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# Multi-locus phylogeny reveals three new species of *Diaporthe* from Thailand

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**Abstract** – Species of *Diaporthe* are important phytopathogens with wide host ranges and a global distribution. In the present study multi-locus phylogeny based on combined sequences of rDNA ITS, and partial sequences from the translation elongation factor 1-α (EF 1-α), β tubulin (TUB) and calmodulin (CAL) genes, reveal three new species from fresh collections made in northern Thailand. The new species *Diaporthe siamensis*, *D. thunbergii* and *D. pterocarpicola* are introduced in this paper with full descriptions and comparison with similar taxa. *Phomopsis pterocarpi* which is epitypified and synonymised under *Diaporthe pterocarpi* is described based on a collection from northern Thailand.

**Endophyte / Host diversity / New combination / *Phomopsis* / Phytopathogen / Taxonomy**

## INTRODUCTION

*Diaporthe* Nitschke (asexual state *Phomopsis* (Sacc.) Bubák) comprises important phytopathogens, often with wide host ranges and distributions (Uecker, 1988; Rossman *et al*., 2007; Crous, 2005; Udayanga *et al*., 2011, 2012, Cowley *et al*., 2012). Species recognition criteria in *Diaporthe* have historically been based on morphology, culture characteristics and host affiliation (Rehner and Uecker, 1994; Mostert *et al*., 2001; van Niekerk *et al*., 2005; Murali *et al*., 2006; Santos & Phillips, 2009). The current state of taxonomic knowledge of *Diaporthe* effectively means that strains can be identified to species level only if molecular techniques are employed (Santos *et al*., 2010). Udayanga *et al*. (2012) provided a multi-locus phylogenetic study of *Diaporthe* using combined sequences of rDNA ITS, and partial sequences of EF1-α, TUB and CAL genes.

We are restudying and carrying out inventories of the plant pathogens of northern Thailand and China based on morphology and molecular sequence analysis (KoKo *et al.*, 2011) and are discovering many new cryptic species in

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genera such as *Colletotrichum* (e.g. Yang *et al.*, 2011, Wikee *et al.*, 2011a) and *Cryptosporiopsis* (e.g. Cheewangkoon *et al.*, 2011). In this study we place fresh collections of *Diaporthe* from Thailand in the rDNA ITS, and partial sequences of EF1-a, TUB and CAL gene backbone tree and as a result describe three novel cryptic species and one species combination. We opt to use the older name *Diaporthe* over the asexual "Phomopsis" name concerning the one name for one biological species (Hawksworth 2012), as already applied for the genus by Santos *et al.* (2010) and Udayanga *et al.* (2011, 2012).

## MATERIALS AND METHODS

***Collection and isolation of fungi:*** Plant pathogenic, endophytic and saprobic strains of *Diaporthe* were collected in field surveys in different locations from various hosts in Chiang Rai and Chiang Mai Provinces in northern Thailand (Table 1). Specimens with disease symptoms were observed under a stereo microscope and conidia from sporulating pycnidia were used for single spore isolation following a modified spore suspension method as described for different fungal groups (Choi *et al.*, 1999; Chomnunti *et al.*, 2011). The sporulating pycnidia (of asexual state) or ascomata (for sexual state) were excised using a sterile needle, placed in a few drops of sterile distilled water on a glass slide, and broken with the needle. The spore suspension was then transferred to water agar (WA) plates. The inoculated WA plates were incubated for 24 hours and germinating single spores were then transferred to malt extract agar (MEA) plates and incubated at 25ºC in the dark. Endophytic *Diaporthe* strains were isolated using the protocol outlined by Murali *et al.* (2006). All fresh cultures were deposited in Mae Fah Luang University Culture Collection (MFLUCC) and herbarium material in MFLU. Duplicate cultures were deposited in BCC and CBS, the latter under Material Transfer Agreement (MTA: C27/2011). Details of nomenclatural novelties were added to MycoBank (Crous *et al.*, 2004).

***Morphology:*** Morphological descriptions are based on sporulating pycnidia on WA+alfalfa stem, and for the sterile cultures as observed on the original host. Wherever possible, 20-30 measurements (× 40 and × 100 magnication) were made of structures mounted in 5% KOH, using a compound light microscope (Nikon Elipse 80i). The extremes of measurements are given in parentheses. Three duplicate cultures of each isolate were used for determining colony characters on potato-dextrose agar (PDA, Difco) at 25ºC in the dark following the methods of Brayford (1990). Colony diameters on PDA and MEA were recorded at intervals of 24 hours for 7 days and used to calculate the growth rate of four replicates per isolate. After 7 days, colony size and colour of the colonies (Rayner, 1970) and zonation were recorded.

***Molecular methods and phylogenetic analysis:*** DNA extraction, gene amplification, sequencing, sequence alignment are as described in Udayanga *et al.* (2012). Combined sequences of rDNA ITS, and partial sequences of EF1-α, TUB and CAL genes were used in phylogenetic analysis. PAUPv 4.0b10 was used to conduct the parsimony analysis to obtain the phylogenetic trees. Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree

Table 1. Isolates and GenBank accessions used in this study

| *Collection Code* | *Identity* | *Host* | *Country of Origin* | *GenBank Accession numbers* | | | |
|---|---|---|---|---|---|---|---|
| | | | | *rDNA ITS* | *EF1-α* | *TUB* | *CAL* |
| CBS 439.82 | *D. cotoneastri* | *Cotoneaster* sp. | UK, Scotland | FJ889450 | GQ250341 | JX275437 | JX197429 |
| DNP 128 | *D. castaneae-mollissimae* | *Castanea mollissima* | China | JF57786 | JX275401 | JX275438 | JX197430 |
| DNP 129 | *D. castaneae-mollissimae* | *Castanea mollissima* | China | JQ619886 | JX275402 | JX275439 | JX197431 |
| CBS 160.32 | *D. vaccinii* | Oxycoccus macrocarpus | USA | AF317578 | GQ250326 | JX275436 | n.d. |
| MFLUCC 10- 0576a | *D. thunbergii* | *Thunbergia laurifolia* | Thailand | JQ6198893 | JX275409 | JX275449 | JX197440 |
| MFLUCC 10- 0576b | *D. thunbergii* | *Thunbergia laurifolia* | Thailand | JQ619894 | JX275410 | JX275450 | JX197441 |
| MFLUCC 10- 0576c | *D. thunbergii* | *Thunbergia laurifolia* | Thailand | JQ619895 | JX275411 | JX275451 | JX197442 |
| CBS 109745 | *D. perjuncta* | *Ulmus glabra* | Austria | AY485785 | GQ250323 | JX275453 | JX197444 |
| CBS 161.64 | *D. phoenicicola* | *Areca catechu* | India | FJ889452 | GQ250349 | JX275440 | JX197432 |
| MFLUCC 10-0609 | *Diaporthe* sp. | *Mangiferra* sp. | Thailand | JQ619892 | JX275408 | JX275446 | JX197437 |
| MFLUCC 10-0587 | *Diaporthe* sp. | *Tectona grandis* | Thailand | JQ619890 | JX275406 | JX275444 | JX197436 |
| MFLUCC 10-0590 | *Diaporthe* sp. | *Cassia spectabilis* | Thailand | JQ619891 | JX275407 | JX275445 | n.d. |
| MFLUCC 10-0580a | *D. pterocarpicola* | *Pterocarpus indicus* | Thailand | JQ619887 | JX275403 | JX275441 | JX197433 |
| MFLUCC 10-0580b | *D. pterocarpicola* | *Pterocarpus indicus* | Thailand | JQ619888 | JX275404 | JX275442 | JX197434 |
| MFLUCC 10-0583 | *Diaporthe* sp. | *Tectona grandis* | Thailand | JQ619889 | JX275405 | JX275443 | JX197435 |
| MFLUCC 10-0571 | *D. pterocarpi* | *Pterocarous indicus* | Thailand | JQ619899 | JX275416 | JX275460 | JX197451 |
| MFLUCC 10-0575 | *D. pterocarpi* | *Pterocarous indicus* | Thailand | JQ619901 | JX275418 | JX275462 | JX197453 |
| MFLUCC-10-0588 | *D. pterocarpi* | *Magnolia* sp. | Thailand | JQ619900 | JX275417 | JX275461 | JX197452 |
| CBS 187.27 | *D. neotheicola* | *Camelia sinesis* | Italy | DQ286287 | DQ286261 | JX275463 | n.d. |
| CBS 123208 | *D. neotheicola* | *Foeniculum vulgare* | Portugal | EU814480 | GQ250315 | JX275464 | n.d. |
| MFLUCC 10-0601 | *Diaporthe* sp. | *Coffea arabica* | Thailand | JQ619902 | JX275419 | JX275466 | JX197455 |
| MFLUCC 10-0584 | *Diaporthe* sp. | *Tectona grandis* | Thailand | JQ619884 | JX275398 | n.d. | n.d. |
| MFLUCC10-0582 | *Diaporthe* sp. | *Aeschynanthus radicans* | Thailand | JQ619885 | JX275399 | JX275433 | JX197426 |
| MFLUCC 10-0570 | *Diaporthe* sp. | Dead wood | Thailand | JQ619877 | JX275391 | JX275428 | JX197421 |
| DEN 009 | *Diaporthe* sp. | *Tectona grandis* | Thailand | JQ619882 | JX275397 | JX275432 | JX197425 |
| MFLUCC 10-0573a | *D. siamensis* | *Dasymaschalon* sp. | Thailand | JQ619879 | JX275393 | JX275429 | JX197423 |
| MFLUCC 10-0573b | *D. siamensis* | *Dasymaschalon* sp. | Thailand | JQ619880 | JX275395 | JX275430 | JX197423 |
| MFLUCC 10-0573c | *D. siamensis* | *Dasymaschalon* sp. | Thailand | JQ619881 | JX275396 | JX275431 | JX197424 |
| MFLUCC 10-0589 | *Diaporthe* sp. | *Magnolia* sp. | Thailand | JQ619878 | JX275392 | n.d. | n.d. |
| MFLUCC 10-0581 | *Diaporthe* sp. | *Rhapis* sp. | Thailand | JQ619883 | JX275394 | n.d. | n.d. |

MFLUCC: Mae Fah Luang University Culture Collection CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands DNP/DEN: First author's personal collection (deposited in MFLUCC), n.d.: not determined, References for the sequences Udayanga *et al.*, 2012.

Length [TL], Consistency Index [CI], Retention Index [RI], Related Consistency Index [RC] and Homoplasy Index [HI]) were calculated for trees generated under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino & Hasegawa 1989) were performed in order to determine whether trees were significantly different. Trees were figured in Treeview (Page, 1996). We analyzed the newly generated sequences with all available type derived sequences listed in Udayanga *et al*., (2011) and (2012) a sub-set of cryptic taxa were selected to represent the combined phylogenetic tree presented here (Fig. 1). Bayesian analysis was performed as described in Phillips *et al*., (2007), setting burn-in at 2000 generations.

## RESULTS

***Combined ITS, EF1-α, CAL and β- tubulin gene analysis:*** The combined gene data matrix contains 31 taxa including the out group. The statistics for the parsimony analysis revealed that from the remaining 1824 characters 996 characters are constant, 539 characters are parsimony informative while 289 variable characters are parsimony-uninformative (126 characters are excluded). The parsimony analysis of alignment yielded one equally parsimonious trees and which was recognized as the best tree and presented here (TL = 1721, CI = 0.668, RI =, 0.822, RC = 0.549, HI = 0.332). Based on the combined phylogenetic tree, we recognized that all the ex-type derived taxa are placed in terminal clades with higher bootstrap values without any conflict between phylogenetic species delimitation. Three distinct species were distinguished based on phylogenetic inferences coupled with morphological features and are described below. A modern description of *Diaporthe pterocarpi* (≡ *Phomopsis pterocarpi*) is provided.

## TAXONOMY

***Diaporthe siamensis*** Udayanga, X. Z. Liu & K.D. Hyde, **sp. nov.** **Figs 2a-o**

*MycoBank:* MB 800826.

*Etymology:* Siam, former name for Thailand where fungus was first recognized.

***Pycnidia*** associated with necrotic leaf tissue; stem, up to 70 µm diam, 50 µm high, erumpent, with slightly elongated black necks, mostly submerged in tissue; walls consisting of 2-4 layers of medium dark brown *textura angularis*; yellowish white, spiral conidial cirri exuding from ostioles; walls consisting of 2-4 layers of medium dark brown *textura angularis*. ***Conidiophores*** hyaline, branched, densely aggregated, cylindrical-filiform, straight to sinuous, 7-18 × 1-1.5 µm. ***Conidiogenous cells*** phialidic, cylindrical, terminal and lateral, with slight taper towards apex, 0.5-1 µm diam, with visible periclinal thickening; collarette not seen. ***Paraphyses*** present, hyaline, sub-cylindrical, septate, extending above conidiophores, straight, flexuous, branched, up to 20 µm long, 1-1.5 µm wide at base. ***Alpha conidia*** aseptate, hyaline, smooth, mono- or biguttulate, ellipsoidal-fusiform, base subtruncate, (3.5-)4-5(-6) × (2-)2.5(-3) µm.

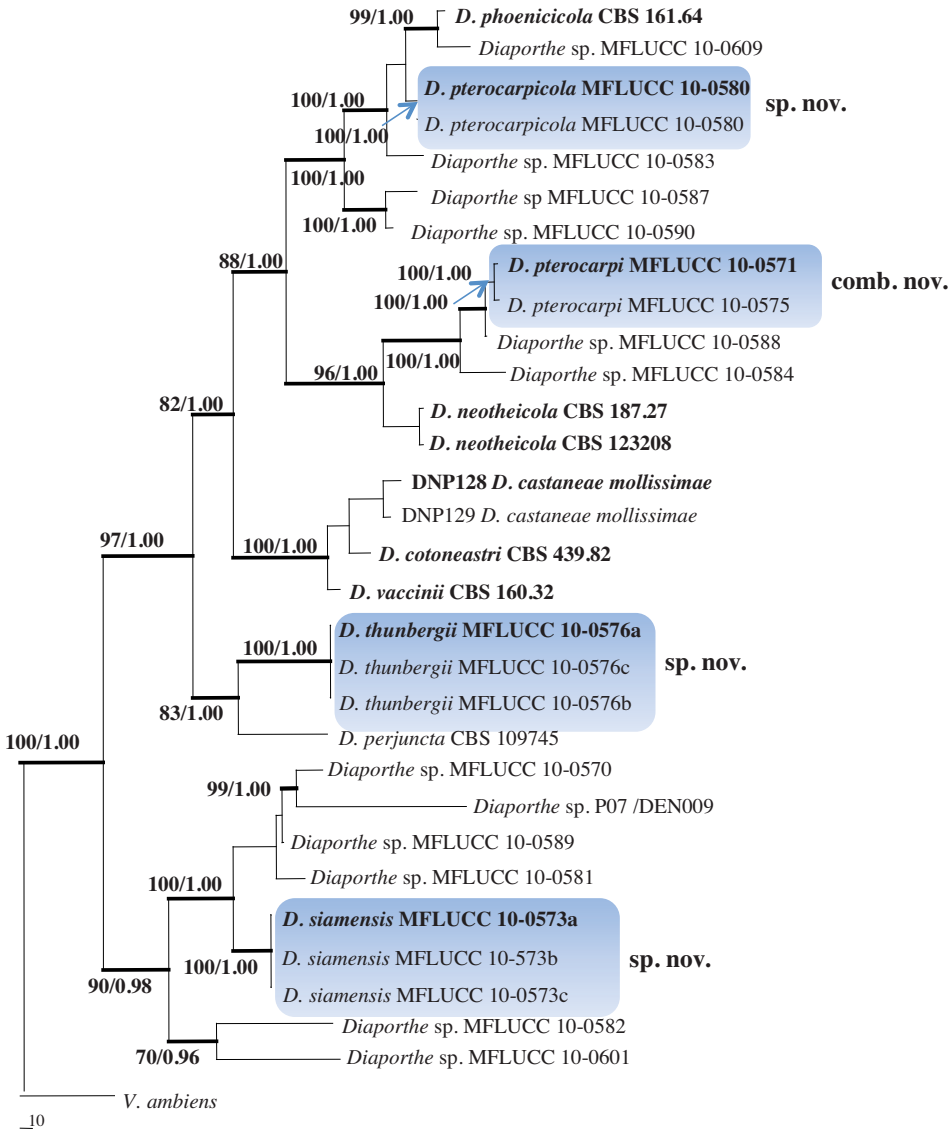
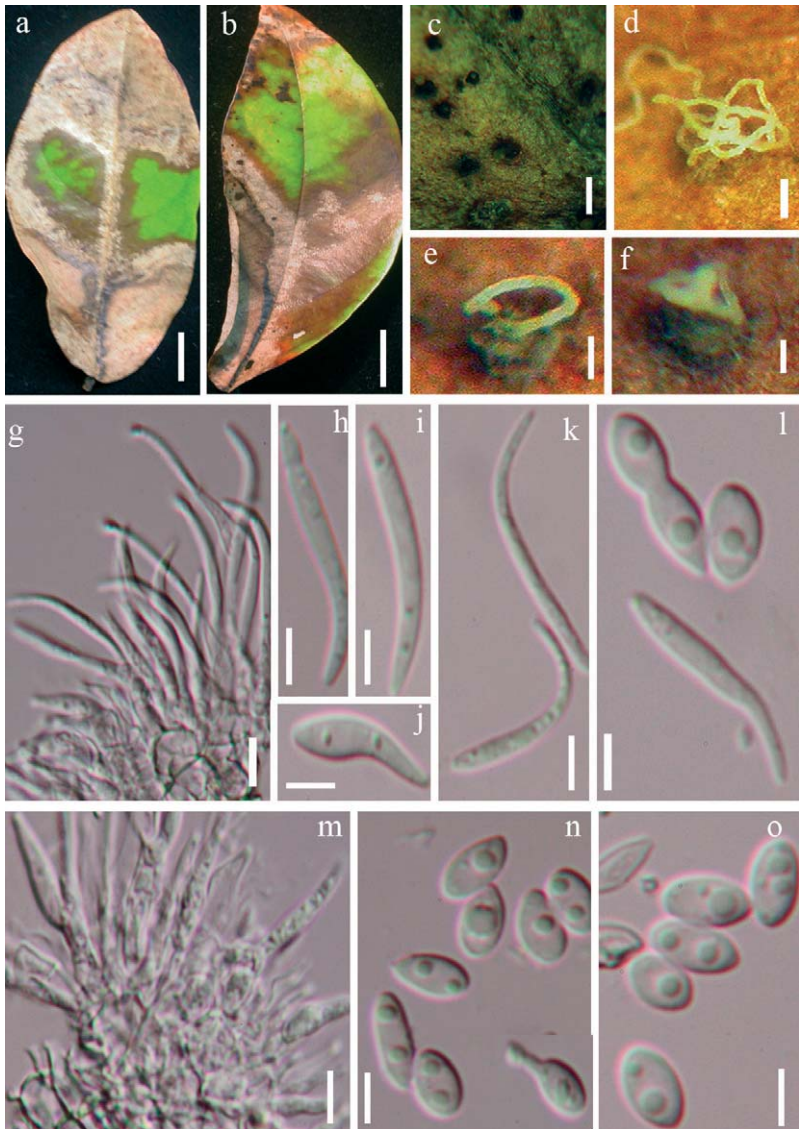



Fig. 1. One of the most parsimonious trees generated from analysis of four combined genes (rDNA ITS, EF1-α, CAL and β- tubulin partial sequences) of *Diaporthe* species. Ex-type cultures are in bold. The bootstrap support values > 70% from 1000 replicates are shown below or above the thickened branches followed by Bayesian posterior probabilities. Novel species and combinations are highlighted. The tree is rooted with *Valsa ambiens*.

***Gamma conidia*** aseptate, hyaline, smooth, fusiform, eguttulate or biguttulate, apex acutely rounded, base subtruncate, 12-13 × 1.5-2 µm. ***Beta conidia*** present on the host, conidia aseptate, hyaline, hamate or curved, apex acutely rounded, base truncate, (14-)15-18(-19) × (1.5-)2 µm.




Figs 2 **a-o.** *Diaporthe siamensis* (holotype). **a-b.** Necrotic leaves of *Dasymaschalon* sp. **c.** Pycnidia on infected areas. **d-f.** Conidia in cirri exuding from ostioles. **g.** Conidiophores and beta conidia. **h-k.** Beta conidia. **j.** Gamma conidia **l.** Alpha and beta conidia **m.** Paraphyses **n-o.** Alpha conidia Scale bars : **a-b** = 2 cm, **c-f** = 1 mm, **g-o** = 5 µm.

*Culture characteristics*: (in the dark, 25°C, after 2 wk): Colonies on PDA, and MEA with moderate growth rate (7.8 mm/day), colonies on PDA with white to cream, cottony, smooth, margin lobate, reverse of the culture greenish yellow at the centre due to pigment formation.

*Material examined*: THAILAND, Chiang Rai Province, Thasud, Muang District, Mae Fah Luang University Park, N 18° 05’ 59.1”, E 102° 40’ 02.9”, on

leaves of *Dasymaschalon* sp. (Annonaceae), 11 March 2010, D. Udayanga DPH 004 (MFLU 12-0121 **holotype**); ex-type culture MFLUCC 10-0573a, ex- isotype cultures10-573b,10-573c = BCC = CBS.

*Notes:* Three *Diaporthe* species (as *Phomopsis*) are known from *Annonaceae*, but none are from *Dasymaschalon*. *Diaporthe siamensis* has paraphyses among conidiophores and intermediate gamma conidia, which also distinguishes it from other species reported from *Annonaceae*. *Diaporthe annonae* Speg. associated with *Annona cherimolia* in Argentina was described with only a sexual state (type LPS 2468). However, *D. siamensis* did not produce a sexual morph in culture or on the host. *Phomopsis annonacearum* Bond.-Mont. described from a green-house in Russia on living leaves of *Annona cherimolia* and *A. squamosa* has 120-140 µm conidiomata, 12-15 × 2.5-3 µm conidiophores and 5-8 × 2-2.5 µm alpha conidia, while *P. annonae* Urries, described from Spain associated with *A. cherimolia* has 300-1000 µm conidiomata, 8-14 × 2 µm conidiophores, 6.5-9.5 × 1.5-2.5 µm alpha conidia and 20-30 × 1-1.5 µm beta conidia.

Based on a Blast search of NCBI's GenBank nucleotide database, the closest matches for the ITS sequence of *D. siamensis* are an endophytic undescribed *Phomopsis* sp. 122AC/L from a fruit tree in Malaysia (GU066685; Identities = 497/501 (99%), Gaps = 2/501 (0%), and an endophytic *Phomopsis* sp. 45GP/T from *Garcinia parvifolia* from Malaysia (GQ352480; Identities = 485/487 (99%), Gaps = 0/487 (0%)). The association with *Phomopsis*/*Diaporthe* was confirmed by the homology of EF, CAL and TUB gene sequences. MAT1-2-1 gene is detected in PCR amplification of all three isolates used. A sexual stage was not formed in culture suggesting the fungus is heterothallic or asexual.

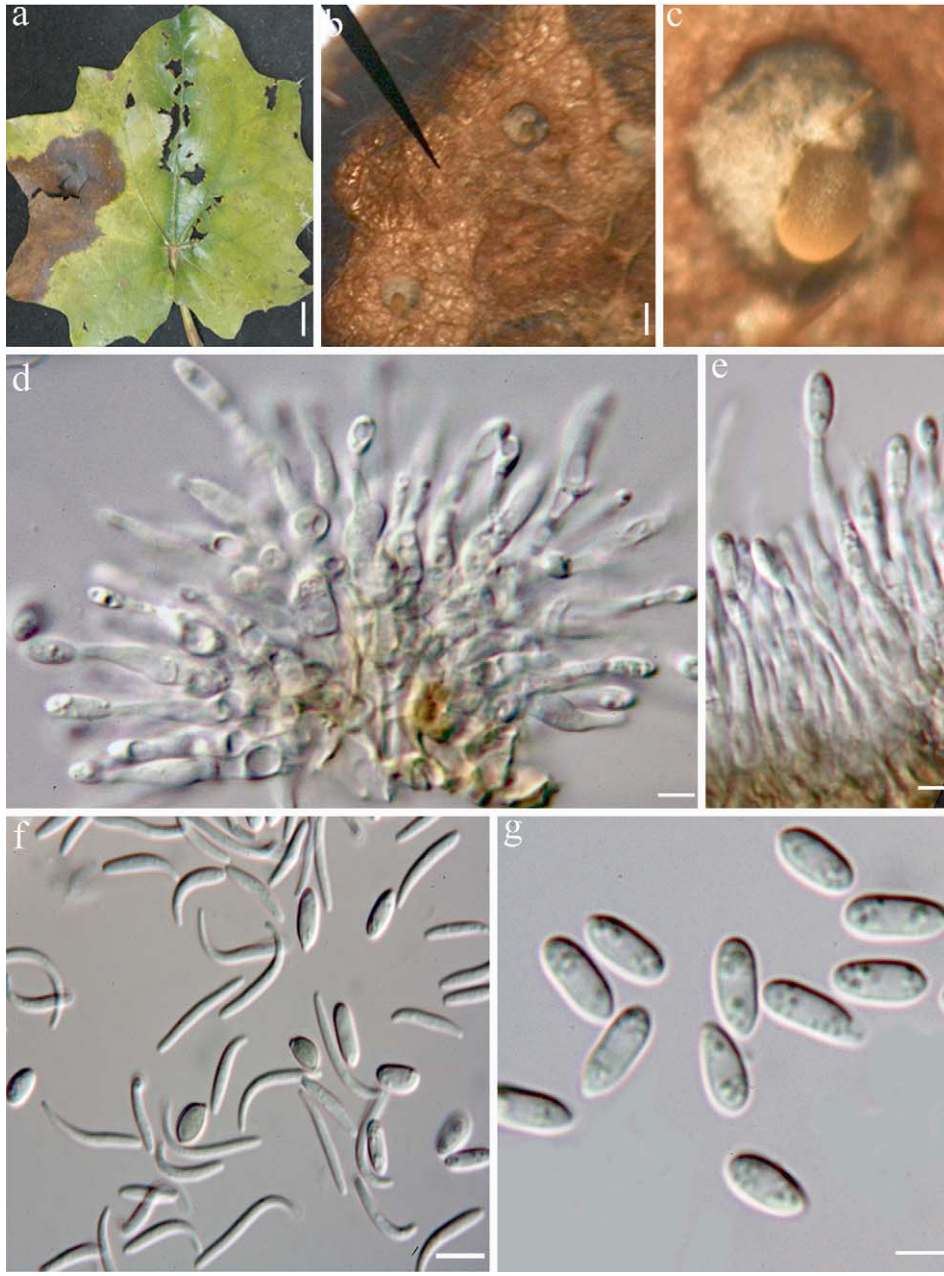
***Diaporthe thunbergii*** Udayanga, X.Z. Liu & K.D. Hyde, **sp. nov.** **Figs 3a-g**

*MycoBank:* MB 800959.

*Etymology:* Named after the host from which it was isolated, *Thunbergia laurifolia* (Acanthaceae).

***Pycnidia*** associated with necrotic leaf tissue, globose, up to 90-100 µm diam, erumpent, with slightly elongated black necks, mostly submerged in the host tissue; yellowish translucent conidial droplets exuded from the ostioles; walls consisting of 5-6 layers of medium brown *textura globosa-angularis*. ***Conidiophores*** hyaline, unbranched, densely aggregated, subcylindrical, straight to sinuous, 5-20 × 0.8-2.8 µm. ***Conidiogenous cells*** phialidic, sub-cylindrical, terminal, slightly tapering towards the apex, 0.5-1 µm long, with visible periclinal thickening; collarette not seen. ***Paraphyses*** hyaline, smooth, cylindrical, septate, extending above conidiophores, straight, flexuous, unbranched, or branched below, up to 10-20 µm long, 2-2.5 µm wide at the base. ***Alpha conidia*** aseptate, hyaline, smooth, multi-guttulate, ovoid-, base subtruncate, (5-)6-7(-7.5) × (1.5-)2-2.5(-2.6) µm. ***Beta conidia*** present on the host, aseptate, hyaline, smooth, straight to hamate, base truncate (14-)15-17(-18) × (1-)1.7 µm. ***Gamma conidia*** aseptate, hyaline, smooth, fusiform, multi-guttulate, apex acutely rounded, base subtruncate, 8-11 × 1-2 µm.

*Culture characteristics*: (in the dark, 25°C, after 1 wk): Colonies on PDA, fast growing (11.4 mm/day), but on malt extract agar (MEA) with moderate growth rate (7.1 mm/day), colonies on PDA with abundant dirty white, fluffy aerial mycelium, concentric zonation, margin fimbriate, and in reverse the centre of the culture is greenish yellow, colour development due to pigment formation.




Figs 3 **a-g.** *Diaporthe thunbergii* (holotype). **a.** Necrotic leaf of *Thunbergia laurifolia* with irregular, brown leaf spot. **b-c.** Conidial droplets exuding from ostioles. **d-e.** Conidiophores. **f-g.** Alpha, beta and gamma conidia : Scale bars: **a** = 2 cm, **b** = 200 µm, **c** = 20 µm, **d-e** = 5 µm, **f-g** = 8 µm.

*Material examined* : THAILAND, Chiang Mai Province, Doi Suthep-Pui National Park, Medicinal Garden, 18°48.62N 98°54.60E, on leaves of *Thunbergia laurifolia*, 7 April 2010, D. Udayanga (DPH 008a/MFLU 12-0117, **holotype**); ex-type culture MFLUCC 10-0576a; THAILAND, Chiang Mai Province, Doi Suthep-Pui National Park, Medicinal Garden, 18°48.62N 98°54.60E, on leaves of *Thunbergia laurifolia*, 16 May 2010, D.S. Manamgoda (DPH 008b/MFLU 12-0118), ex- isotype cultures 10-576b.10-0576c = BCC = CBS.

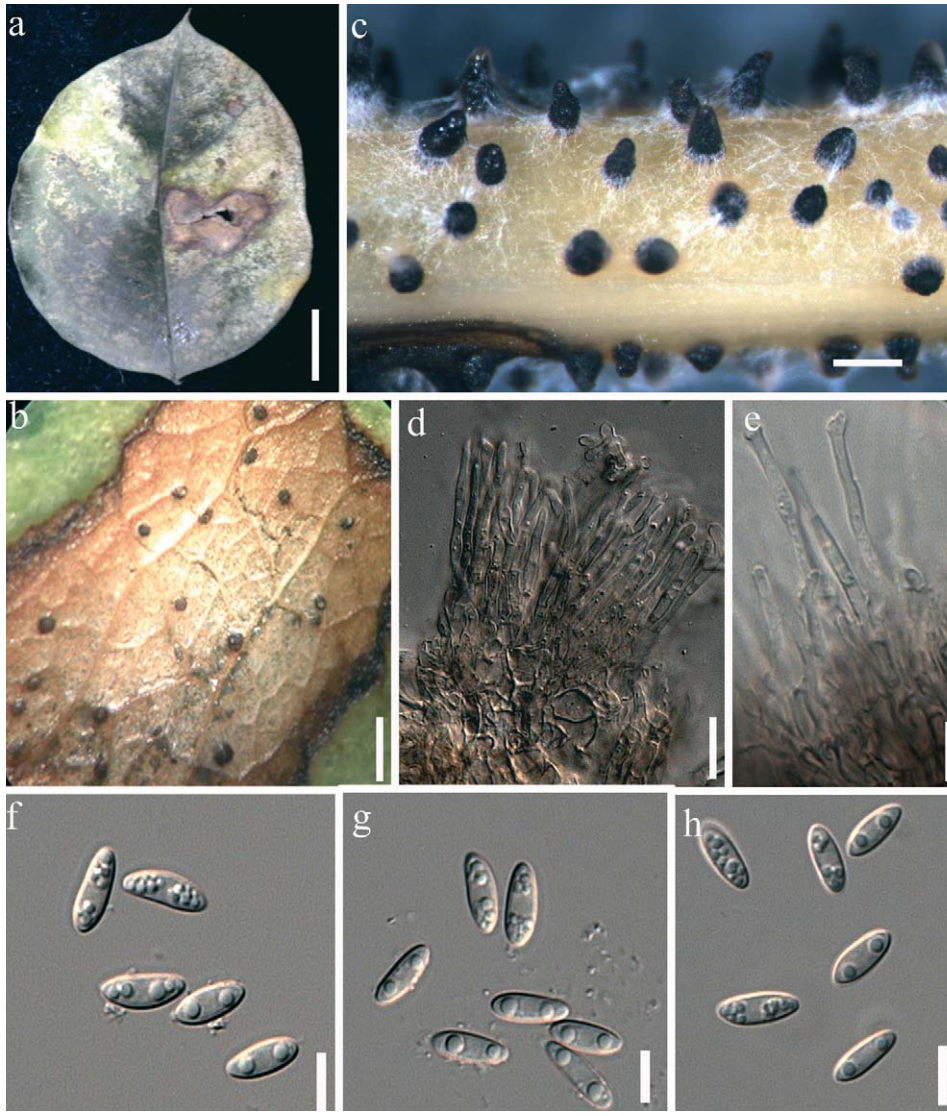
*Notes – Diaporthe* (or *Phomopsis*) species have not been previously reported from *Thunbergia* spp. (*Acanthaceae*). The present species has distinct morphological characters including multi-guttulate, small, bacillus-like alpha conidia on the host and in forming gamma conidia. The conidiophores are also remarkably long and densely aggregated. *Diaporthe thunbergii* sp. nov. was compared with herbarium specimens of two morphologically similar species, *Phomopsis ampelopsidis* Petr., and *P. elaeidis* Punith. and with the ITS sequence analysis of available *Diaporthe/Phomopsis* type sequences. The pycnidia of *P. ampelopsidis* on stems of *Ampelosis quinquefolia* (PR 7579) were 1000 × 500 µm diam. Alpha conidia were biguttulate and 6-11 × 2-3 µm, but beta and gamma conidia were not observed. *Phomopsis elaeidis* on *Elaeidis guineensis* (K: IMI 172622), had pycnidia up to 2000 µm in diam., conidiophores 8-12 × 3 µm, and beta conidia 18-24 × 0.5-1 µm. Most anamorphic *Diaporthe* species have overlapping conidial dimensions, therefore, other distinct morphological characters such as paraphyses, conidiophore aggregation, guttulation and presence of gamma conidia, were compared. However, it has a phylogenetic affinity (99% similarity in standard BLAST search) to an unidentified endophyte from *Magnolia liliflora* from the vicinity of the type location in a different study. These endophytes, (MS24, and MS28) from *Magnolia liliflora* (Magnoliaceae) from Doi-Suthep Pui National Park, Thailand (DQ485957; Identities = 488/490 (99%), Gaps = 1/490 (0%)) and DQ485961: Identities = 485/488 (99%), Gaps = 1/488 (0%), respectively). MAT 1-2-1 gene is detected in PCR amplification of all three isolates used. The sexual stage did not form in culture and one mating type was detected in all isolates used, therefore, *D. thunbergii* is presumed to be heterothallic or asexual.

***Diaporthe pterocarpicola*** Udayanga, X.Z. Liu and K.D. Hyde, **sp. nov. Figs. 4a-h**

*MycoBank* : MB 801053.

*Etymology*: Named after the host from which it was isolated, *Pterocarpus indicus* (Fabaceae).

***Pycnidia*** associated with infected leaf tissue; hemi-spherical, up to 120 µm diam, 75 µm high, immersed, with slightly elongated black necks, mostly submerged into tissue; yellowish translucent conidial droplets emerging from ostioles; walls consisting of 3-4 layers of medium dark brown *textura angularis*. ***Conidiophores*** hyaline, guttulate, unbranched, densely aggregated, subcylindrical to cylindrical, wide at the base, straight to sinuous, 7-18 µm × 1.5-3.5 µm, 2.5-3.5 µm wide at the base. ***Conidiogenous cells*** phialidic, cylindrical, terminal, slightly tapering towards apex, 1-2 µm diam, with visible periclinal thickening; collarette not clearly observed. ***Paraphyses*** occasionally present, hyaline, smooth, cylindrical, septate, extending above the conidiophores, straight, flexuous, unbranched, up to 25 µm long, 1.5-2 µm wide at base. ***Alpha conidia*** unicellular, hyaline, multi-guttulate, ellipsoid or clavate, and base subtruncate, (5-)6-7(-8) (2-)2.5(-3.5) µm. ***Beta or gamma conidia*** not observed on host or on alfalfa stem in culture.




Figs 4 **a-h.** *Diaporthe pterocarpicola* (holotype). **a.** Infected leaf of *Pterocarpus indicus*. **b.** Visible pycnidia on leaf spot. **d-e.** Conidiophores. **h.** Alpha conidia. Scale bars: **a** = 1 cm, **b** = 500, µm, **c** = 200 µm, **d-e** = 10 µm **f-h** = 6 µm.

*Culture characteristics* – (in the dark, 25ºC, after 1 wk): Colonies on PDA and MEA fast growing (12.1 mm/day), on PDA with abundant white fluffy aerial mycelium, with concentric zonation, margin fimbriate, reverse of the culture slightly greenish yellow with pigment formation.

*Material examined*: THAILAND, Chiang Rai Province, Thasud, Muang District, Chiang Rai Arboretum, N 18º 05' 59.1", E 102º 40' 02.9", on leaves of *Pterocarpus indicus*, 14 May 2010, D. Udayanga DPH 013 (MFLU 12-0129,

**holotype**); ex-type culture MFLUCC 10-0580a = BCC = CBS; THAILAND, Chiang Mai province, on leaves of *Pterocarpus indicus*, 18 May 2010, D. Udayanga DNP 008 (MFLU 12-0126, isotype); ex-isotype culture MFLUCC 10-0580b; THAILAND, Chiang Rai province, Doi Luang, on leaves of *Pterocarpus indicus*, 28 May 2010, D. Udayanga DNP 008 (MFLU 12-0127); THAILAND, Chiang Rai, Mae Fah Luang garden, on leaves of *Pterocarpus indicus*, 30 June 2010, D. Udayanga DNP 005 (MFLU 12-0123).

*Notes*: *Diaporthe pterocarpi* (as *Phomopsis pterocarpi* ) is recorded from *Pterocarpus erinaceus*. This species is redescribed below (as *Diaporthe pterocarpi*) based on a study of type material (on leaves of *Pterocarpus erinaceus* PDD 14878) and a new authentic collection from Thailand. *Diaporthe pterocarpi* has 2-3 guttulate, 6-9 ×2.5-3 µm alpha conidia , which is different to *D. pterocarpicola,* which has mostly multi-guttulate conidia and guttulate conidiophores. However these characters are dependent on culture conditions and environmental factors.

Based on a Blast search of NCBI's GenBank nucleotide database, the closest sequence to *D. pterocarpicola* is that of *Phomopsis* sp. 40GP/S an endophyte from *Garcinia parvifolia* from Malaysia (GQ352478; Identities = 485/486 (99%), Gaps = 0/486 (0%) and endophytic fungus *Phomopsis* sp. from *Garcinia* sp. from Malaysia 89CN/F. (GU066658 ; Identities = 494/499 (99%), Gaps = 4/499 (1%). MAT1-2-1 gene is detected in PCR amplification of all three isolates used. The sexual stage did not form in culture and one mating type was detected in all isolates used. Therefore, this species is also heterothallic or asexual.

***Diaporthe pterocarpi*** (Hughes) Udayanga, X.Z. Liu & K.D. Hyde, **comb. nov.**
**Figs 5 a-g**

≡ *Phomopsis pterocarpi* S. Hughes, *Mycol. Pap.* **50**: 54 (1953).

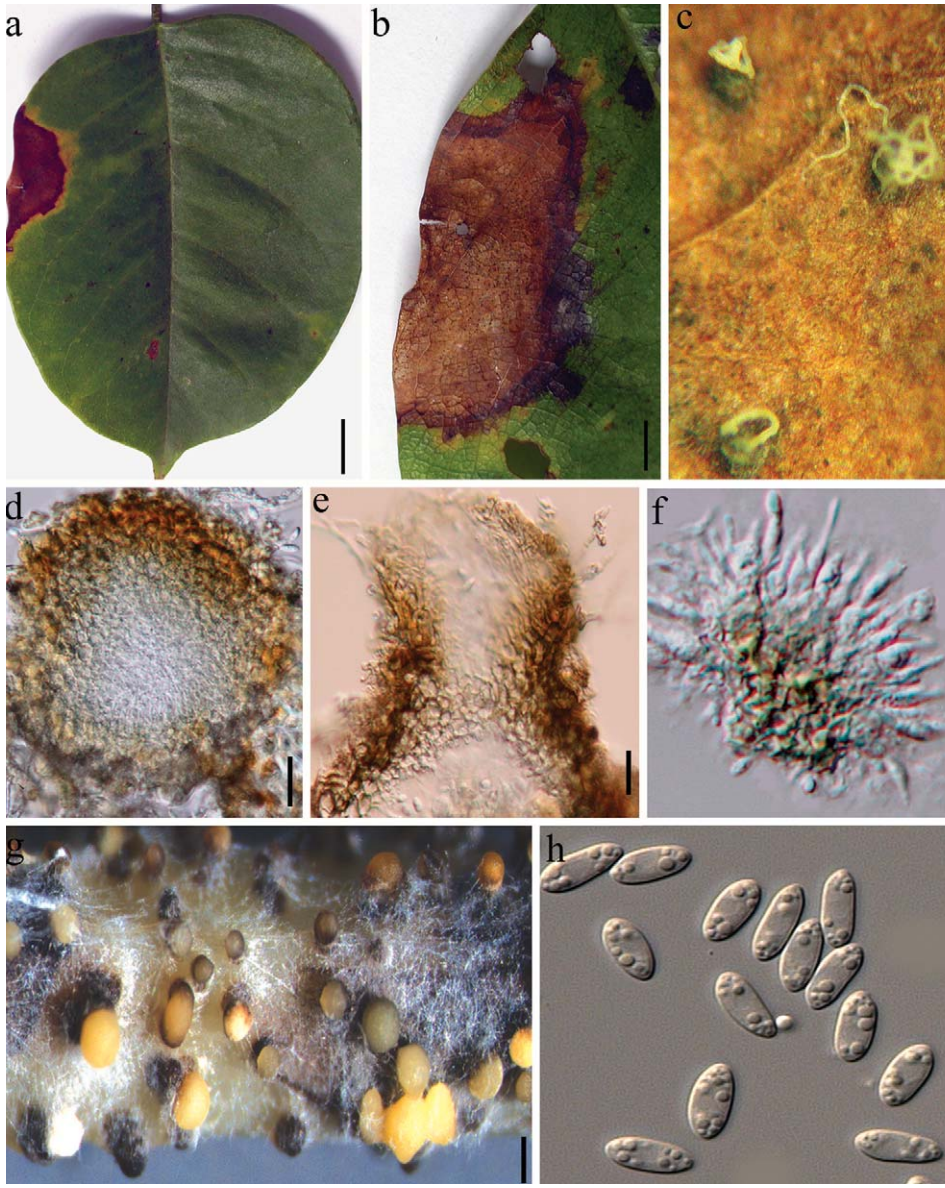
*MycoBank:* MB 801055.

***Pycnidia*** associated with infected leaf tissue; globose 100-120 µm diam, later conical, up to 100 µm diam, 65-100 high, somewhat erumpent, with a slightly elongated black neck, mostly submerged in tissue; dirty white, spiral conidial masses (cirri) extruding from ostioles; walls consisting of 3-4 layers of medium brown *textura globosa-angularis*. ***Conidiophores*** hyaline, smooth, unbranched, ampulliform, straight to sinuous, 10-15 × 1-2 µm. ***Conidiogenous cells*** phialidic, cylindrical, terminal, with slight taper towards apex, 0.5-1 µm diam; collarettes not seenobserved., ***Paraphyses*** absent. ***Alpha conidia*** aseptate, hyaline, smooth, fusiform, biguttulate, rarely 3-guttulate and base subtruncate, (5-)6-7(-9) × (2-)2.5(-3) µm. ***Gamma and beta conidia*** not observed on the host or in culture.

*Culture characteristics:* (in the dark, 25ºC, after 1 wk): Colonies on PDA and MEA slow growing (4.2 mm/day), Cultures on PDA with white, fluffy aerial mycelium, underside greenish yellow pigmentation developing in the centre.

*Material examined*: TOGOLAND (Togo, West Africa), Kete Krachi, on leaves of *Pterocarpus erinaceus,* 18 April 1949, S Hughes (PDD 14878, **isotype**); THAILAND, Chiang Rai Province, Mae Fah Luang University Garden, leaves of *Pterocarpus indicus*, 12 April 2010, D. Udayanga, DPH 002 (MFLU 12-0120, **epitype designated here**); ex-epitype culture MFLUCC 10-0572, 10-0575 = BCC = CBS.

*Known hosts and distribution*: *Aloe vera, Jatropha curcas, Ougeinia dalbergioides, Pterocarpus santalinoides, P. angolensis, P. erinaceus, P. Indicus, P. violaceus* from Brazil, Ghana, Hong Kong, India, Sierra Leone, Thailand, Togo, Zambia.




Figs 5 **a-g.** *Diaporthe pterocarpi* (epitype). **a.** Infected leaf of *Pterocarpus indicus*. **b.** Necrotic lesion on the leaf. **c.** Cirri exuding from ostioles. **d.** Section of pycnidia in early stage (immature). **e.** section through matured pycnidia with ostiole. **f.** Conidiophores. **g.** Sporulation on alfalfa stem on WA. **h.** alpha conidia. Scale bars: **a** = 1 cm, **b** = 0.5 cm, **c** = 200 µm, **d** =20 µm, **e** = 50 µm, **f, h** = 5 µm, **g** = 6 µm.

*Notes*: A fresh isolate collected in Thailand and the isotype specimen (PDD 14878) were compared for the symptoms on the host and micromorphology and are considered to be the same species. We therefore epitypify *Phomopsis pterocarpi* with our new collection from Thailand, and synonymise the taxon under *Diaporthe pterocarpi.* The species is known to be common in tropical Asia and Africa.

Based on a Blast search of NCBI's GenBank nucleotide database for ITS sequence, the closest to *D. pterocarpi* are an endophytic "*Phomopsis*" sp. CML 1936 from *Phoradendron perrottetii* from Brazil (JN153068; Identities = 473/478 (99%), Gaps = 0/478 (0%)) and an unnamed endophyte P1808A from unknown tropical plant from Peru (EU977317; Identities = 479/490 (98%), Gaps = 8/490 (2%)). MAT1-2-1 gene is detected in PCR amplification of all three isolates used. The sexual stage did not form in culture and one mating type was detected in all isolates used, therefore, the taxon is heterothallic or asexual.

## DISCUSSION

Udayanga *et al.* (2012) showed that all four gene regions (rDNA ITS, EF, TUB, CAL) used in this study are useful markers to infer phylogenetic relationships and identify species of *Diaporthe*. The combined analysis of these four loci gives more robust support to resolve the tips of branches in phylogeny. Recognition of new species, mainly from woody plants as pathogens and endophytes based on molecular data, suggests that many more species of *Diaporthe* remain to be discovered. A similar situation is occurring in other important plant pathogenic genera such as *Bipolaris, Colletotrichum*, *Curvularia, Fusarium*, *Phyllosticta* and *Pestalotiopsis* (Manamgoda *et al.*, 2011; Summerell *et al.*, 2010, 2011; Wikee *et al.*, 2011a,b; Maharachchikumbura *et al.*, 2011).

The species described here were discovered during study of a wide range of hosts including mostly tropical woody and herbaceous plants in wild and ornamental plants in common in the tropics. The cryptic new species introduced herein, were originally identified as pathogens or secondary invaders on particular hosts, but may exhibit wide host ranges, geographic distribution and switching of life modes (e.g. to endophytes or saprobes, Promputtha *et al.*, 2007, Dai *et al.*, 2010). Therefore, the host diversity and geographic distributions should be reassessed and updated in future studies with the availability of more isolates and better defined taxa. With a large number of unidentified endophytic *Diaporthe/ Phomopsis* sequences in GenBank (Murali *et al.*, 2006, Abreu *et al.*, 2012), there is a need to identify more species with wide range of hosts sharing variable ecological niches.

Based on the current knowledge of *Diaporthe* it is challenging to identify a strain isolated from a host for which a species has not previously been described. This is because both host-specific as well as non-host specific generalist species reported from one host could represent more than one taxa (Udayanga *et al.*, 2011, Santos *et al.*, 2011, Thompson *et al.*, 2011, Crous *et al.*, 2011, 2012). However, the multi-locus backbone phylogenetic tree in Udayanga *et al.* (2012) provides an alternative approach to identify and describe different species occurring on one particular host as well as cryptic taxa found on wide range of hosts.

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