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The diverse community of leaf-inhabiting fungal endophytes from Philippine natural forests reflects phylogenetic patterns of their host plant species *Ficus benjamina*, *F. elastica* and *F. religiosa*

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ABSTRACT

Leaf-inhabiting endophytes belong to a diverse and active group of plant-associated fungi harboured in plant-rich tropical environments. Employing dilution-to-extinction cultivation and ITS sequencing, we assessed species richness, phylogeny and community composition of fungal endophytes within healthy leaves of three *Ficus* tree species (*F. religiosa*, *F. benjamina*, and *F. elastica*) naturally growing in the two Philippine forest reserves Mt. Makiling in Laguna and Mt. Palay–Palay in Cavite. Apart from a few basidiomycetes (3 orders, 6 genera), fungal isolates were abundantly ascomycetes (11 orders, 16 genera) and predominated by commonly known endophytic genera, such as *Pseudocercospora*, *Phyllosticta*, or *Penicillium*. Phylogenetic analysis revealed Capnodiales and Eurotiales as most OTU-rich clades and suggesting a high potential pathogen load in the investigated trees. Biodiversity analyses further revealed a higher similarity between the fungal species composition in the leaves of *F. benjamina* and *F. elastica* than to the one in *F. religiosa*. The observed higher abundance, species richness and similarity of the fungal community assemblage in the closely related host species *F. benjamina* and *F. elastica*, suggests an effect of host identity in structuring fungal endophytes community in the tropics.

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1. Introduction

Endophytic fungi are a diverse, polyphyletic group of primarily ascomycete species living within asymptomatic tissues of all known plant lineages in a broad range of natural and anthropogenic communities (Bazzicalupo et al. 2013; Matsumura and Fukuda 2013; Chen et al. 2015; Sato et al. 2015) either as permanent or temporary endophytic residents (Márquez et al. 2012; Hodgson et al. 2014). In the latter, fungi may switch between endophytic and necrotrophic lifestyles (Delaye et al. 2013; Stergiopoulos and Gordon 2014) or later on persist as saprobes (e.g., xylariaceous species) during leaf litter decomposition while demonstrating the capacity to utilize various carbon sources (i.e., lignin, cellulose) (Osono 2006; Osono et al. 2013). Although the extent and scale of their ecological roles across biogeographical environments are not fully known (Wani et al. 2015), most studies have synonymized fungal endophytes as primarily mutualists conferring various benefits to its host (e.g., resistance to microbial pathogens, deterrence of herbivore attacks, increase fitness against stressful environments, enhanced physiology and biomass production) (Yuan et al. 2010; Saikkonen et al. 2013).

Ikeda et al. (2014) recently reported the increases of fungal endophyte diversity across a latitudinal gradient from boreal to subtropical forests in Asia. Such a trend was previously observed along the boreal-tropical latitudes of the neotropics (Arnold and Lutzoni 2007). Surveyed neotropical forests revealed the hyperdiversity of fungal endophytes (Arnold et al. 2000, 2001, Arnold and Herre 2003; Gamboa and Bayman 2001; Arnold and Lutzoni 2007), however paleotropic forests showed contrasting diversity (Fröhlich and Hyde 1999; Suryanarayanan et al. 2002; Murali et al. 2007). Tropical fungal endophytic communities typically list a few but frequently-occurring host generalists while the majority as rare specialists (Gamboa and Bayman 2001; Cannon and Simmons 2002; Arnold and Lutzoni 2007; Suryanarayanan et al. 2011). Poor host density in tropical forests accounts for the large disproportion between fungal specialists and host species (May, 1991). Consequently, host-specificity in investigated tropical fungal guilds are generally absent (Murali et al. 2007; Parfitt et al. 2010; Tedersoo et al. 2010). Often, abiotic factors (e.g., geographic distance, climate, seasonal and spatial variations, microclimates, disturbances) are described as drivers of fungal diversity in tropical environments. For example, dissimilarity among endophyte community assemblages depended on the forest types (Suryanarayanan et al. 2011) and temperature and rainfall (Zimmerman and Vitousek 2012). Endophyte abundance and leaf fragment preferences were influenced by elevation, temperature and precipitation (Vaz et al. 2014b), while distribution patterns over local (0–100 km) and regional scales (101–5000 km) were due to geographic distance and environmental factors respectively (Vaz et al. 2014a).

The Philippine archipelago is among the most important repositories of global biodiversity with a very high concentration of endemic plant species (Myers et al. 2000). In the face of potentially novel plant-associated fungal communities and patterns of diversity persisting in these underexplored regions of the paleotropics, the knowledge pertaining to forest species diversity is virtually unknown. In addition, the high abundance of plant species typically found in forests makes host selection an important criteria for testing diversity hypothesis (i.e., host influence, site influence). Fig trees are among the largest, globally-distributed group of plants (Berg 2003). Among the hundreds of *Ficus* species, three species (*F. religiosa*, *F. elastica*, *F. benjamina*) display multiple common attributes: high distribution and abundance in local forests, relevant roles in forest ecology, high tolerance and adaptability to stress conditions, growth in several types of environments (e.g., forests, indoor environments, highly urbanized habitats, tropical greenhouses), global distribution, and phylogenetic positions within the *Ficus* genera to assess host phylogeny/identity influences on plant-associated fungal communities. To date, leaf-inhabiting fungal endophytes are unexplored in these host species. This study was conducted to elucidate the community, including diversity and richness composition of leaf-inhabiting endophytes of native *Ficus* tree species from tropical areas in the Philippines. Among the available hosts, three species were chosen to contain a pair of the closely related taxa *F. benjamina* and *F. elastica* (both sect. *Conosycea*, Rønsted et al. 2005) and a more distantly related one (*F. religiosa*, sect. *Urostigma*, Cruaud et al. 2012). Specifically, we seek to shed light on the fundamental aspect of fungal host preferences on the community level, or, in other words we want to address the following questions: does host identity influence species diversity and community composition of leaf-inhabiting fungal endophytes?

2. Materials and methods

2.1. *Host species*

The fig trees (*Ficus* spp.) belong to a comparatively large plant genus of predominantly tropical distribution. Its ca. 750 species have been grouped into six subgenera (i.e., *Ficus*, *Urostigma*, *Pharmacosycea*, *Sycomorus*, *Sycidium* and *Synoecia* (Berg 2003)). Within the subgenera *Urostigma*, *F. benjamina* and *F. elastica* belong to subsection *Conosycea* while *F. religiosa* grouped separately under subsection *Urostigma* (Rønsted et al. 2008; Cruaud et al. 2012). The three *Ficus* hosts (i.e., *F. benjamina*, *F. elastica*, *F. religiosa*) are naturally distributed within mostly tropical and a few subtropical Asia–Pacific regions (e.g., Nepal, Bhutan, India, Indonesia, China, Philippines, in other parts of Southeast Asia, North Australia, South Pacific Islands). These plant species preferably grow either on loam or sand soil but vary under varying soil pH. *Ficus elastica* adapts well to broad range tolerances (acidic to alkaline soil), however *F. benjamina* and *F. elastica* prefer slightly acidic to slightly alkaline and neutral to highly alkaline soil respectively. *Ficus* leaves are distinctly evergreen; however habit and morphology (i.e., height and leaf dimensions) differ among species. *Ficus elastica* (thick, oblong leaves, 35 × 17 cm) and *F. religiosa* (broadly ovate to ovateorbiculate leaves, 7 × 4 cm) typically grow up to 30 m high while *F. benjamina* (oblong, elliptic, lanceolate, or ovate leaves, 4 × 1.5 cm) reach heights of only 10 m. Male and female flowers of all *Ficus* host species occur separately (monoecious) in individual trees.