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ORIGINAL RESEARCH ARTICLE

Pseudoteratosphaeria supramediana sp. nov. (Teratosphaeriaceae, Mycosphaerellales), a New Foliar Pathogen on *Eucalyptus* in Indonesia

Nam Q. Pham[1] · **Seonju Marincowitz**[1] · **Brenda D. Wingfield**[1] · **Pedro W. Crous**[1,2] · **Samuel A. Santos**[3] · **Alvaro Durán**[3] · **Marthin Tarigan**[3] · **Michael J. Wingfield**[1]

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Abstract

The *Eucalyptus* plantation industry in Indonesia has expanded rapidly during the last few decades. This growth is primarily attributed to the replacement of *Acacia mangium*, that was severely damaged by diseases. The rapid shift to large-scale monoculture plantations of *Eucalyptus* has introduced new challenges, particularly the emergence of new disease and pest problems. During routine *Eucalyptus* disease surveys in Indonesia, symptoms of a new leaf and shoot disease were observed on a single hybrid *Eucalyptus grandis* × *pellita* clone in Riau and Kalimantan. Leaf samples were collected, and isolations were made from the disease symptoms. Isolates were identified based on their morphological characteristics and DNA sequence data for seven loci. Phylogenetic analyses of the isolates revealed a novel species of *Pseudoteratosphaeria*, described here as *Pseudoteratosphaeria supramediana* sp. nov. A greenhouse pathogenicity test resulted in symptoms similar to those found under field conditions, and the inoculated fungus was consistently reisolated from the resulting lesions. Its appearance in two geographically distinct locations of Indonesia raises the possibility of multiple independent introductions of the pathogen.

Keywords Emerging fungal pathogen · Leaf spot · Mycosphaerella-like · Plantation forestry · Tree disease

Introduction

Eucalyptus plantation forestry has expanded rapidly in Indonesia during the last two decades. This is largely in response to the devastation of *Acacia mangium* plantations caused by *Ceratocystis manginecans* (Tarigan et al. 2011; Wingfield et al. 2023). As a result, species such as *E. pellita, E. grandis, E. urophylla, E. brassiana*, and clones of their interspecific hybrids have been extensively planted in the region (Harwood and Nambiar 2014). This rapid shift towards large-scale *Eucalyptus* monocultures has resulted in significant new challenges, most notably, the increasing emergence of pests and diseases (Wingfield et al. 2008, 2015).

Leaf and shoot diseases pose a significant threat to the sustainability of non-native planted *Eucalyptus* forests. In Indonesia, notable examples of foliar pathogens include species of *Calonectria*, which cause shoot and leaf blight (Pham et al. 2019; Tarigan et al. 2023a), Eucalyptus scab and shoot malformation caused by *Elsinoe necatrix* (Pham et al. 2021, 2023, 2024, 2025; van Heerden et al. 2024), and Quambalaria leaf and shoot blight (Tarigan et al. 2023b). In addition to these pathogens, diseases caused by fungi in the families Mycosphaerellaceae and Teratosphaeriaceae have become increasingly prevalent (Andjic et al. 2019; Havenga et al. 2020; Old et al. 2003; Wingfield et al. 1996).

Species in the Mycosphaerellaceae and Teratosphaeriaceae are responsible for Mycosphaerella leaf disease (MLD) and Teratosphaeria leaf blight (TLB), respectively. These pathogens can cause severe defoliation, reduced photosynthetic capacity, retarded growth, and significant losses to productivity in non-native *Eucalyptus* plantations (Hunter

✉ Nam Q. Pham
nam.pham@fabi.up.ac.za

1 Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

2 Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

3 Research and Development, Asia Pacific Resources International Limited (APRIL), Pangkalan Kerinci 28300, Riau, Indonesia

et al. 2011; Crous et al. 2019b). At least 150 species residing in various genera (Andjic et al. 2019; Videira et al. 2017; Quaedvlieg et al. 2014) are associated with MLD and TLB (Hunter et al. 2011), including both obligate and secondary pathogens (Crous et al. 2019b; Maxwell et al. 2003). Given their largely similar disease symptoms and mycosphaerella-like sexual morphs, the identification of species in these groups relies on a combination of morphological characteristics of their reproductive structures and DNA-sequence based phylogenetic inference data (Videira et al. 2017; Quaedvlieg et al. 2014).

During routine surveys of *Eucalyptus* diseases in Indonesia, symptoms of a new leaf and shoot disease were observed on a hybrid clone of *Eucalyptus grandis* × *pellita* in Riau and Kalimantan. The objectives of this study were to identify the cause of the disease.

Materials and Methods

Sample Collection and Fungal Isolations

Leaf disease symptoms were observed on a single hybrid *Eucalyptus grandis* × *pellita* clone in clonal screening plots in Kalimantan and Riau during November 2022 and December 2023, respectively (Fig. 1). The disease was characterised by distinct necrotic lesions surrounded by yellow halos that were amphigenous, polygonal to irregular in shape, and dark olive-brown in colour. The lesions commonly had thin, raised, corky edges with necrotic tissues abscising to produce a shot-hole symptom (Fig. 2). Severe infection resulted in defoliation and crown thinning (Fig. 2).

Leaf samples were collected, stored in brown paper bags, and transferred to the laboratory for isolations. Fungal isolates were obtained either by direct isolation from symptomatic tissues, or by ascospores shot from ascomata (Crous 1998). For direct isolations, small pieces of leaf tissue were surface disinfested with 70% ethanol, washed with sterile distilled water, and dried with sterile paper towel. Leaf lesions were removed from the leaf lamina using a sterile hypodermic needle and transferred to the surface of malt extract agar (MEA; 20 g malt extract, 20 g Difco agar, 1 L ionized water) supplemented with 1% streptomycin sulphate (Sigma-Aldrich). Isolation plates were incubated for 5–7 days at 25 °C for fungal growth. Pure cultures were established by transferring hyphal tips of the emerging fungi to fresh MEA plates.

For the spore "shooting" method, excised lesions with visible ascomata were placed onto a piece of double-sided tape and fixed to the inner side of the Petri dish lid, with the ascomata facing the agar surface. The plates were incubated at room temperature in the dark for 24–48 h. Germinating ascospores were located using a dissection microscope and transferred to fresh MEA plates.

Pure cultures were deposited in the research culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The holotype was deposited in the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, and the ex-holotype in the culture collection (CMW-IA) of Innovation Africa at the University of Pretoria, South Africa.

DNA Extraction, PCR Amplification, and Sequencing

DNA was extracted from 7-day-old isolates grown on MEA at 25 °C, using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's protocols. The nuclear internal transcribed spacer regions 1 and 2 (ITS), the nuclear large subunit (LSU) of the rRNA, a fragment of the actin (*ACT*) gene region, calmodulin (*CMDA*), DNA-directed RNA polymerase II second largest subunit (*RPB2*), translation elongation factor 1-alpha (*TEF1*) and β-tubulin (*TUB2*), were used for polymerase chain reaction (PCR) amplification and subsequent sequencing (Table 1). PCR amplification was performed in 13 µL reactions containing 1 µL of genomic DNA, 0.5 µL of each primer (10 µM), 2.5 µL of 5 × MyTaq buffer (Bioline, London, UK), 0.25 µL MyTaq DNA polymerase (Bioline), and sterile deionised water. Amplified fragments were cleaned using an ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and sequenced in both directions. Geneious Prime v. 2025.0.3 (https://www.geneious.com) was used to assemble and edit the raw sequences.

Phylogenetics Analyses

Sequences of species related to those emerging from this study were sourced from the GenBank database (http://www.ncbi.nlm.nih.gov/) (Table 2). Alignments of all sequences were generated using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server) (Katoh and Standley 2013), then confirmed manually in MEGA v. 7 (Kumar et al. 2016). A concatenated data set was generated comprising ITS, LSU*, ACT, CMDA, RPB2, TEF1,* and *TUB2* sequences. Maximum likelihood (ML) analyses were conducted using RaxML v. 8.2.4 (Stamatakis 2014) on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010) with default GTR substitution matrix and 1 000 rapid bootstraps. BI analyses were performed using MrBayes v. 3.2.6 (Ronquist et al. 2012) on the CIPRES Science Gateway v. 3.3. (Miller et al 2010). The most appropriate models were obtained using the software jModeltest v. 1.2.5 (Posada 2008). Four Markov Chain Monte Carlo (MCMC) chains were run from a random starting tree for

Fig. 1 Geographic location of the surveys conducted in Sumatra and Kalimantan, Indonesia. Yellow dots on the map represent the sampling sites in each region

5 million generations, and trees were sampled every 100th generation. The first 25% of trees sampled were eliminated as burn-in, and the remaining trees were used to determine the posterior probabilities. Sequences for *Staninwardia suttonii* (CBS 120061) were used as the outgroup in all phylogenetic analyses. Final consensus trees were viewed using MEGA v. 7 (Kumar et al. 2016) and FigTree v. 1.4.5 (https://github.com/rambaut/figtree/).

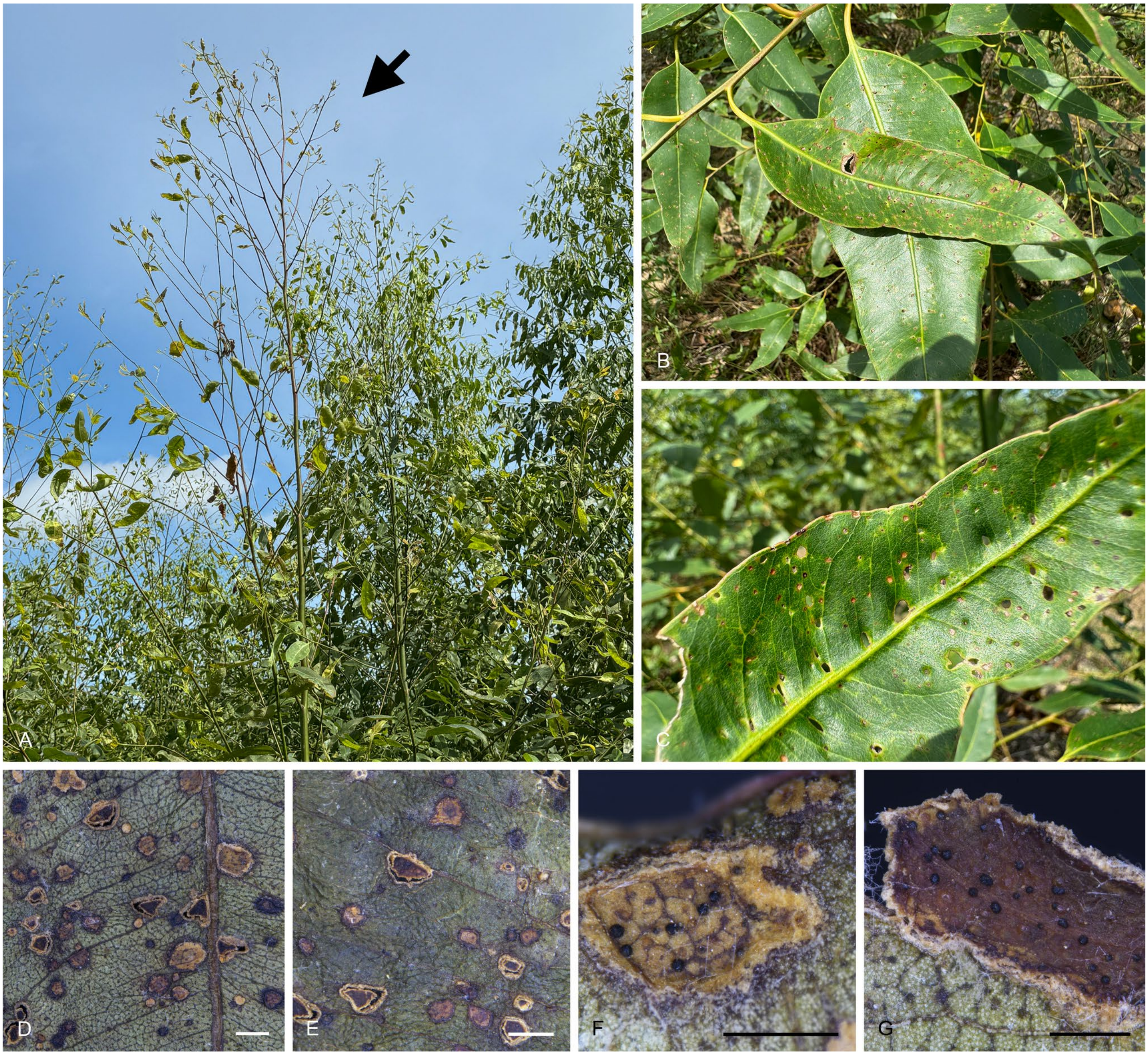


Fig. 2 Disease symptoms of infected *Eucalyptus* in Riau and Kalimantan. **A**. Infected tree in the field resulting in defoliation and crown thinning (arrow). **B, C**. Leaf spots with thin, raised, corky edges and frequent leaf abscission leading to perforations. **D, E**. Close-up of necrotic leaf spots. **F, G**. Pseudothecia on leaf spot. Scale bars D–E = 1 mm, F–G = 500 µm

Microscopy and Culture Characteristics

Ascomata isolated from symptomatic leaves were mounted on microscope slides in water, which was replaced with 85% lactic acid for further study and preservation. Microscopic structures were studied using microscopes (Nikon SMZ18, Eclipse Ni, Nikon Corporation, Tokyo, Japan) mounted with a camera (Nikon DS-Ri2, Nikon Corporation, Tokyo, Japan). An image program (NIS Elements v. 4.30, Nikon Corporation, Tokyo, Japan) was used to capture images and measure structures. Dimensions of characteristic structures are presented as minimum–maximum (average ± standard deviation, n = number of observations).

Pieces of symptomatic leaf tissue (5 × 5 mm) containing ascomata were mounted on a stub in a tissue freezing medium. These were sectioned vertically to a thickness of 10–12 µm using a cryomicrotome (CM1520, Leica Biosystems, Wetzlar, Germany). The cut sections were mounted on glass slides in 85% lactic acid for further study.

Mycelial growth characteristics were assessed on MEA. Mycelial plugs (5 mm diam.) were excised from the actively growing edges of 30-day-old cultures and placed at the

Table 1 Primers used in this study, with sequences and sources

Region	Primer	Direction	Sequence	Annealing temperature	References
Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA (ITS)	ITS-1F ITS-4	Forward Reverse	CTTGGTCATTTAGAGGAAGTAA TCCTCCGCTTATTGATATGC	56 °C	Gardes and Bruns (1993); White et al. (1990)
Nuclear large subunit of the ribosomal RNA (LSU)	LR0R LR5	Forward Reverse	ACCCGCTGAACTTAAGC TCCTGAGGGAAACTTCG	55 °C	Rehner and Samuels (1994); Vilgalys and Hester (1990)
DNA-directed RNA polymerase II second-largest subunit (*RPB2*)	fRPB2-5F fRPB2-7cR	Forward Reverse	GAYGAYMGWGATCAYTTYGG CCCATRGCTTGYTTRCCCAT	58 °C	Liu et al. (1999)
Translation elongation factor 1-α (*TEF1*)	EF1-728F EF1-986R	Forward Reverse	CATCGAGAAGTTCGAGAAGG TACTTGAAGGAACCCTTACC	52 °C	Carbone and Kohn (1999)
β-Tubulin 2 (*TUB2*)	BT2a BT2b	Forward Reverse	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	52 °C	Glass and Donaldson (1995)
Calmodulin (*CMDA*)	CAL-228F CAL2Rd	Forward Reverse	GAGTTCAAGGAGGCCTTCTCCC TGRTCNGCCTCDCGGATCATCTC	55 °C	Carbone and Kohn (1999); Groenewald et al. (2013)
Actin (*ACT*)	ACT-512F ACT-783R	Forward Reverse	ATGTGCAAGGCCGGTTTCGC CATCGAGAAGTTCGAGAAGG	61 °C	Carbone and Kohn (1999)

centre of 90 mm diam Petri dishes containing. Five replicate plates were incubated at seven temperatures ranging from 5–35 °C, at 5 °C intervals. Colony diameters were measured every 7th day for 28 d after which the average diameters were calculated.

Pathogenicity Tests

To test pathogenicity, two isolates (CMW 60296 and CMW 63637) were selected for an inoculation trial. They were grown for two weeks on MEA at 22 °C and a mycelial suspension was prepared for inoculation following the method described by Pham et al. (2021). The mycelial suspension was prepared by adding sterile distilled water to the cultures and gently scraping their surfaces with a sterilised micro-spatula. The mixture was homogenised using a magnetic stirrer at high speed for five minutes. The concentration of the suspension was adjusted to approximately 10^6 mycelial fragments per mL with a haemocytometer and supplemented with a drop of Tween 20 (Sigma-Aldrich).

One-year-old plants of a *E. grandis* clone, planted in 5 L black polyethylene bags containing composted bark medium, were maintained in a greenhouse at 25 °C under natural day-night light conditions. To promote the development of multiple young shoots and partially expanded leaves suitable for inoculation, the plants were pruned three weeks prior to the experiment. Small branchlets 15–20 cm in length with young leaves, were cut from the plants and inserted into moistened florist foam submerged in sterile distilled water. Five branchlets were inoculated per isolate by spraying both upper and lower leaf surfaces with fine droplets of mycelial suspension until run-off. An equal number of branchlets were sprayed with sterile distilled water to serve as controls. The branchlets were enclosed in clear plastic bags to maintain high humidity and leaf wetness. Inoculated plants were monitored for the development of symptoms over a four-week period. Re-isolations were made from the necrotic spots on MEA as described above, and the resulting isolates were identified based on colony morphology and DNA sequencing for the ITS region as described above.

Results

Fungal Isolations

Twelve isolates having a similar morphology were obtained from both direct isolation from symptomatic tissues (five isolates) and active ascospore discharge (seven isolates). Of these, five were collected from Kalimantan in 2022 and seven were from the 2023 sampling in Riau (Fig. 1).

Phylogenetic Analyses

Six representative isolates were chosen for further study. Amplicons of approximately 560 bp were generated for

Table 2 Collection details and GenBank accessions of isolates included in the phylogenetic analyses

Species	Isolate	Substrate/Host	Locality	LSU	*ACT*	*CMDA*	ITS	*RPB2*	*TEF1*	*TUB2*	References
Araucasphaeria foliorum	CPC 33084^{T}	*Araucaria araucana*	Chile	MH327829	N/A	N/A	MH327793	N/A	N/A	N/A	Crous et al. (2018)
Batcheloromyces alistairii	CBS 120035^{T}	*Protea repens*	South Africa	KF937220	N/A	N/A	DQ885901	KF937253	N/A	N/A	Quaedvlieg et al. (2014)
Batcheloromyces leucadendri	CBS 111577^{T}	*Leucadendron laureolum*	South Africa	KF937221	N/A	N/A	AY260101	KF902195	N/A	N/A	Quaedvlieg et al. (2014)
Penidiella columbiana	CBS 486.80^{T}	*Paepalanthus columbianus*	Colombia	KF901965	KF903587	KF902594	KF901630	KF902272	KF903158	KF902860	Quaedvlieg et al. (2014)
Pseudoteratosphaeria africana	CBS 144595^{T}	Leaf of unknown host	Angola	MK442558	MK442645	N/A	MK442622	N/A	MK442713	MK442745	Crous et al. (2019a)
	CBS 144596	Leaf of unknown host	Angola	MK442559	MK442646	N/A	MK442623	N/A	MK442714	MK442746	Crous et al. (2019a)
Pseudoteratosphaeria supramediana	**CMW 60294**	*Eucalyptus grandis × pellita*	Riau, Indonesia	PX651744	PX597597	PX597603	PX651738	PX597609	PX597615	PX597621	This study
	CMW 60295	*Eucalyptus grandis × pellita*	Riau, Indonesia	PX651745	PX597598	PX597604	PX651739	PX597610	PX597616	PX597622	This study
	CMW 60296^{T}	*Eucalyptus grandis × pellita*	Riau, Indonesia	PX651746	PX597599	PX597605	PX651740	PX597611	PX597617	PX597623	This study
	CMW 63637	*Eucalyptus grandis × pellita*	Kalimantan, Indonesia	PX651747	PX597600	PX597606	PX651741	PX597612	PX597618	PX597624	This study
	CMW 63641	*Eucalyptus grandis × pellita*	Kalimantan, Indonesia	PX651748	PX597601	PX597607	PX651742	PX597613	PX597619	PX597625	This study
	CMW 63642	*Eucalyptus grandis × pellita*	Kalimantan, Indonesia	PX651749	PX597602	PX597608	PX651743	PX597614	PX597620	PX597626	This study
Pseudoteratosphaeria flexuosa	CBS 110743	*Eucalyptus globulus*	Colombia	KF902098	KF903403	KF902653	KF901745	KF902345	KF903228	KF902931	Quaedvlieg et al. (2014)
	CBS 111012^{T}	*Eucalyptus globulus*	Colombia	KF902110	KF903421	KF902654	KF901755	KF902346	N/A	KF902932	Quaedvlieg et al. (2014)
Pseudoteratosphaeria gamsii	CBS 118495^{T}	*Eucalyptus* sp.	India	KF901990	KF903494	KF902655	KF901650	KF902347	KF903229	KF902933	Quaedvlieg et al. (2014)
Pseudoteratosphaeria ohnowa	CBS 112896^{T}	*Eucalyptus grandis*	South Africa	KF901946	KF903457	KF902656	KF901620	KF902348	KF903230	KF902934	Quaedvlieg et al. (2014)
	CBS 112973	*Eucalyptus grandis*	South Africa	GU214511	N/A	N/A	AF173299	N/A	N/A	N/A	Quaedvlieg et al. (2014)
Pseudoteratosphaeria perpendicularis	CBS 118367^{T}	*Eucalyptus eurograndis*	Colombia	KF901972	KF903491	N/A	KF901637	KF902350	KF903232	KF902936	Quaedvlieg et al. (2014)
Pseudoteratosphaeria secundaria	CBS 118507^{T}	*Eucalyptus* sp.	Brazil	KF901947	KF903503	KF902661	KF901621	KF902354	KF903236	KF902940	Quaedvlieg et al. (2014)
	CBS 111002	*Eucalyptus grandis*	Colombia	KF901858	KF903420	KF902659	KF901536	KF902352	KF903234	KF902938	Quaedvlieg et al. (2014)
Pseudoteratosphaeria stramenticola	CBS 118506^{T}	*Eucalyptus* sp.	Brazil	KF901948	KF903502	KF902662	KF901622	KF902355	KF903237	KF902941	Quaedvlieg et al. (2014)

Table 2 (continued)

Species	Isolate	Substrate/Host	Locality	LSU	*ACT*	*CMDA*	ITS	*RPB2*	*TEF1*	*TUB2*	References
	CBS 120737	*Eucalyptus urophylla*	Venezuela	KF902167	KF903530	KF902657	KF901806	KF902349	KF903231	KF902935	Quaedvlieg et al. (2014)
Teratosphaeria destructans	CBS 111370	*Eucalyptus grandis*	Indonesia	KF901898	KF903447	KF902716	KF901574	KF902427	KF903301	KF903000	Quaedvlieg et al. (2014)
Teratosphaeria nubilosa	CBS 116005 ^{T}	*Eucalyptus globulus*	Australia	KF902031	KF903479	KF902749	KF901686	KF902460	KF903336	KF903033	Quaedvlieg et al. (2014)
Teratosphaericola pseudoafricana	CBS 114782 ^{T}	*Eucalyptus globulus*	Zambia	KF902084	KF903473	KF902783	KF901737	KF902500	KF903371	KF903067	Quaedvlieg et al. (2014)
	CBS 111168	*Eucalyptus globulus*	Zambia	KF902045	KF903435	KF902782	KF901699	KF902499	KF903370	KF903066	Quaedvlieg et al. (2014)
Teratosphaericola leucadendri	CBS 146993 ^{T}	*Leucadendron* sp.	South Africa	MZ064478	MZ078147	MZ078164	NR_173039	N/A	MZ078224	MZ078262	Crous et al. (2021)
Teratosphaeriopsis pseudoafricana	CBS 111171 ^{T}	*Eucalyptus* sp.	South Africa	KF902085	KF903436	KF902784	KF901738	KF902501	KF903372	KF903068	Quaedvlieg et al. (2014)
Staninwardia suttonii	CBS 120061 ^{T}	*Eucalyptus robusta*	Australia	KF901874	KF903517	KF902693	KF901552	KF902392	KF903270	KF902974	Quaedvlieg et al. (2014)

N/A represents information that is not available. Isolates obtained in this study are indicated in bold

^{T} denotes ex-type strain

CBS, the culture collection of Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CMW, the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC, Pedro Crous working collection housed at Westerdijk Fungal Biodiversity Institute

ACT, Actin; *CMDA,* Calmodulin; ITS, internal transcribed spacer regions 1 and 2 including the 5.8S region of ribosomal RNA; LSU, Nuclear large subunit of the ribosomal RNA; *RBP2*, DNA-directed RNA polymerase II second largest subunit; *TEF1*, translation elongation factor 1-alpha; *TUB2*, β-tubulin the ITS region, 850 bp for the LSU, 220 bp for the *ACT*, 420 bp for the *CMDA*, 740 bp for the *RPB2*, 420 bp for the *TEF1*, and 380 bp for the *TUB2*. The seven-locus combined sequence data set used in the phylogenetic analyses included 28 ingroup taxa and 3364 characters with alignment gaps. Based on the results of jModeltest, BI analysis was performed applying the TrN+I+G substitution model for ITS region, the TIM2+I+G for LSU, the TIM1+I+G for *RPB2*, the TIM1+G for *TEF1*, the TIM3+I+G for *TUB2*, GTR+I+G for *CMDA*, and the TrN+G for *ACT*. The ML and BI analyses resulted in phylogenetic trees with concordant topologies and showed similar phylogenetic relationships between taxa. The ML tree with bootstrap support values of the ML and the posterior probabilities obtained from the BI analysis is presented in Fig. 3. All six isolates had identical sequences and formed a well-supported (ML/BI=100/1.00) monophyletic clade in the phylogenetic tree (Fig. 3), clearly distinct from the two most closely related species, *Pseudoteratosphaeria secunda* and *P. flexuosa*, and thus represent a novel taxon.

Taxonomy

Pseudoteratosphaeria supramediana N.Q. Pham, Marinc. & M.J. Wingf. Figures 4–5. MycoBank: MB861397.

Etymology: The name refers to the supramedian ascospore septation.

Diagnosis: Closely related to *P. flexuosa* and *P. secundaria* but differs in larger ascospores (11–15 × 3–4 µm), more rapid growth in culture and having supramedian septation.

Type: INDONESIA: Kalimantan. From leaf of a diseased *Eucalyptus grandis* x *E. pellita* hybrid clone with necrotic spots, November 2022, *M.J. Wingfield* (Holotype PRU(M) 4634; ex-holotype culture CMW-IA 7208, CMW 60296). GenBank: PX651740 (ITS); PX651746 (LSU), PX597599 (*ACT*), PX597605(*CMDA*), PX597611 (*RPB2*), PX597617 (*TEF1*), PX597623 (*TUB2*).

Description: *Leaf spots* amphigenous, polygonal, irregular, strong yellow to dark olive brown, with thin raised edges, often abscising, resulting in perforations, 322–1405 (700 ± 236.17, n=50) µm long, 260–1124 (631.1 ± 191.58, n=50) µm wide, ascomata present. *Ascomata* epiphyllous, pseudothecial, unilocular, minute, gregarious, subepidermal, subglobose, becoming erumpent, ostiolate, 41–85 (57.3 ± 9.93, n=25) µm high, 48–81 (61.0 ± 9.72, n=25) µm wide, olive green when immature, becoming dark brown; wall of 3–4 layers of brown textura angularis. *Asci* bitunicate, 8-spored, multiseriate, obovoid, 26–34 × 9–12 (29.0 ± 2.35 × 10.16 ± 0.88, n=12) µm, with minute ocular chamber. *Ascospores* hyaline, guttulate, granular, obovoid, tapering uniformly towards base from septum, tapering abruptly near subobtuse apex, straight or slightly curved near the base, 1-septate, supramedian, occasionally constricted at the septum, base obtuse, 11–15 × 3–4 (12.8 ± 1.07 × 3.8 ± 0.20, n=49) µm. No asexual structures found.

Fig. 3 Phylogenetic tree based on maximum likelihood (ML) analysis of a combined DNA data set of ITS, LSU, *ACT, CMDA, RPB2, TEF1*, and *TUB2* sequences. Bootstrap values ≥ 60% for ML analyses and posterior probability values ≥ 0.90 obtained from Bayesian inference (BI) are indicated at the nodes as ML/BI. Bootstrap values < 60% or probability values < 0.9 are marked with "*", and nodes lacking the support values are marked with "−". Isolates representing ex-type material are marked with "T" and novel isolates from this study are presented in bold. *Staninwardia suttonii* (isolate CBS 120061) represents the outgroup ▶

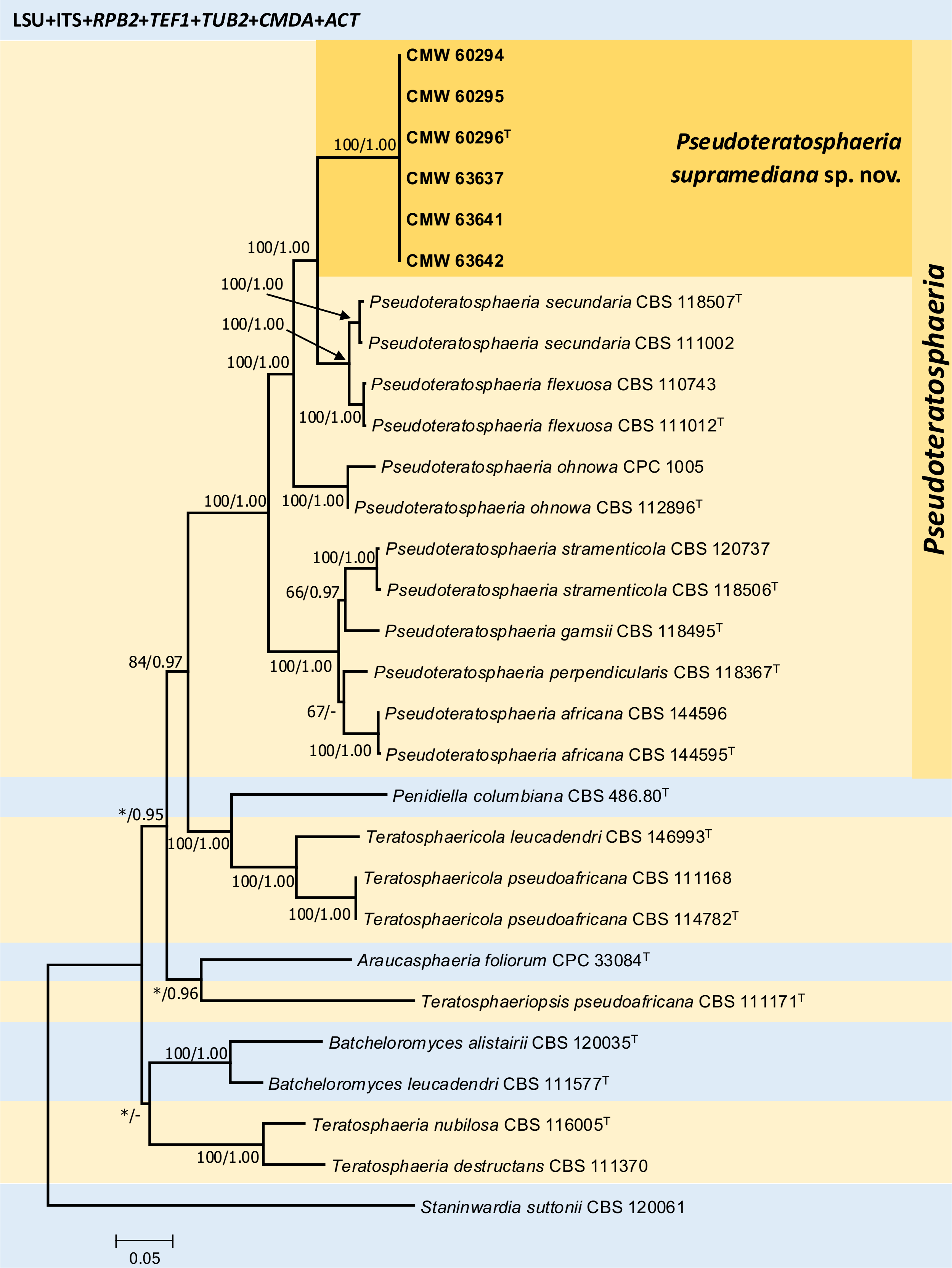
Culture characteristics on MEA, circular, elevation raised, texture velvety, edge entire to undulate, density dense, no zonation. Sterile. Colour above light olive grey to olive grey, reverse dark greyish olive to olive black. Optimum temperature 25 °C, reaching 50.1 mm diam in 28 d, followed by 20 °C (39.5 mm), 15 °C (23.5 mm), 30 °C (14.4 mm) and 10 °C (9.8 mm). No growth shown at 5 °C and 35 °C.

Host: Hybrid *Eucalyptus grandis* × *pellita* clone.

Distribution: Riau and Kalimantan, Indonesia.

Additional materials examined: INDONESIA: Riau. A symptomatic leaf of *Eucalyptus grandis* x *E. pellita* with necrotic spots, December 2023, *M.J. Wingfield* (PRU(M) 4635, cultures CMW-IA:7209, CMW:63637). GenBank: PX651741 (ITS); PX651747 (LSU), PX597600 (*ACT*), PX597606 (*CMDA*), PX597612 (*RPB2*), PX597618 (*TEF1*), PX597624 (*TUB2*). (PRU(M) 4636, cultures CMW-IA:7210, CMW:63641). GenBank: PX651742 (ITS); PX651748 (LSU), PX597601 (*ACT*), PX597607 (*CMDA*), PX597613 (*RPB2*), PX597619 (*TEF1*), PX597625 (*TUB2*).

Notes: *Pseudoteratosphaeria supramediana* is closely related to *P. secundaria* and *P. flexuosa*. Those species are known from *Eucalyptus* in South America, specifically in Brazil and Colombia (Crous 1998; Crous et al. 2004, 2006, 2019b). They were considered secondary colonists on leaf spots caused by the pathogen *Suberoteratosphaeria suberosa* (≡ *Mycosphaerella suberosa*), although *P. flexuosa* was also observed on young leaves (Crous 1998; Crous et al. 2006). Apart from the differences in DNA sequence data, *P. supramediana* can be distinguished from the closely related species based on its larger ascospores (*P. supramediana*: 11–15 × 3–4 µm, *P. flexuosa*: 9–13 × 2–3 µm, *P. secundaria*: 8–10 × 2.5–3 µm), and more rapid growth at 25 °C (*P. supramediana*: 1.79 mm/d, *P. flexuosa*: 0.73–0.83 mm/d; *P. secundaria*: 1.19–1.66 mm/d). The supramedial placement of the ascospore septa also distinguishes *P. supramediana* from *P. flexuosa* and *P. secundaria,* which have medianly septate ascospores.


LSU+ITS+RPB2+TEF1+TUB2+CMDA+ACT
CMW 60294
CMW 60295
CMW 60296^{T}
CMW 63637
CMW 63641
CMW 63642
100/1.00
Pseudoteratosphaeria supramediana sp. nov.
Pseudoteratosphaeria
100/1.00
100/1.00
100/1.00
100/1.00
Pseudoteratosphaeria secundaria CBS 118507^{T}
Pseudoteratosphaeria secundaria CBS 111002
Pseudoteratosphaeria flexuosa CBS 110743
100/1.00
Pseudoteratosphaeria flexuosa CBS 111012^{T}
Pseudoteratosphaeria ohnowa CPC 1005
100/1.00
100/1.00
Pseudoteratosphaeria ohnowa CBS 112896^{T}
100/1.00
Pseudoteratosphaeria stramenticola CBS 120737
66/0.97
Pseudoteratosphaeria stramenticola CBS 118506^{T}
Pseudoteratosphaeria gamsii CBS 118495^{T}
84/0.97
100/1.00
Pseudoteratosphaeria perpendicularis CBS 118367^{T}
67/-
Pseudoteratosphaeria africana CBS 144596
100/1.00
Pseudoteratosphaeria africana CBS 144595^{T}
Penidiella columbiana CBS 486.80^{T}
*/0.95
Teratosphaericola leucadendri CBS 146993^{T}
100/1.00
100/1.00
Teratosphaericola pseudoafricana CBS 111168
100/1.00
Teratosphaericola pseudoafricana CBS 114782^{T}
Araucasphaeria foliorum CPC 33084^{T}
*/0.96
Teratosphaeriopsis pseudoafricana CBS 111171^{T}
100/1.00
Batcheloromyces alistairii CBS 120035^{T}
Batcheloromyces leucadendri CBS 111577^{T}
*/-
Teratosphaeria nubilosa CBS 116005^{T}
100/1.00
Teratosphaeria destructans CBS 111370
Staninwardia suttonii CBS 120061
0.05

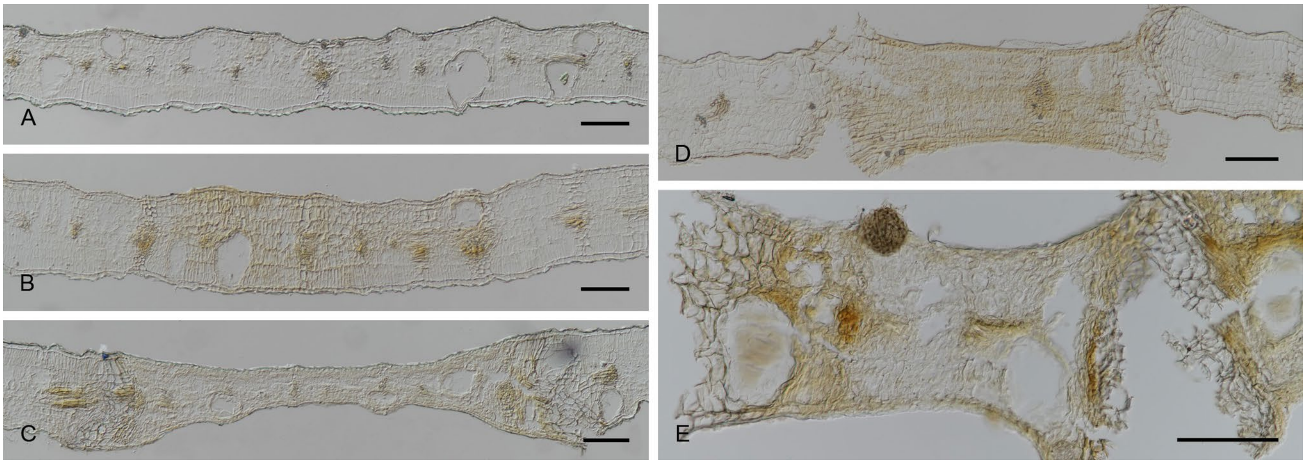


Fig. 4 Cross-section of symptomatic leaves of *Eucalyptus grandis* × *pellita* with leaf spots. **A.** Healthy tissue. **B–E.** Infected leaves. **B.** Colonised by fungal hyphae. **C.** Collapsed parenchyma tissue. **D.** Abscising leaf spot. **E.** Pseudothecium on abscising leaf spot. Scale bars: A–E = 100 µm

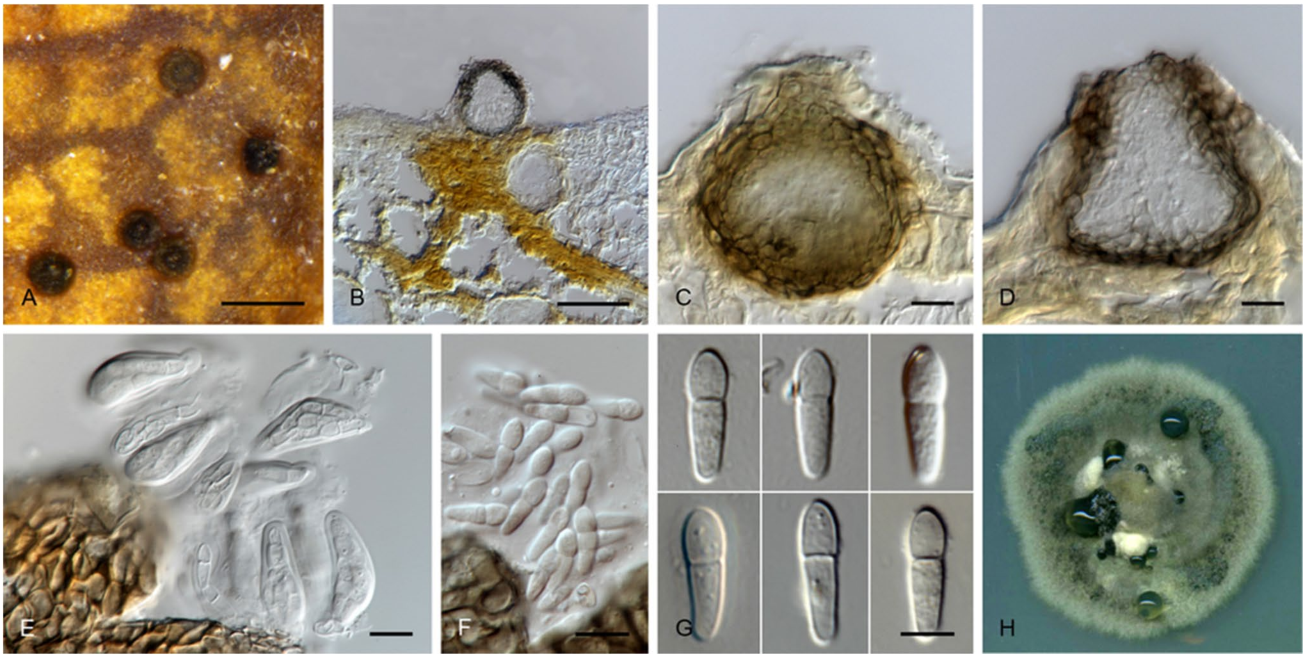


Fig. 5 Microscopic images of *Pseudoteratosphaeria supramediana* sp. nov. (Holotype: PRU(M):4634; ex-holotype: CMW-IA 7208, CMW 60296). **A.** Pseudothecia on a leaf spot. **B–D.** Section through a pseudothecium. **E.** Asci. **F, G.** Ascospores. **H.** Culture grown on MEA at 25 °C in the dark for 14 d. Scale bars: A = 100 µm; B = 50 µm; C–F = 5 µm; G = 2.5 µm

Pathogenicity tests

The two isolates (CMW 60269 and 63637) used in the pathogenicity trial produced small, necrotic, brown leaf spots with yellow halos starting at seven days after inoculation. The necrotic spots became more abundant on the leaf laminas two weeks after inoculation and became raised and corky at the edges as the lesions aged (Fig. 6). No symptoms developed in the control inoculations (Fig. 6). *Pseudoteratosphaeria supramediana* was re-isolated on MEA from the lesions on all the inoculated *E. grandis* branchlets and the isolates were identical to those used to test pathogenicity.

Discussion

This study resulted in the identification of a new species of *Pseudoteratosphaeria* causing a severe leaf disease on a *E. grandis* × *pellita* hybrid clone in two areas of Indonesia reasonably distant from each other. The fungus provided with

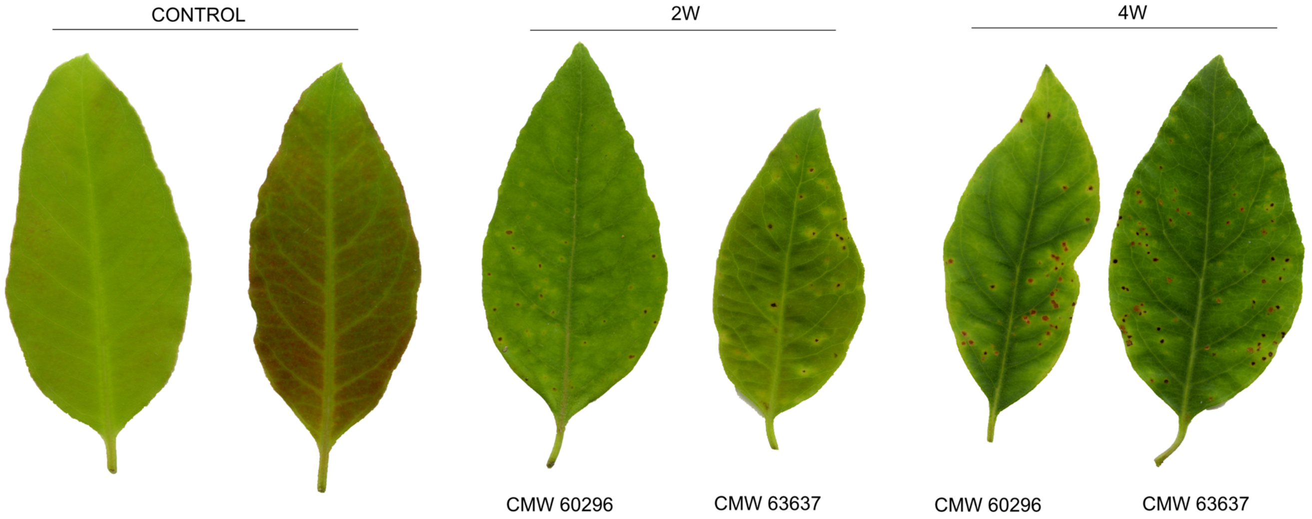


Fig. 6 Results of inoculations with *Pseudoteratosphaeria supramediana* on detached *Eucalyptus* branchlets

the name *P. supramediana* was consistently isolated from the necrotic leaf spots observed on all affected trees and resulted in similar symptoms in a pathogenicity trial. The disease occurred only on a single clone, with no symptoms observed in numerous other clones grown in close proximity.

The isolates of *P. supramediana* originated from both single ascospores and from infected tissues. These were identified based on DNA sequence comparisons for seven regions (LSU, ITS, *RPB2, TEF1, TUB2, CMDA,* and *ACT*), confirming their distinct position within the genus *Pseudoteratosphaeria*. The name *P. supramediana* sp. nov. was provided for the fungus, referring to its distinct ascospore morphology where the septum characteristically occurs at a supramedial position, unlike those of other species in the genus.

Pathogenicity trials conducted on young branchlets of a *E. grandis* clone under laboratory conditions resulted in symptoms identical to those observed in the field. Consistent re-isolation of *P. supramediana* from infected tissue confirmed the pathogenicity of the fungus. Although *Pseudoteratosphaeria* includes several species that appear to be primary pathogens, some species are considered secondary colonists or saprophytic where they occur in association with other disease symptoms (Crous et al. 2006, 2019b; Quaedvlieg et al. 2014). As far as we are aware, this study provides the first experimental evidence that a *Pseudoteratosphaeria* species can act as a primary pathogen.

Pseudoteratosphaeria was first introduced to accommodate various cryptic *Teratosphaeria* species lacking any known asexual morphs (Quaedvlieg et al. 2014). Morphologically, the two genera are broadly indistinguishable, thus DNA sequence data are required to identify them with confidence (Quaedvlieg et al. 2014). Initially, six species, all isolated from *Eucalyptus* in either Brazil, Colombia, India, South Africa, and Venezuela, were reassigned to the genus (Crous et al. 2019b; Quaedvlieg et al. 2014). More recently, the seventh taxon, *P. africana*, was described from leaves of an unidentified tree species in Angola (Crous et al. 2019a). *Pseudoteratosphaeria supramediana* represents the first species in the genus recorded from Southeast Asia.

The origin of *P. supramediana* is unknown. As is true for many other species in the Mycosphaerellaceae and Teratosphaeriaceae associated with *Eucalyptus* outside their native range (Andjic et al. 2019; Burgess and Wingfield 2017; Hunter et al. 2011; Wingfield et al. 2015)*,* it is plausible that the pathogen originated from where these trees are native in Australia or the surrounding New Guinea islands (Thornhill et al. 2019; Turnbull 2000). Its appearance in two geographically distinct locations of Indonesia (Sumatra and Borneo islands), raises the possibility of multiple independent introductions. Alternatively, a single introduction into one of the sampled regions may have served as a source for subsequent spread to other areas, consistent with a "bridgehead effect" (Lombaert et al. 2010). Clarifying the pathways of introduction and distribution of *P. supramediana* will require more extensive sampling and investigations using population genetic approaches.

The leaf spot and shot-hole disease symptoms caused by *P. supramediana* occurred only on a single clone of a *E. grandis* × *E. pellita* hybrid, growing in close proximity to numerous other *Eucalyptus* clones including various hybrids. The disease was also found on the same clone growing in two different islands of Indonesia relatively widely separated from each other (approx. 1000 km). The susceptible clone has since been removed from commercial deployment. These observations suggest that the fungus is highly host specific and is not likely to be particularly important to the extensive *Eucalyptus* clonal forest plantation industry of Indonesia.

Host specificity is a common feature of many *Eucalyptus* pathogens and especially those in the Teratosphaeriaceae (Aylward et al. 2019; Crous et al. 2008; Hunter et al. 2009; Pérez et al. 2009). This provides excellent opportunities to select planting stock with high levels of tolerance to infection and thus to avoid serious disease problems from emerging. An interesting aspect of contemporary *Eucalyptus* forestry lies in the utilisation of clones of interspecific hybrids (Potts and Dungey 2004). Other than providing planting stock with traits, specifically suited to particular environments and industrial requirements, they have emerged as important sources of tolerance to diseases (Balmelli et al. 2014; Dungey et al. 1997). However, they also expose unknown and little understood genetic backgrounds linked to pathogen susceptibility (Solís et al. 2025). The host-specificity of *P. supramediana* underscores the importance of intensive screening of *Eucalyptus* clones prior to commercialisation (Alfenas et al. 2016; van Heerden et al. 2005; van Heerden and Wingfield 2002; Solís et al. 2023).

Authors' Contribbution

Conceptualization: Nam Q. Pham, Seonju Marincowitz, Michael J. Wingfield; Investigation: Nam Q. Pham, Seonju Marincowitz, Michael J. Wingfield; Writing – original draft: Nam Q. Pham; Seonju Marincowitz; Methodology: Nam Q. Pham, Seonju Marincowitz, Michael J. Wingfield; Visualization: Nam Q. Pham, Seonju Marincowitz; Writing – review & editing: Nam Q. Pham, Seonju Marincowitz, Brenda D. Wingfield, Pedro W. Crous, Samuel A. Santos, Alvaro Durán, Marthin Tarigan, Michael J. Wingfield; Software: Nam Q. Pham, Seonju Marincowitz; Formal analysis: Nam Q. Pham, Seonju Marincowitz; Data curation: Nam Q. Pham, Seonju Marincowitz; Project administration: Nam Q. Pham, Brenda D. Wingfield, Michael J. Wingfield; Funding acquisition: Brenda D. Wingfield, Michael J. Wingfield; Validation: Nam Q. Pham, Seonju Marincowitz, Pedro W. Crous, Michael J. Wingfield; Resources: Nam Q. Pham, Brenda D. Wingfield, Samuel A. Santos, Alvaro Durán, Marthin Tarigan, Michael J. Wingfield.

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Data Availability The data that support the findings of this study are openly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers as listed in Table 2.

Declarations

Ethical Approval Not Applicable.

Competing interest The authors declare that there is no conflict of interest.

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