantly polyporoid. Only four of the taxa identified in the *T. cacao* study

Table 3 – Summary table of *Ascomycota* isolates from *Theobroma cacao* (adapted from Crozier et al. 2006)

Identification	Location	Ecosystem[a]	Tissue	
			Stem	Pod
Xylariaceae sp. 1	Brazil	Exotic	−	+
Pleosporaceae sp.	Cameroon	Exotic	+	−
Xylaria sp. 6	Cameroon	Exotic	+	−
Hypocreales sp. 1	West Ecuador	Exotic	+	−
Hypocreales sp. 2 (cf. *Leucosphaerina* sp.)	West Ecuador	Exotic	+	−
Pleosporales sp.	West Ecuador	Exotic	−	+
Xylaria sp. 2	West Ecuador	Exotic	+	−
Xylaria sp. 4	West Ecuador	Exotic	+	−
Xylaria sp. 5	West Ecuador	Exotic	+	−
Xylariaceae sp. 2	West Ecuador	Exotic	−	+
Nectriaceae sp. (cf. *Stephanonectria* sp.)	Mexico	Exotic/forest	+	−
Xylaria sp. 1	Mexico	Exotic/forest	+	−
Bionectria sp.	East Ecuador	Forest	+	−
Clavicipitaceae sp.	East Ecuador	Forest	+	−
Hypocreaceae sp.	East Ecuador	Forest	+	−
Xylaria sp. 2	East Ecuador	Forest	+	−
Xylaria sp. 3	East Ecuador	Forest	+	−

[a] Exotic = cultivated cacao (farm, germplasm collection) outside the centre of origin; exotic/forest = naturalised cacao in a forest habitat outside the centre of origin; forest = wild cacao within the centre of origin, growing as an understorey tree.

Table 4 – Summary table of *Basidiomycota* isolates from *Theobroma cacao* (adapted from Crozier et al. 2006)

Identification	Location	Ecosystem[a]	Tissue	
			Stem	Pod
Corticioid sp. 2 (3)	Brazil	Exotic	+	−
Auriculariales sp.	Cameroon	Exotic	+	−
Corticioid sp. 1 (4)	Cameroon	Exotic	+	−
Corticioid sp. 3	Cameroon	Exotic	+	−
Corticioid sp. 5	Cameroon	Exotic	+	−
Corticioid sp. 7	Cameroon	Exotic	+	−
Podoscypha sp.	Cameroon	Exotic	+	−
Coprinellus sp. 1	Costa Rica	Exotic	+	−
Corticioid sp. 4	Costa Rica	Exotic	+	−
Hymenochaetoid sp. 1 (4)	Costa Rica	Exotic	+	−
Inonotus sp.	Costa Rica	Exotic	+	−
Polyporaceae sp. 1	Costa Rica	Exotic	+	−
Polyporaceae sp. 2 (phylotype 1)	Costa Rica	Exotic	+	−
Polyporaceae sp. 2 (phylotype 2) (2)	Costa Rica	Exotic	+	−
Coprinellus sp. 2 (2)	West Ecuador	Exotic	+	−
Corticioid sp. 8	West Ecuador	Exotic	+	−
Corticioid sp. 9	West Ecuador	Exotic	+	−
Phanerochaete sp.	West Ecuador	Exotic	+	−
Pycnoporus sp. 1	West Ecuador	Exotic	−	+
Pycnoporus sp. 2 (2)	West Ecuador	Exotic	−	+
Oxyporus sp.	Mexico	Exotic/forest	+	−
Polyporaceae sp. 2 (phylotype 1)	West Ecuador	Exotic/forest	+	−
Byssomerulius sp.	East Ecuador	Forest	+	−
Coprinellus sp. 2 (2)	East Ecuador	Forest	+	−
Hymenochaetoid sp. 2	East Ecuador	Forest	+	−
Lentinus sp.	East Ecuador	Forest	+	−
Gloeosterioid sp.	Brazil	Forest/exotic	+	−
Phlebioid sp.	Brazil	Forest/exotic	−	+
Coprinellus sp. 2	East Ecuador	Forest/exotic	+	−
Corticioid sp. 6	East Ecuador	Forest/exotic	+	−

[a] Exotic = cultivated cacao (farm, germplasm collection) outside the centre of origin; exotic/forest = naturalised cacao in a forest habitat outside the centre of origin; forest = wild cacao within the centre of origin, growing as an understorey tree; forest/exotic = cultivated cacao within the centre of origin but outside the forest ecosystem.

(*Coprinellus* sp., *Lentinus* sp., *Polyporaceae* sp., and *Ganoderma* sp.) were found in the *T. gileri* basidiomycete isolates. Although these appear in the collections from forest-inhabiting *T. cacao*, some seem more ubiquitous, being isolated from cacao in agroforestry systems, outside the centre of origin.

It would be presumptuous to make any assumptions about host or site specificity from these limited data. From this study, although it is presumed that they once were part of a continuum, the *T. gileri* sampled sites differ in the profile of the basidiomycetes identified, indeed only *Coprinellus* and the Polypores were isolated from both sites.

The rationale for the overarching study, of which this forms one part, was to identify novel biocontrol agents of the basidiomycete pathogen of *T. cacao, Moniliophthora roreri*. There is limited information in the literature on the use of basidiomycetes as biocontrol agents, although there are reports of basidiomycetes as antagonists of insects, such as *Scleroderma citrinum* against *Mythimna separata* (Wei *et al.* 2005) and nematophagous basidiomycetes, such as *Coprinus comatus* (Luo *et al.* 2004). The most successful use of a basidiomycete as a biocontrol agent to date is *Phlebiopsis gigantea* for control of the root-rotting pathogen *Heterobasidion annosum* of *Pinus* spp. (Asiegbu *et al.* 2005; Vasiliauskas *et al.* 2005). The endophytic basidiomycetes reported here could be a potential source of biocontrol agents. These endophytic basidiomycetes could compete with the basidiomycetous pathogen *M. roreri* through direct competition for the same ecological niche, the strategy utilised by *Phlebiopsis gigantea*. Alternatively, they could displace the invading pathogen or induce the host's own defence system. Basidiomycetes are known to produce a range of bioactive metabolites, similar to those of ascomycetes (Schulz *et al.* 2002). Valdivia *et al.* (2005) described seven diterpenoid compounds produced by *Coprinellus* (syn. *Coprinus*) *heptemerus,* a basidiomycete with the ability to inhibit spore germination of the fungal rice blast pathogen, *Magnaporthe grisea*. Therefore, it is not unreasonable to speculate that the endophytic basidiomycetes associated with *T. gileri* could produce anti-fungal metabolites that may be effective against the pathogen *M. roreri*.

Although not prolific sporulators, basidiomycetes may be able to offer a biological control solution for fungal pathogens, such as frosty pod rot. Production of fruiting bodies of basidiomycete fungi may be possible with the use of *in-vitro* techniques. Griffith & Hedger (1994) developed a technique that used a bran and vermiculite medium to produce basidiomes of *M. perniciosa*. A similar method was used by Ohta & Fujiwara (2003) to produce ectomycorrhizal fungi with media that consisted of sawdust and barley grains. These techniques and others used for the commercial production of edible fungi (Oei 2003) could be developed to further aid both identification and production of basidiomycete fungi as biocontrol agents.

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