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Pestalotiopsis species occur as generalist endophytes in trees of Western Ghats forests of southern India

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ABSTRACT

One hundred tree species from 4 different forest types (25 from each forest) of the Western Ghats were studied for the occurrence of *Pestalotiopsis* as foliar endophytes. Morphological and ITS sequence study confirmed that species of *Pestalotiopsis* are generalist endophytes infecting taxonomically unrelated tree hosts. Furthermore, a single tree species harboured more than one species of this endophyte. Considering the leaf as a microhabitat, such a wide ecological amplitude of *Pestalotiopsis* as a foliar endophyte appears to be the result of accumulation of a suite of traits which are governed by the environment. This could be one of the reasons for the existence of generalist fungi among endophytes transcending host taxonomic and habitat restrictions.

1. Introduction

Pestalotiopsis (*Amphisphaeriaceae*, Ascomycota) is an anamorphic (coelomycete) and monophyletic genus (Jeewon et al., 2004) characterized by multicellular conidia bearing appendages (Lee et al., 2006); it is pantropical and temperate in distribution (Bate-Smith and Metcalfe, 1957). The genus *Pestalotiopsis* has numerous (at least 253) species represented as the asexual form with 13 species having the sexual morph (Maharachchikumbura et al., 2014). Species of *Pestalotiopsis* are phytopathogenic causing various diseases such as leaf spots, grey blights, leaf blights, fruit rots as well as post-harvest damage (Hyde and Fröhlich, 1995; Keith et al., 2006; Espinoza et al., 2008; Crous et al., 2011; Zhang et al., 2013), sometimes resulting in considerable economic loss (Maharachchikumbura et al., 2014). They also commonly occur as saprotrophs in the leaf litter of many plant species (Osono and Takeda, 2006; Suryanarayanan et al., 2011a; Govinda Rajulu et al., 2014; Prakash et al., 2015). Recent studies have shown that several species of *Pestalotiopsis* cause symptomless infection of plant tissues (Watanabe et al., 2010; Maharachchikumbura et al., 2012; Debbab et al., 2013) including the bark of trees (Murali et al., 2013). Such endophytic *Pestalotiopsis* have been reported in plants of different taxonomic lineages from different parts of the world including mangroves (Suryanarayanan et al., 1998, 2002), hemiparasitic plants (*Dendrophthoe falcata*) (Kumaresan et al., 2002), trees of dry thorn, dry deciduous, moist deciduous and stunted montane evergreen forests from southern India (Suryanarayanan et al., 2002), *Phyllanthus reticulatus* and *Zizyphus jujuba* (Suryanarayanan et al., 2000), ethnopharmaceutically important trees (Tejesvi et al., 2009), *Anacardium occidentale* (Suryanarayanan et al., 2005a), *Mangifera indica* (Mohandoss and Suryanarayanan, 2009) and lichens from India (Suryanarayanan et al., 2005b), orchids from Costa Rica (Tempesta et al., 2011), plants of Podocarpaceae, Theaceae and Taxaceae from China (Wei et al., 2007), and *Cocos nucifera* from Brazil (Ramos-Mariano et al., 1998). Apart from exhibiting such a wide host range and geographic distribution, *Pestalotiopsis* dominates the endophyte assemblage in many plants in terms of isolation frequency (Tejesvi et al., 2009). Species of *Pestalotiopsis* surviving as endophytes in plant tissue elaborate many novel biologically active metabolites

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(Aly et al., 2010; Xu et al., 2014; Liu et al., 2015; Wang et al., 2015) and novel industrial enzymes (Govinda Rajulu et al., 2011; Nagarajan et al., 2014). In our long term study on fungi associated with tree species of different types of forests *viz.* dry thorn (DT), dry deciduous (DD), stunted montane evergreen (EG) and moist deciduous (MD) in the Western Ghats, southern India, species of *Pestalotiopsis* were frequently encountered as foliar endophytes (Murali et al., 2007, 2013; Govinda Rajulu et al., 2014). To understand the diversity and host preference of foliar endophytic *Pestalotiopsis* in these forests, we undertook molecular characterization of 28 isolates of this fungus. These isolates were chosen to represent host tree species (i) belonging to various families and (ii) growing in four different types of forests and (iii) isolates from one tree species (*Cordia dichotoma*) growing in two different forests (DT and DD).

2. Materials and methods

2.1. Collection sites

The DT is situated in the Nilgiri Biosphere Reserve (NBR) (Latitude 11°32′ and 11°43′ N, Longitude 76°22′ and 76°45′ E) and receives an annual rainfall of about 800 mm. To its east lies the DD which is the major forest type of the Mudumalai Wildlife Sanctuary; it receives about 1000–1500 mm of rainfall per annum. Trees in DD shed their leaves seasonally and remain dormant through January and May (Kodandapani et al., 2009). The EG forests are largely restricted to the sheltered folds of the mountains and receive an annual rainfall of 1300–3000 mm (Suresh and Sukumar, 1999). The MD forests constitute about 10% of the Nilgiri landscape area. The annual rainfall here is generally above 1800 mm (Kodandapani, 2013). It has both deciduous and evergreen tree species. Collections were from sites situated immediately around NBR in private tracts of forests.

2.2. Tree hosts studied

From each of the 4 forests, 25 trees species were chosen and their leaves were screened for the presence of *Pestalotiopsis* endophytes. Trees with high and medium frequencies of occurrence were chosen for the study. All the leaves screened for endophytes were collected from a single individual tree of each species.

2.3. Fungal isolates used in the study

Foliar endophytes were isolated by following the standard procedure of surface sterilization of leaf tissues using bleach and ethanol (Suryanarayanan et al., 2003, 2011b). For each tree species, 100 surface sterilized leaf segments (0.5 cm^2) were plated on antibiotic-amended PDA medium and incubated at 26 ±1 °C for 21 d under near UV illumination (12 h dark: 12 h light regime) to induce sporulation in the fungi (Mani and Swamy, 1983; Suryanarayanan, 1992). All the endophytes which grew out from the leaf segments and having the characteristic conidia of *Pestalotiopsis* were isolated and maintained in PDA slants. Their colonization frequency was calculated following the method of Suryanarayanan and Thennarasan (2004) as follows:

$$CF\,\% = \frac{\text{Number of segments colonized by each endophyte}}{\text{Total number of segments observed}} \times 100$$

Twenty eight foliar endophyte isolates of *Pestalotiopsis* identified at the genus level based on conidial morphology from the above study were selected for molecular characterization. These had been isolated from leaves of 19 tree hosts belonging to 13 dicotyledonous families and growing in the four different types of forests; three isolates were from the leaves of three individual trees of DD, nine were from nine individual trees of DT; six isolates were obtained from six individual trees of EG and 10 isolated from 10 individual trees of MD forest (Table 2).

2.4. Fungal genomic DNA extraction

For fungal genomic DNA extraction, the phenol-chloroform method was followed. Briefly, fresh mycelia were collected from 7d old fungal cultures grown on Czapek Dox Agar medium and transferred to a sterile microfuge tube. To the mycelium, 500 μL of DNA extraction buffer (0.1 M NaCl, 50 mM Tris, 10 mM Na_2EDTA, 2% SDS, pH 8.0) was added and ground for 15 min using a sterile glass rod. 500 μL of chilled phenol was added to this mix and centrifuged at 15,300 *g* for 15 min at 4 °C. From this, the upper aqueous phase (200–300 μL) was collected in a fresh tube and an equal volume of chilled chloroform: isoamylalcohol (24: 1) was mixed. The tubes were again centrifuged at 15,300 *g* for 15 min at 4 °C and the aqueous phase was collected in a fresh microfuge tube. To this aqueous phase, 0.6 volume of chilled isopropanol and 1/10th volume (of the aqueous phase) of 3 M sodium acetate was added and gently mixed. The tubes were kept at −80 °C for 2 h and centrifuged at 15,300 *g* for 15 min at 4 °C and the resultant supernatant was discarded. The pellet was washed with 200 μL of 70% ethanol followed by centrifugation at 15,300*g* for 10 min at 4 °C. The pellet was air-dried and resuspended with 50 μL of sterile distilled water before storing at −20 °C.

2.5. PCR amplification and sequencing of ITS region

The 5.8S rDNA and its flanking ITS regions were amplified using fungal specific primers ITS4 and ITS5 (White et al., 1990). The reaction volume consisted of 10X PCR buffer, forward and reverse primers (10 μM each), 4 mM dNTPs, 1 Unit of Taq DNA Polymerase, 1% DMSO, 25 mM $MgCl_2$ and approx. 50 ng of fungal DNA as template. The polymerase chain reaction was carried out in a Master Cycler Thermocycler (Eppendorf, USA) with the following cycling conditions: 3 min of initial denaturation at 94 °C, 34 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 10 min. The amplified products were run on a 1.5% agarose gel to determine the size and purity of the product. The PCR products were purified using gel elution method and sequenced using ITS4 primer in ABI 3130 Genetic Analyzer. The obtained sequences were edited manually and searched for the closest match in the NCBI (BLAST algorithm) and CBS databases. The sequences were submitted to GenBank and accession numbers were obtained (Table 2) A BLASTn search was performed to find similar sequences in the GenBank of NCBI database relative of the newly sequenced endophytic isolates. Sequences retrieved from GenBank were added to the alignments. Sequences were aligned by using the software MAFFT program (http://mafft.cbrc.jp/alignment/server/) and alignment was manually corrected. A phylogenetic tree was reconstructed using MEGA6 (Tamura et al., 2013) software. The Kimura two-parameter model was used to estimate evolutionary distance. The phylogenetic tree was reconstructed using maximum parsimony with bootstrap values calculated from 1000 replicate runs.

3. Results

Of the 100 tree species screened from all the 4 forests (25 from each forest), *Pestalotiopsis* spp. were isolated as foliar endophytes

Table 1
Endophytic *Pestalotiopsis* isolated from leaves of trees growing in four different types of forests (n = 100).

DT	CF%	DD	CF%	EG	CF%	MD	CF%
Acacia ferruginea	–	*Anogeissus latifolia*	27.3	*Cinnamomum malabatrum*	–	*Butea monosperma*	16.0
Anogeissus latifolia	–	*Careya arborea*	4.7	*Cryptocarya bourdillonii*	6.7	*Careya arborea*	16.0
Bauhinia racemosa	–	*Casearia esculenta*	–	*Daphniphyllum neilgherrense*	–	*Casearia esculenta*	1.0
Bridelia retusa	4.7	*Cassia fistula*	–	*Euonymus angulatus*	2.0	*Cassia fistula*	–
Butea monosperma	2.0	*Catunaregam spinosa*	–	*Eurya nitida*	0.7	*Cinnamomum malabatrum*	4.0
Cassia fistula	1.3	*Cordia obliqua*	–	*Glochidion zeylanicum*	0.7	*Cordia obliqua*	1.0
Cordia dichotoma	0.7	*Cordia dichotoma*	0.7	*Ilex denticulata*	0.7	*Dalbergia latifolia*	1.0
Dalbergia lanceolaria	–	*Dalbergia oojeinensis*	–	*Ilex wightiana*	–	*Dalbergia oojeinensis*	1.0
Diospyros montana	–	*Gmelina arborea*	–	*Isonandra candolleana*	16.0	*Diospyros montana*	4.0
Ehretia canarensis	–	*Grewia tiliifolia*	4.7	*Lasianthus venulosus*	–	*Elaeocarpus serratus*	9.0
Elaeodendron glaucum	0.7	*Helicteres isora*	–	*Ligustrum roxburghii*	0.7	*Kydia calycina*	–
Erythroxylum monogynum	–	*Kydia calycina*	–	*Litsea floribunda*	–	*Lagerstroemia microcarpa*	41.0
Givotia rottleriformis	–	*Lagerstroemia microcarpa*	–	*Litsea stocksii*	4.7	*Murraya paniculata*	1.0
Gmelina asiatica	–	*Lagerstroemia parviflora*	17.3	*Meliosma simplicifolia*	2.0	*Olea dioica*	3.0
Ixora nigricans	16.0	*Phyllanthus emblica*	–	*Memecylon malabaricum*	–	*Olea glandulifera*	2.0
Maytenus emarginata	4.7	*Premna tomentosa*	–	*Michelia nilagirica*	–	*Persea macrantha*	3.0
Pongamia pinnata	1.3	*Radermachera xylocarpa*	–	*Neolitsea zeylanica*	1.3	*Salix tetrasperma*	1.0
Premna tomentosa	–	*Schrebera swietenioides*	2.7	*Phoebe lanceolata*	4.0	*Semecarpus anacardium*	–
Pterocarpus marsupium	–	*Shorea roxburghii*	16.0	*Psychotria bisulcata*	–	*Schrebera swietenioides*	1.0
Randia dumetorum	0.7	*Stereospermum personatum*	2.7	*Rhodomyrtus tomentosa*	–	*Stereospermum tetragonum*	2.0
Stereospermum angustifolium	–	*Syzygium cummini*	8.0	*Symplocos cochinchinensis*	0.7	*Syzygium cumini*	20.0
Strychnos potatorum	0.7	*Tectona grandis*	0.7	*Symplocos obtusa*	1.3	*Terminalia bellirica*	9.0
Terminalia chebula	–	*Terminalia alata*	8.7	*Syzygium densiflorum*	3.3	*Terminalia crenulata*	6.0
Ziziphus jujuba	–	*Terminalia crenulata*	–	*Turpinia nepalensis*	–	*Viburnum punctatum*	2.0
Zizyphus xylopyrus	1.3	*Vitex altissima*	0.7	*Vepris bilocularis*	–	*Withania somnifera*	–

– = *Pestalotiopsis* absent; CF% = colonization frequency %.

Table 2
Pestalotiopsis fungal isolates used in this study, their hosts, habitat and NCBI accession numbers.

Fungal isolates	Host	Family	Forest type	Accession no.
P. vismiae EGRC1	*Cryptocarya wightiana*	Laureaceae	Evergreen	KT589393
P. vismiae DTRC2	*Bridelia retusa*	Euphorbiaceae	Dry thorn	KT589394
P. vismiae EGRC3	*Elaeocarpus serratus*	Elaeocarpaceae	Evergreen	KT589395
P. vismiae EGRC4	*Glochidion zeylanicum*	Euphorbiaceae	Evergreen	KT589396
P. parva EGRC5	*Macaranga peltata*	Euphorbiaceae	Evergreen	KT589397
P. vismiae EGRC6	*Ligustrum roxburghii*	Oleaceae	Evergreen	KT589398
P. vismiae EGRC7	*Symplocos obtusa*	Symplocaceae	Evergreen	KT589399
N. mesopotamica DTRC8	*Bridelia retusa*	Euphorbiaceae	Dry Thorn	KT589400
P. microspora MDRC9	*Butea monosperma*	Fabaceae	Moist deciduous	KT589401
N. cubana MDRC10	*Careya arborea*	Barringtoniaceae	Moist deciduous	KT589402
P. microspora MDRC11	*Casearia esculenta*	Flacourtiaceae	Moist deciduous	KT589403
N. piceana MDRC12	*Cinnamomum malabatrum*	Lauraceae	Moist deciduous	KT589404
P. menezesiana DD1RC13	*Cordia dichotoma*	Boraginaceae	Dry deciduous	KT589405
N. cubana DD2RC14	*Cordia dichotoma*	Boraginaceae	Dry deciduous	KT589406
N. mesopotamica DD3RC15	*Cordia dichotoma*	Boraginaceae	Dry deciduous	KT589407
N. saprophytica DT1RC16	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589408
P. microspora DT2RC17	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589409
P. microspora DT3RC 18	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589410
N. australis DT4RC19	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589411
N. mesopotamica DT5RC 20	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589412
P. theae DTRC21	*Cordia dichotoma*	Boraginaceae	Dry thorn	KT589413
N. ellipsospora MDRC22	*Diospyros montana*	Ebenaceae	Moist deciduous	KT589414
P. microspora DTRC23	*Gymnosporia emarginata*	Celastraceae	Dry thorn	KT589415
P. microspora MDRC24	*Olea dioica*	Oleaceae	Moist deciduous	KT589416
P. microspora MDRC25	*Dalbergia oojeinensis*	Fabaceae	Moist deciduous	KT589417
P. microspora MDRC26	*Persea macrantha*	Lauraceae	Moist deciduous	KT589418
N. cubana MDRC27	*Stereospermum tetragonum*	Bignoniaceae	Moist deciduous	KT589419
P. microspora MDRC28	*Viburnum punctatum*	Caprifoliaceae	Moist deciduous	KT589420

from 11, 12, 14, and 21 tree species of DT, DD, EG and MD forest respectively (Table 1). The minimum and maximum CF% of the endophytes ranged from 0.7 (*C. dichotoma, Tectona grandis*, *Vitex altissima*) to 27.3 (*Anogeissus latifolia*) in DD, 0.7 (*C. dichotoma*, *Elaeodendron glaucum*, *Randia dumetorum*, *Strychnos potatorum*) to 16.0 (*Ixora nigricans*) in DT, 0.7 (*Eurya nitida*, *Glochidion zeylanicum*, *Ilex denticulata*, *Ligustrum roxburghii*, *Symplocos cochinchinensis*) to 16.0 (*Isonandra candolleana*) in EG and 1.0 (*Casearia esculenta*, *Cordia obliqua*, *Dalbergia latifolia*, *Dalbergia oojeinensis*, *Murraya paniculata*, *Salix tetrasperma, Schrebera swietenioides)* to 41.0 (*Lagerstroemia microcarpa*) in MD forest trees (Table 1).

Identification of selected isolates at the species level using molecular method helped in discerning the status of foliar endophytic *Pestalotiopsis* in these forests. ITS sequencing approach revealed that the 28 isolates assumed to be species of *Pestalotiopsis* based on spore characteristics actually constituted two genera *viz*. *Pestalotiopsis* (18 isolates) and *Neopestalotiopsis* (10 isolates) (Table 2). Phylogenetic analysis clustered all the sequences into four

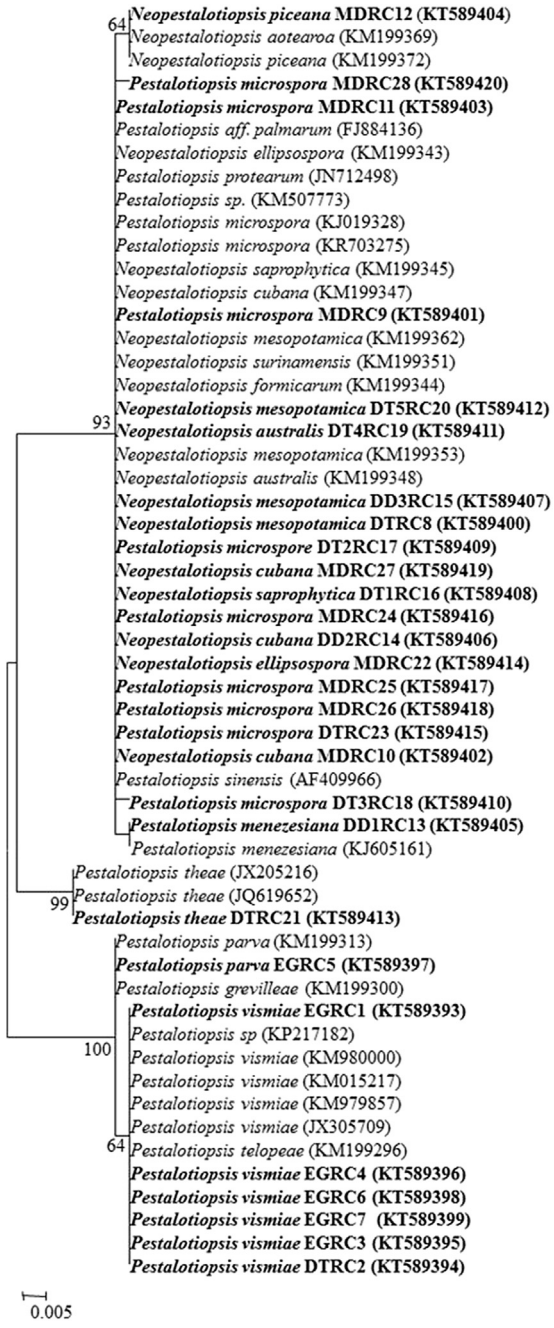

Fig. 1. Phylogenetic tree based on ITS sequences of *Pestalotiopsis* showing the relationship between isolates of the present study and representatives of other related taxa. Numerical values indicate bootstrap percentile from 1000 iterations.

groups (Fig. 1). Group I consisted of different species of *Pestalotiopsis* and *Neopestalotiopsis*. Of the 28 isolates, 20 isolates clustered in this group. *Pestalotiopsis theae* DTRC21 formed group II. *Pestalotiopsis parva* EGRC5 formed group III, and *Pestalotiopsis vismiae* clustered as group IV which included isolates of EGRC1, 3, 4, 6, 7 and DTRC2.

Of the 18 isolates of *Pestalotiopsis*, six were from MD forest, six from EG, five from DT and one from DD forest. Of the 10 isolates of *Neopestalotiopsis* identified, two were from DD forests and 4 each were from DT and MD forests. Nine of the 18 *Pestalotiopsis* isolates belonged to *Pestalotiopsis microspora* and were endophytic in the leaves of trees belonging to different families such as Boraginaceae, Caprifoliaceae, Celastraceae, Fabaceae, Flacourtiaceae, Lauraceae and Oleaceae (Table 2). Six of the isolates were identified as belonging to *P. vismiae* and were isolated from trees of families such as Elaeocarpaceae, Euphorbiaceae, Lauraceae, Oleaceae and Symplocaceae. There was one isolate each belonging to *Pestalotiopsis menezesiana* (isolated from *C. dichotoma* of Boraginaceae), *P. parva* (from *Macaranga peltata* of Euphorbiaceae) and *P. theae* (from *C. dichotoma* of Boraginaceae). Of the 10 *Neopestalotiopsis* isolates, three belonged to *Neopestalotiopsis cubana* (obtained from the leaves of *Careya arborea* of Barringtoniaceae, *C. dichotoma* of Boraginaceae and *S. tetragonum* of Bignoniaceae) and three were *Neopestalotiopsis mesopotamica* (from *C. dichotoma* of Boraginaceae and *Bridelia retusa* of Euphorbiaceae). There was one species each of *Neopestalotiopsis australis* (from *C. dichotoma*), *Neopestalotiopsis ellipsospora* (from *Diospyros montana* of Ebenaceae), *Neopestalotiopsis piceana* (from *Cinnamomum malabatrum* of Lauraceae), and *Neopestalotiopsis saprophytica* (from *C. dichotoma*). Six individual trees of *C. dichotoma* growing in DT forest had *P. microspora, P. theae, N. saprophytica, N. australis* and *N. mesapotomica* as foliar endophytes. Similarly three individuals of this tree from DD forest supported *P. menezesiana, N. cubana* and *N. mesopotomica* as endophytes. *P. vismiae* and *N. mesopotamica* were present in two individuals of *B. retusa* of DT forest; similarly, *P. menezesiana, P. microspora* and *P. theae* were isolated from *C. dichotoma* individuals from DT and *N. australis, N. cubana, N. mesopotamica,* and *N. saprophytica* were present in this individual from DT and DD forests. Species of *Neopestalotiopsis* and *P. microspora*, although most prevalent, were absent in EG.

4. Discussion

In our long term study extending over 25 y on the foliar endophytes of trees of different types of forests of the southern Western Ghats, it was observed that certain species of fungi including *Colletotrichum* (Suryanarayanan et al., 2011b), *Phomopsis* (Murali et al., 2006), *Phyllosticta* (Pandey et al., 2003) and *Xylaria* (Govinda Rajulu et al., 2013) had a wide host affiliation and occurred in taxonomically disparate plant host species. Here, we report that species of *Pestalotiopsis* are also recurrent as foliar endophytes in these forests. We observed that the CF% of *Pestalotiopsis* endophytes varied from low (0.7%) to high (41.0%) depending on the forest type and tree host. This is consistent with the observation of Wei et al. (2007) on endophytic *Pestalotiopsis* of Podocarpaceae, Theaceae and Taxaceae in southern China.

Earlier studies have also shown that *Pestalotiopsis* species are not host specific (Jeewon et al., 2004; Wei et al., 2005; Tejesvi et al., 2009) and hence its identification based on host association is not desirable (Wei et al., 2007; Maharachchikumbura et al., 2011). Conidial characters are more reliable in distinguishing species of *Pestalotiopsis*; ITS-RFLP has been used for identifying *Pestalotiopsis* species (Liu et al., 2010) and for distinguishing strictly endophytic species from pathogenic ones (Jeewon et al., 2002, 2003, 2004; Wei et al., 2007; Tejesvi et al., 2009). Hence we identified these endophytes based on both conidial morphology and ITS sequences. Although the conidial features were characteristic of *Pestalotiopsis*, we could not distinguish between *Pestalotiopsis* and *Neopestalotiopsis* based on morphology. The ITS methodology indicated that some of these isolates are *Neopestalotiopsis*. More detailed molecular studies are needed to confirm this.

Our results show that species of *Pestalotiopsis sensu lato* (including the newly established *Neopestalotiopsis*) are wide spread as foliar endophytes in the four different forests we studied. This indicated the wide ecological amplitude of this genus since the forests from which they were isolated differed in their altitude, rainfall received and tree host species they supported. This was

especially true of *P. microspora* which had a wide host and habitat range. This fungus occurred as an endophyte in tree species belonging to unrelated orders such as Boraginales, Celastrales, Dipsacales, Fabales, Lamiales, Laurales and Malpighiales (according to Angiosperm Phylogeny-APGIII). Furthermore, it is clear that in WG forests a single tree species could support different *Pestalotiopsis* spp., as endophytes since *N. australis, N. cubana, N. mesopotamica, N. saprophytica, P. menezesiana, P. microspora* and *P. theae* were all isolated as foliar endophytes from one plant host species *viz., C. dichotoma*. Indeed *P. microspora* has been isolated from *Ananas comosus*, *Araucaria* sp., *Carya* sp., *Hedera helix, Juniperus bermudiana* and *Platanus occidentalis* earlier (Guba, 1961). Phylogenetic analysis revealed that *P. microspora* clustered into one group though they were isolated from different forests.

Such a wide host range of this endophytic fungus attests its larger ecological amplitude underscored by the ability of its many species to counter a variety of defense chemicals present in these plants. Here, based on our current and previous studies, we attempt to explain this common observation of the wide host range of some endophytes. We observed a suite of ecophysiological traits especially for *P. microspora* which explain this. The DD, DT and MD forests of the Western Ghats in the study area experience periodic ground fires due to prolonged dry periods and accumulation of dry biomass in the form of leaf litter on the forest floor. The fire regimes affect significantly the plant species diversity and their regeneration in these forests (Kodandapani et al., 2009). Several *Pestalotiopsis* species including *P. microspora* produce thermotolerant conidia which survive exposure to dry heating (temperature above 100 °C) for several hours (Suryanarayanan et al., 2011a). We observed that several endophytic *Pestalotiopsis* species including *P. microspora* like other endophytes (Kumaresan and Suryanarayanan, 2002; Okane et al., 2008) shift to a saprotrophic mode of life style and continue to survive in the leaf litter; they also produce cell wall degrading enzymes to facilitate their survival as saprotrophs in the plant litter (Prakash et al., 2015). Furthermore, they could utilize toxic furaldehydes, the most common and most abundant organic volatiles released during biomass burning, as a carbon source (Govinda Rajulu et al., 2014). It is conceivable that accumulation of such traits by endophytes of dry seasonal forests subjected to periodic ground fires, aids them in amplifying their ecological niche by infecting taxonomically disparate plant species (Govinda Rajulu et al., 2014). Suryanarayanan et al. (2011a) proposed that the multi host endophytes of the fire-prone Western Ghats forests have an active saprobic stage of life cycle while functioning as pioneer litter decomposers during which they sporulate and infect the plants to reinitiate the endophyte stage.

Two isolates of *P. theae* from different hosts from two different forest types (MD and DT) clustered into two groups probably indicating some host specificity; however, increased sampling is needed to confirm this. Though in general, host sharing by a fungus should decline as a function of phylogenetic distance between the plant hosts (Webb et al., 2008), it is now established that overlapping ecological niches rather than the taxonomy of the host determines the distribution of endophytes in an ecosystem (Mohali et al., 2005; Suryanarayanan et al., 2011b; Zimmerman and Vitousek, 2012; Rojas-Jimenez et al., 2016) and may even be responsible for inter-kingdom host jumping among fungi (Nikoh and Fukatsu, 2000). Detailed phylogenetic analyses of both the endophytic *Pestalotiopsis* spp. and their plant hosts are needed to discern the mode of cospeciation after host switching in these fungi (Herrera et al., 2016).

Our study indicates that *Pestalotiopsis* species have evolved as generalist endophytes owing to the development of certain traits as a response to the environment and such traits have aided the amplification of their plant host range.

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