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Barriopsis tectonae sp. nov. a new species of Botryosphaeriaceae from *Tectona grandis* (teak) in Thailand

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Abstract

Tectona grandis (teak) is an increasingly important timber resource globally. It is native to Asia, including Thailand. In this paper a new species of *Barriopsis, B. tectonae sp. nov.*, is described from a dead *T. grandis* branch collected in Thailand. *Barriopsis tectonae* can be differentiated from the two previously described species of *Barriopsis*; *B. fusca* and *B. iraniana,* by its ascospore and conidial dimensions. Phylogenetic evaluation of the ITS, TEF1-α and BT genomic regions provide further evidence that *B. tectonae* is a novel species.

Key words: Botryosphaeriales, Dothideomycetes, multigene phylogenetics

Introduction

Tectona grandis L.f. (teak) is an important timber resource native to India, Lao PDR, Myanmar and Thailand (Gyi & Tint 1998, Kollert & Cherubini 2012). Ancient stands of teak still occur in Thailand, although it now also occupies large areas of land as a commercial forestry species (Kollert & Cherubini 2012). Individual teak trees from natural forests are reported to be worth up to USD 20 000 (Swerdlow 1995). The domestic price of logs from planted teak forests in Thailand ranges from 245.00-796.50 USD/m^3 depending on the size of the logs (Kollert & Cherubini 2012).

During studies of fungi associated with *T. grandis* in Thailand, an isolate of Botryosphaeriaceae, resembling a species of *Barriopsis* was obtained. *Barriopsis* A.J.L. Phillips *et al*. was introduced as a new genus in the family Botryosphaeriaceae, with *B. fusca* (N.E. Stevens) A.J.L. Phillips *et al*. as the type species (Phillips *et al.* 2008). There are currently two species in *Barriopsis*, *B. fusca*, and *B. iraniana* Abdoll. *et al*. (Phillips *et al.* 2013, Abdollahzadeh *et al*. 2009). The sexual morph of *Barriopsis* is defined as having bitunicate asci, containing brown ascospores that are widest in the middle, and that lack terminal apiculi (Phillips *et al.* 2008). *Barriopsis* has a *Lasiodiplodia*-like asexual morph with conidia that are initially hyaline, aseptate and thick-walled, becoming dark brown and septate with irregular longitudinal striations (Stevens 1926, Phillips *et al.* 2008). However, in contrast to *Lasiodiplodia*, conidia are striate at an earlier stage of development and striations are already visible on hyaline conidia, even while attached to conidiogenous cells (Abdollahzadeh *et al*. 2009).

The understanding of the order Botryosphaeriales has rapidly evolved with the use of molecular data (Phillips *et al*. 2008, Lumbsch & Huhndorf 2010, Lui *et al*. 2012, Hyde *et al.* 2013, Phillips *et al*. 2013). The order currently comprises six families, namely the Aplosporellaceae, Botryosphaeriaceae, Melanopsaceae, Phyllostictaceae, Planistromellaceae and Saccharataceae (Minnis *et al.* 2012, Monkai *et al*. 2012, Slippers *et al.* 2013, Wikee *et al.* 2013). The family Botryosphaeriaceae was recently redefined by Slippers *et al.* (2013) using morphological and

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molecular data, and now comprises seventeen well defined genera. They include important pathogens of trees and other plants (Mehl *et al.* 2013).

The aim of this study was to clarify the identity of the *Barriopsis* species from *T. grandis* in Thailand. A combination of multi-gene phylogenetics (ITS, TEF1-α, BT), in combination with sexual and asexual morphology, was used to compare the isolate from *T. grandis* with previously described species of *Barriopsis*.

Materials and methods

Collection, isolation and morphological characterization

A specimen resembling a species of Botryosphaeriaceae was collected from a dead *T. grandis* branch at a plantation in Phayao Province, Muang District, Thailand. Isolation was made from single ascospores following the method described in Doilom *et al*. (2013).

Colony colour was determined with the Methuen handbook of colour (Kornerup & Wanscher 1967). The production of asexual fruiting bodies was induced by transferring isolates to water agar (WA) overlaid with sterilized toothpicks as a substrate and incubated for 2–3 months at 25°C. Cardinal temperatures and growth studies were completed following the protocol described in Slippers *et al.* (2014).

Sections of the sexual and asexual fruiting structures were made by hand with the aid of an Olympus SZ40 stereo microscope, and mounted in 10% lactoglycerol. Morphological observations and photomicrographs were made with a Nikon ECLIPSE 80i compound microscope fitted with a Cannon 550D digital camera. Morphological measurements were made with the Tarosoft (R) Image Frame Work. Images used for figures were processed with Adobe Photoshop CS5 (Adobe Systems, U.S.A.).

After observation the sections were preserved on slides in 10% lactoglycerol, and stored at the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU). The type herbarium specimen was also deposited at MFLU. Cultures were deposited in the Mae Fah Luang Culture Collection (MFLUCC). A duplicate of the ex-type culture was deposited in the culture collection of the Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa (CMW) and Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS).

DNA extraction, PCR amplification and sequencing

The Botryosphaeriaceae isolate from *T. grandis* was grown on MEA for one week at 25°C. DNA was extracted from actively growing mycelia using a PrepMan™ Ultra Kit (Applied Biosystems, Foster City, CA, U.S.A.), following the manufacturer's protocol. The internal transcribed spacer (ITS) operon of the ribosomal DNA was amplified and sequenced using primers ITS1 and ITS4 (White *et al*. 1990). A fragment of translation elongation factor 1-α (TEF1-α) was amplified and sequenced using EF1F and EF2R (Jacobs *et al*. 2004) and a fragment of the β-tubulin gene (BT) was amplified and sequenced using primers Bt2a and Bt2b (Glass & Donaldson 1995).

PCR reactions were completed in 25 µL final volumes and consisted of 5 µL 5x MyTaq Reaction Buffer (Bioline, U.S.A.), 0.15 µL MyTaq™ DNA Polymerase (Bioline, U.S.A.), 0.5 µL of each primer (10 mM stock) (Whitehead Scientific, Cape Town, South Africa), 1 µL DNA, made up to 25 µL with sterile Sabax Pour Water (Adcock Ingram Critical Care, Johannesburg, South Africa). PCR reactions were run on a 2720 Thermal Cycler PCR (Applied Biosystems, Foster City, CA, U.S.A.) machine with an initial denaturation step of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s annealing at 52°C, 55°C, 56°C for ITS, β-tubulin, and TEF1-α respectively, then 1 min at 72°C, and a final extension of 7 min at 72°C. Amplification was confirmed by staining 4 µL aliquots of PCR products with 3 µL of GelRed™ Nucleic Acid Gel stain (Biotium, Hayward, CA, U.S.A.) and separated on a 1 % agarose gel before being visualized on a Bio-Rad GelDoc EZ system. PCR products were cleaned using an ExoSAP-IT PCR clean-up kit (USB Corporation, Cleveland, OH, U.S.A.), according to the manufacturer's instructions.

Purified PCR products were used for sequencing in both directions using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.) with the same primers used for the PCR reactions. Each reaction contained 2 µL sequencing buffer, 0.5 µL BigDye, 1 µL of each primer and 2 µL of purified PCR product. Final volumes were adjusted to 12µL by adding sterile Sabax Pour Water. Sequence reactions were run under the following conditions, 25 cycles of 10 s at 96°C, 5 s at 54°C, 4 min at 60°C and were precipitated using the

ethanol/sodium acetate precipitation method (http://www.bi.up.ac.za/seqlab/download/dna_sequencing.pdf). Sequencing was completed by the DNA sequencing facility, Bioinformatics and Computational Biology Unit, University of Pretoria, South Africa.

Consensus sequences using forward and reverse stands were generated using BioEdit (Hall 1999). Sequence similarities were determined using the GenBank BLAST option.

Phylogenetic analyses

Reference sequences of type species, used in the phylogenetic analyses, were obtained from relevant literature and GenBank. The sequences generated in this study were deposited in GenBank (Table 1). Individual datasets of the ITS, TEF1-α, and the BT genomic regions were aligned individually and in a concatenated dataset online with MAFFT version 7.110. (http://mafft.cbrc.jp/alignment/ server/). The individual datasets were combined as this has been previously found to increase phylogenetic accuracy (Cunningham 1997, Bull *et al.* 1993). The combined ITS, TEF1-α, BT dataset contained 38 sequences, including two isolates of *Pseudofusicoccum stromaticum* as the outgroup. This outgroup was selected based on information from Phillips *et al.* (2008) and Abdollahzadeh *et al.* (2009). Alignments were checked visually and manually adjusted for errors. Congruence and combinability of the individual datasets was tested using the partition homogeneity test (PHT), also known as the Incongruence Length Difference (ILD) test (Farris *et al.* 1995a, b) with 1,000 heuristic search replicates in PAUP v. 4.0b10 (Swofford 2003).

Phylogenetic trees were inferred with maximum parsimony (MP) and Bayesian inference (BI). MP trees were calculated with PAUP. Uninformative characters were excluded. All characters were unordered and of equal weight. The heuristic search function was used with 1,000 random sequence addition replicates and tree bisection-reconnection (TBR) as the branch-swapping algorithm. Alignment gaps were treated as missing data. Statistical supports for branches were estimated using maximum parsimony bootstrap (BS) analysis with 1,000 replicates. Phylogenetic trees were visualized and annotated using Treeview (Page 1996) or FigTree v1.3.1 (Rambaut 2009), and formatted using PowerPoint 2010. Relative support of branches was determined with maximum parsimony bootstrap (MPBS) using 1,000 replications (Felsenstein 1985).

Bayesian inference was made using the Markov Chain Monte Carlo (MCMC) method using the MrBayes v. 3.2.1 plug-in (Huelsenbeck & Ronquist 2001) of Geneious® R7 (Biomatters, New Zealand). jModelTest v.2.2 (Posada 2008) was used to select the best-fit nucleotide substitution models under the Akaike information criterion (AIC). The model selected for the individual datasets were all GTR+I+G, and GTR+G was selected for the combined dataset. Four chains were run for the individual and combined data sets. One million generations were selected with a sub-sampling frequency every 1,000 generations. The first 100,000 generations were eliminated as burn-in. Posterior probabilities (Rannala & Yang 1996) were calculated in Geneious from a majority-rule consensus tree generated with the remaining 900,000 trees.

TABLE 1. Isolates used in this study.

Taxon	Culture No[1]	Isolates	GenBank Accession No.[2]		
			ITS	TEF1-α	BT
Barriopsis fusca	CBS 174.26	ex-type	EU673330	EU673296	EU673109
B. iraniana	CBS 124698/ IRAN 1448C	ex-type	FJ919663	FJ919652	KF766127
B. iraniana	IRAN 1451C		FJ919668	FJ919657	N/A
B. tectonae	MFLUCC 12-0381/ CMW 40687	ex-type	**KJ556515**	**KJ556516**	**KJ556517**
Diplodia mutila	CBS 112553		AY259093	AY573219	DQ458850
D. mutila	CBS 230.30		DQ458886	DQ458869	DQ458849
D. seriata	CBS 112555	ex-epitype	AY259094	AY573220	DQ458856
D. seriata	CBS 119049		DQ458889	DQ458874	DQ458857
Dothiorella moneti	MUCC 505/ WAC 13154	ex-type	EF591920	EF591971	EF591954
Do. moneti	MUCC 507		EF591922	EF591973	EF591956
Do. sarmentorum	IMI 63581b	ex-type	AY573212	AY573235	EU673102
Do. sarmentorum	CBS 115038		AY573206	AY573223	EU673101

..... *continued on the next page*

TABLE 1 (*continued*)

Taxon	Culture No[1]	Isolates	GenBank Accession No.[2]		
			ITS	TEF1-α	BT
Lasiodiplodia gonubiensis	CBS 115812/ CMW 14077	ex-type	AY639595	DQ458877	DQ458860
L. gonubiensis	CBS 116355/ CMW 14078		AY639594	DQ103567	EU673126
L. margaritacea	CBS 122519/ CMW 26162	ex-type	EU144050	EU144065	N/A
L. margaritacea	CBS 122065/ CMW 26163		EU144051	EU144066	N/A
L. pseudotheobromae	CBS 116459	ex-type	EF622077	EF622057	EU673111
L. pseudotheobromae	CBS 447.62		EF622081	EF622060	EU673112
L. theobromae	CBS 164.96		AY640255	AY640258	EU673110
L. theobromae	CBS 124.13		DQ458890	DQ458875	DQ458858
Neodeightonia phoenicum	CBS 122528	ex-type	EU673340	EU673309	EU673116
N. phoenicum	CBS 169.34		EU673338	EU673307	EU673138
N. subglobosa	CBS 448.91	ex-type	EU673337	EU673306	EU673137
Phaeobotryon cupressi	CBS 124700/ IRAN 1455C	ex-type	FJ919672	FJ919661	N/A
P. cupressi	IRAN 1456C		FJ919670	FJ919659	N/A
P. mamane	CBS 122980/ CPC 12440	ex-type	EU673332	EU673298	EU673121
P. mamane	CPC 12442		EU673333	EU673299	EU673124
Pseudofusicoccum stromaticum	CBS 117448/ CMW 13434		AY693974	AY693975	EU673094
Ps. stromaticum	CBS 117449/ CMW 13435		DQ436935	DQ436936	EU673093
Spencermartinsia viticola	CBS 117009	ex-type	AY905554	AY905559	EU673104
S. viticola	CBS 302.75		EU673319	EU673286	EU673135
Sphaeropsis citrigena	ICMP 16812	ex-type	EU673328	EU673294	EU673140
Sp. citrigena	ICMP 16818		EU673329	EU673295	EU673141
Sp. eucalypticola	CBS 133993/MFLUCC 11-0579	ex-type	JX646802	JX646867	JX646850
Sp. eucalypticola	MFLUCC 11-0654		JX646803	JX646868	JX646851
Sp porosa	CBS 110496/ STE-U 5132	ex-type	AY343379	AY343340	EU673130
Sp. visci	CBS 186.97	ex-type	EU673325	EU673293	EU673128
Sp. visci	CBS 100163		EU673324	EU673292	EU673127

[1]Abbreviation of culture collections: CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC: Collection of Pedro Crous housed at CBS; ICMP: International Collection of Micro-organisms from Plants, Landcare Research, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, U.K; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUCC: Culture Collection, Laboratory of Plant Pathology, Mie University, Tsu, Mie prefecture, Japan; STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; WAC: Department of Agriculture Western Australia Plant Pathogen Collection, South Perth, Western Australia.

[2] Sequence numbers in bold are newly deposited in GenBank

Results

Taxonomy

Barriopsis tectonae Doilom, Shuttleworth & K.D. Hyde, *sp. nov.* MycoBank: MB808202 (Fig. 1)

Etymology:—The name refers to the host genus *Tectona* on which the fungus was collected.

Saprobic on dead branch of *Tectona grandis*. Sexual state: *Ascostromata* (195–) 280–325 (–365) µm high × (230–)265–285(–320) µm diam. ($\bar{x}$ = 280 × 265 µm, n = 10), black, initially immersed, becoming erumpent

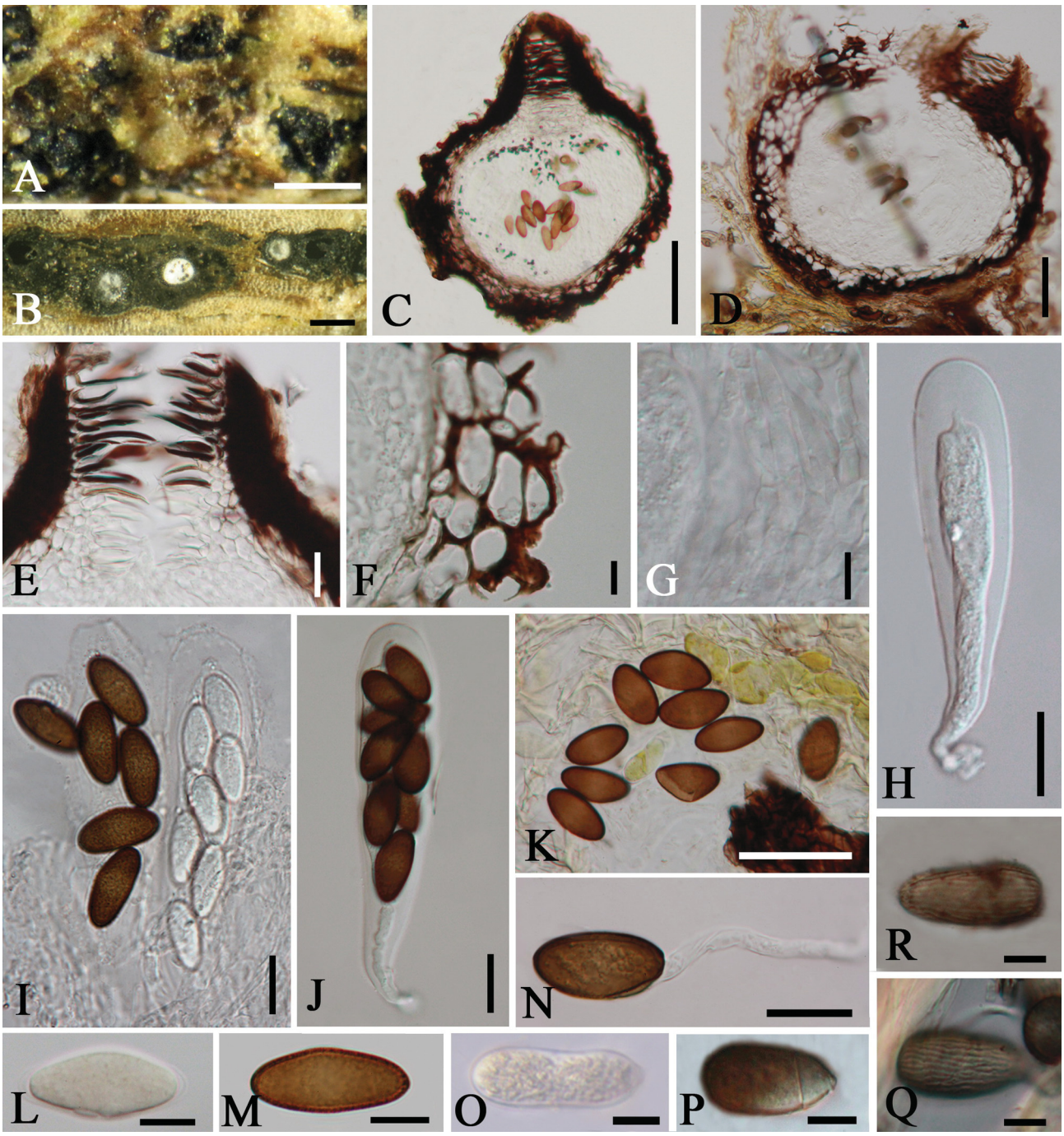

FIGURE 1. *Barriopsis tectonae* (MFLU 14-0024, holotype) A. Ascostromata on host. B. Ascostromata cut through horizontally showing the white contents with dark spots. C–D. Section through ascoma on host. E. Papilla with periphyses. F. Peridium. G. Pseudoparaphyses. H. Immature ascus. I–J. Immature ascus with hyaline ascospores, mature asci with brown ascospores. K, M. Mature ascospore/s without terminal apiculi. L. Immature ascopsore without longitudinal striations. N. Germinated ascospore. O. Immature conidium. P. Mature conidium with two septa. Q, R. Mature conidia with longitudinal striations. Scale bars: A, B = 500 µm, C = 100 µm, D, K = 50 µm, E, H–J, N = 20 µm, F, G, L, M, O–R = 10 µm.

through bark fissures, solitary or gregarious, uniloculate, globose to subglobose, or flask-shaped, when cut horizontally, locules visible with white contents and dark ascospore dots, papillate. *Papilla* 100–115 µm long, 100–120 µm diam, ostiole with periphyses. *Peridium* composed of several layers of dark brown-walled cells of *textura angularis*. *Hamathecium* comprising 2.5–6 µm wide, hyphae-like, numerous, septate, pseudoparaphyses, slightly constricted at septa. *Asci* (120–)167–185(–200) ×(28–)30.5–32(–35) µm ($\overline{x}$=160 × 31µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindro-clavate or clavate, with long or short pedicel, apically rounded with an

ocular chamber. *Ascospores* (26–)29–30 (–33) × (13–)14.5–15(–17) µm ($\bar{x}$ = 29 × 15 µm, n = 30), overlapping biseriate, initially hyaline, becoming pale brown or reddish-brown when mature, aseptate, ellipsoid to ovoid, ends rounded, without terminal apiculi at each end, thick-walled, smooth or verruculose, swollen in the centre. Asexual state: *Conidiomata* pycnidia, superficial on toothpick in agar media, covered with dense olivaceous to olivaceous-black mycelium, solitary, or gregarious, scattered, globose to sub globose, unilocular. *Paraphyses* not observed. *Conidia* (29–)37–37.5(–38) × 15.5–17.5(–18) µm ($\bar{x}$ = 36 × 17 µm, n = 10), initially hyaline and aseptate, becoming pale brown to dark brown and 1–2 septate, thick-walled, smooth, ellipsoid to obovoid, apex broadly rounded, base rounded to truncate, with longitudinal striations when mature, striations not present when immature, granular contents. *Chlamydospores* catenate, intercalary and terminal, hyaline becoming brown to dark brown with maturity, thick-walled, smooth, granular contents.

Culture characteristics:—Ascospores germinating on PDA after 5–12 h. Germ tubes produced from germ pore of ascospores. Only asexual morph produced in culture. Colonies on MEA reaching 35–40 mm diam after 4 days in the dark at 25°C, flattened, velvety, lobate, fimbriate, initially white, after 2 days becoming olive (3F5) in the centre, white at the edge, reaching the edge of the Petri dish after 15 days. Cardinal temperatures for growth after four days and colony diameter (mm): optimum 25°C, no growth at 5°C, 6 mm at 10°C, 16 mm at 15°C, 27 mm at 20°C, 37 mm at 25°C, 13 mm at 30°C, no growth at 35°C.

Material examined:—THAILAND. Phayao Province: Muang District, on dead branch of *Tectona grandis*, 12 March 2012, *M. Doilom* (MFLU 14-0024, **holotype**, ex-type culture MFLUCC 12-0381 = CMW 40687 = CBS 137786).

Phylogenetic analyses

PCR products and sequences were successfully generated for the ITS, TEF1-α and BT loci. The alignment lengths consisted of 526 bp for ITS, 409 bp for TEF1-α, 470 bp for BT and 1405 bp for the combined dataset. The number of parsimony informative sites was 121 for ITS, 216 for TEF1-α, 116 for BT and 453 for the combined dataset. The result from the partition homogeneity test (PHT) was not significant at the 95% level, indicating that the individual data sets were congruent and could be combined. The MP analysis for the individual ITS analysis resulted in six most parsimonious trees with a tree length of 247, four for the TEF1-α, with a length of 552 and 124 for the BT, with a tree length of 238.

Comparison of the polymorphisms of ITS, TEF1-α and BT between *B. tectonae*, and its closest relatives *B. fusca* (CBS 174.26) and *B. iraniana* (IRAN 1448C) showed *B. fusca* with 10, 83 and 13 and *B. iraniana* with 22, 106, 23 (Table 2).

The single isolate of *B. tectonae* (ex-type MFLUCC 12-0381) grouped separate from its closest relative *B. fusca* with strong BS (100%) and PP (1.00) support in the combined phylogeny (Fig. 2), as well as in all the individual phylogenies (BS: ITS= 79%, TEF1-α= 82%, BT= 96%, PP: ITS= 1.00, TEF1-α= 1.00, BT= 1.00).

Discussion

In this study we introduce *Barriopsis tectonae* as a novel species based on sexual and asexual morphology and multi locus sequence data. *B. tectonae* differs from the other two described species of *Barriopsis.* In the asexual morph, conidia are longer and wider than both *B. fusca* and *B. iraniana* (Table 3). Mature conidia of *B. tectonae* also have more numerous longitudinal striations than *B. fusca* and *B. iraniana* (Stevens 1926, Abdollahzadeh *et al*. 2009). *B. iraniana* has longitudinal striations on the immature conidia even while they are still attached to conidiogenous cells, however longitudinal striations are absent on immature conidia of *B. tectonae*, and are not reported for *B. fusca* (Stevens 1926, Phillips *et al.* 2013). In the sexual morph *B. tectonae* has smaller ascospores than *B. fusca*, and the width of asci are also smaller (Table 3). The sexual morph of *B. iraniana* was not reported by Abdollahzadeh *et al.* (2009), and could thus not be compared in this study. Colonies of *B. tectonae* are slower growing than those of *B. iraniana* on MEA after 4 d in the dark at 25°C (Abdollahzadeh *et al.* 2009) and were not described for *B. fusca*.

TABLE 2. Polymorphic nucleotides from sequence data of the ITS, TEF1-α and BT loci for isolates of *Barriopsis tectonae*, *B. fusca* and *B. iraniana*.

Species	Isolate	ITS																								TEF1-α											
		21	60	82	86	135	150	151	153	168	174	334	390	392	398	399	400	402	403	436	437	438	439	440		35	36	37	39	41	42	43	44	45	47	48	49
B. tectonae	MFLUCC 12-0381	G	C	C	C	G	C	T	C	G	C	G	T	C	G	C	G	G	A	C	C	T	G	A		C	G	C	C	C	G	C	T	C	C	G	C
B. fusca	CBS 174.26	A	•	•	•	•	•	T	A	A	T	•	•	•	•	•	•	A	•	-	T	C	T	G		A	C	T	T	-	-	-	-	-	-	-	-
B. iraniana	IRAN 1448C	•	T	T	-	-	T	A	C	A	T	A	G	T	T	-	-	T	T	-	T	C	T	G		A	C	T	T	-	-	-	-	-	-	-	-
	IRAN 1451C	•	T	T	-	-	T	A	C	A	T	A	G	T	T	-	-	T	T	-	T	C	T	G		A	C	T	T	-	-	-	-	-	-	-	-

Species	Isolate	TEF1-α																																			
		50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	73	76	81	82	96	97	101	105	107	108	110	111	112	113	114
B. tectonae	MFLUCC 12-0381	T	C	C	C	C	G	C	A	T	C	C	T	G	C	A	C	C	T	G	C	A	T	A	C	A	C	T	C	G	C	G	T	G	C	C	C
B. fusca	CBS 174.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	•	•	T	C	T	•	•	•	•	•	•	•	•
B. iraniana	IRAN 1448C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	G	G	T	•	T	A	T	C	C	-	-	-	-
	IRAN 1451C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	G	G	T	•	T	A	T	C	C	-	-	-	-

Species	Isolate	TEF1-α																																			
		115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	135	136	145	147	148	149	151	156	157	158	159	160	161	162	167	168	170	182	204	212
B. tectonae	MFLUCC 12-0381	G	C	T	G	C	C	C	G	C	T	G	C	C	C	G	C	C	C	A	G	C	T	G	C	A	C	G	A	C	C	-	C	G	-	G	G
B. fusca	CBS 174.26	•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	T	•	•	•	A	C	C	-	-	-	-	-	-	-	•	-	•	T	C	A
B. iraniana	IRAN 1448C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	G	T	G	C	T	•	G	T	C	G	•	•	C	A	A	T	C	A
	IRAN 1451C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	G	T	G	C	T	•	G	T	C	G	•	•	C	A	A	T	C	A

Species	Isolate	TEF1-α																																			
		221	222	223	231	240	250	251	267	268	269	270	271	272	273	274	282	302	315	316	317	318	325	326	327	340	341	343	344	345	347	356	362	364	369	393	402
B. tectonae	MFLUCC 12-0381	T	G	T	C	C	C	T	C	G	C	C	A	G	G	T	G	T	G	C	T	A	C	A	C	C	T	G	C	A	C	G	C	G	C	C	C
B. fusca	CBS 174.26	•	-	-	•	T	•	-	•	•	T	•	G	•	A	C	•	A	•	•	•	•	-	-	•	A	•	•	•	•	•	•	A	•	T	•	T
B. iraniana	IRAN 1448C	C	•	•	T	•	G	-	T	C	•	G	•	A	•	C	T	•	-	-	-	-	-	•	A	•	A	T	G	G	T	A	A	A	T	T	•
	IRAN 1451C	C	•	•	T	•	G	-	T	C	•	G	•	A	•	C	T	•	-	-	-	-	-	•	A	•	A	T	G	G	T	A	A	A	T	T	•

Species	Isolate	BT																																			
		47	51	57	59	61	65	74	75	82	91	118	121	138	147	150	158	163	164	165	167	172	179	182	183	215	233	341	356	404	469						
B. tectonae	MFLUCC 12-0381	C	T	G	-	T	A	G	T	C	A	T	C	G	C	C	G	G	T	G	T	C	C	T	T	C	T	T	C	C	T						
B. fusca	CBS 174.26	•	•	•	•	C	G	•	C	T	•	C	•	•	T	•	•	•	•	T	C	G	T	•	•	•	•	•	T	T	G						
B. iraniana	IRAN 1448C	-	A	T	C	C	G	A	•	•	G	C	T	A	•	T	C	C	G	T	C	G	•	A	A	G	C	C	•	•	N/A						
	IRAN 1451C	Sequences not available																																			

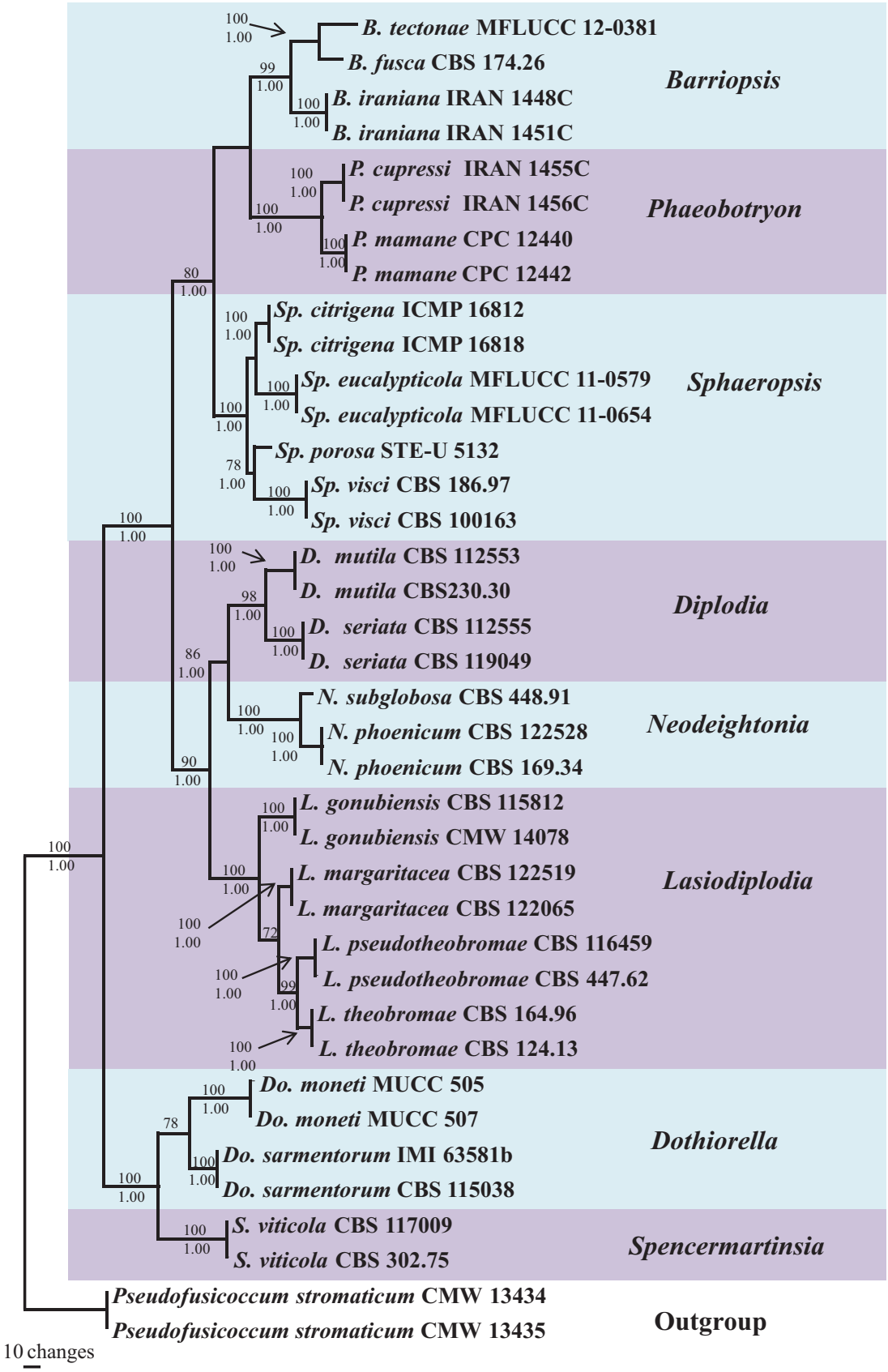

FIGURE 2. The most parsimonious trees (tree length= 1042, CI= 0.651, HI= 0.349, RI= 0.854, RC= 0.556) resulting from a combined ITS, TEF1-α and BT analysis for 38 taxa in the Botryosphaeriaceae. Maximum parsimony bootstrap values ≥70% are given above the nodes. Bayesian posterior probabilities ≥ 0.95 are given below the nodes. The tree was rooted to *Pseudofusicoccum stromaticum.*

TABLE 3. Asci, ascospore and conidia dimensions, and host range of *Barriopsis* species. Measurements for *B. tectonae* are given as minimum and maximum values in parentheses, with second and third quartiles, then the average, standard deviation and sample number e.g. length (min-) Q2–Q3 (-max) x width (min-) Q2–Q3 (-max), av. ± SD, n. All measurements are in µm.

Species	Asci	Ascospores	Conidia	Host	Reference
Barriopsis fusca	(125–180) × (30–36) (av., SD, n= not reported)	(30–)31–36.5(–38.5) × (15.5–)16–18.5(–21) (av. 33.0 ± 1.5 x 17 ± 1.0, n= not reported)	(20–)23–25(–28) × (11–)12–13(–16) (av., SD, n= not reported)	*Citrus* sp. and unidentified hosts	Phillips *et al.* (2008) for asci and ascospores, Stevens (1926) for conidia
B. iraniana	Sexual morph not reported	Sexual morph not reported	(22.7–)24–30 × (12.8–)14–18(–21.5) (av. 27 ± 2.0 × 16.4 ± 1.3, n= ≥50)	*Mangifera indica*, *Citrus* sp., *Olea* sp.	Abdollahzadeh *et al.* (2009)
B. tectonae	(120–)167–185(–200) × (28–)30.5–32(–35) (av. 160 ± 29.5 × 31 ± 2.5, n= 10)	(26–)29–30(–33) × (13–)14.5–15(–17) (av. 29 ±1.5 × 15 ± 1, n= 30)	(29–)37–37.5(–38) × (15–)15.5–17.5(–18) (av. 36 ± 3 × 17 ± 1, n= 10)	*Tectona grandis*	This study

Differences observed in morphology between *B. tectonae* and other *Barriopsis* species are strongly supported in multi-locus sequence data. Both in results of individual gene regions, as well as in the combined analyses, *B. tectonae* grouped separate from the other *Barriopsis* species. The branch that splits *B. tectonae* from its closest relative, *B. fusca,* had strong maximum parsimony bootstrap support and Bayesian posterior probabilities in the individual gene phylogenies and the combined phylogeny. Further scrutiny of sequence data revealed considerable differences in the TEF1-α as well as other gene regions. These fixed differences in multiple, unlinked genomic loci support the Genealogical Concordance Phylogenetic Species concept (Taylor *et al.* 2000) and the description of *B. tectonae* as a unique species.

Species of *Barriopsis* are not currently known as pathogens. *B. tectonae* occurs as a saprobe on teak, *B. fusca* occurs on cut branches of *Citrus* sp. and unidentified hosts (Stevens 1926) and *B. iraniana* occurs as an endophyte in stems of *Citrus* sp., *Mangifera indica* and *Olea* sp. (Abdollahzadeh *et al.* 2009). However, the host ranges of these species will likely increase as further collections are made.

In the current study, a single isolate of *B. tectonae* was used in the phylogenetic analyses to help describe the new species. This is contrary to the informal rules when describing new fungal species (Seifert & Rossman 2010). However, there is compelling morphological, phylogenetic, and host range differences to support the elevation of *B. tectonae* to species level, and it is in the interest of the broader mycological community to be described. Further field collections need to be made to increase the number of cultures of *B. tectonae* and for future taxonomic studies.

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