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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

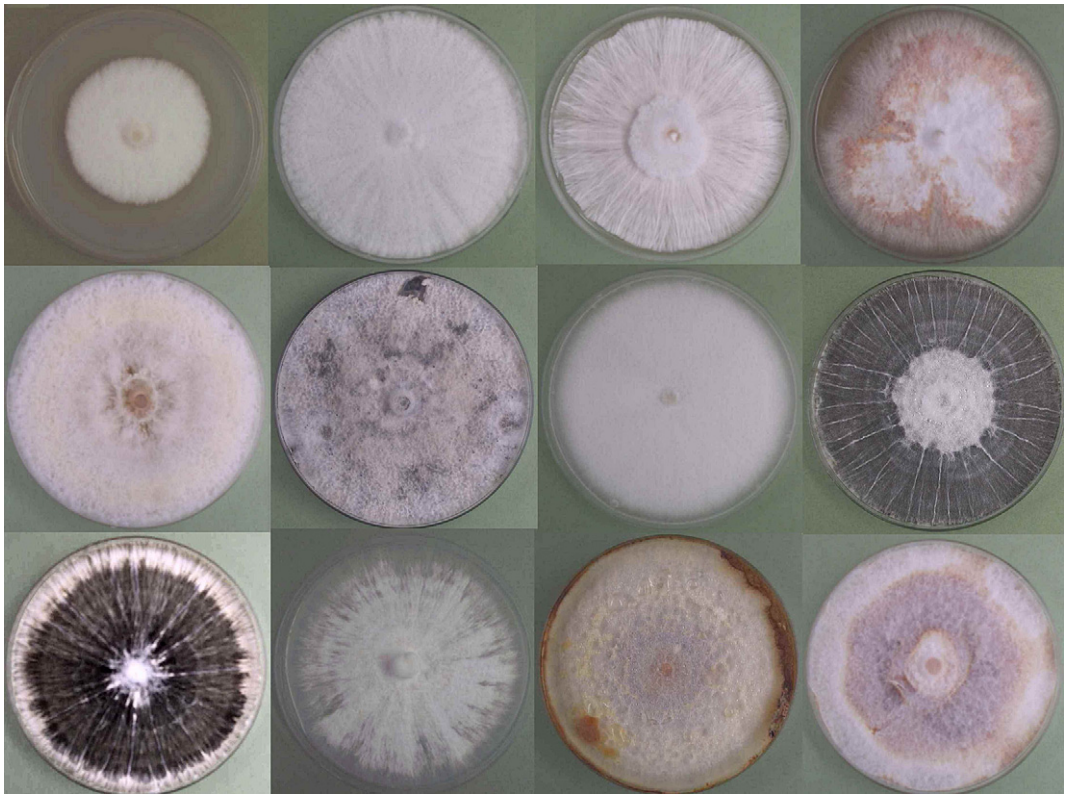

Fig 1 – Morphospecies isolated from stems of *Theobroma gileri*.