

Two *Cryphonectriaceae* species from *Eucalyptus* leaves in North Sumatra and their stem inoculation outcomes

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Abstract: *Cryphonectria* canker, caused by a number of genera and species in the family *Cryphonectriaceae* (*Diaporthales*, *Ascomycota*), is an important disease affecting woody shrubs and trees in the *Melastomataceae* and *Myrtaceae* (*Myrtales*). Initially reported as a stem canker disease on *Eucalyptus* in Cuba, it has since emerged as an important threat, particularly in tropical regions and the Southern Hemisphere where *Eucalyptus* is extensively cultivated for commercial forestry. Many species of *Cryphonectriaceae* can occur as asymptomatic endophytes in different plant parts, causing disease only when the hosts are exposed to biotic or abiotic stresses. During 2018, isolations from healthy *Eucalyptus* leaf tissues adjacent to symptoms caused by *Elsinoe necatrix* in North Sumatra, Indonesia resulted in two isolates resembling species of the *Cryphonectriaceae*. Multi-locus DNA sequence comparisons (ITS+LSU+TUB1+TUB2+TEF1+RPB2), as well as morphological observations, showed that these fungi represented a novel *Celoporthe* (*Cel.*) species, described here as *Cel. endophylla* sp. nov. and the new genus *Aureofoliicola eucalypti* gen. et sp. nov. Inoculation trials on 4-mo-old plants of two *Eucalyptus* genotypes showed that *Cel. endophylla* can cause lesions significantly different to those from the controls, while *A. eucalypti* did not produce lesions on inoculated stems. These findings underscore a biosecurity risk posed by apparently latent fungal pathogens that persist undetected within asymptomatic plant tissues.

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INTRODUCTION

In Southeast Asia, short-rotation plantation forestry has experienced significant expansion over the past few decades, driven by an increasing demand and production capacity of the regional pulp and paper industry (Harwood & Nambiar 2014, Healey *et al.* 2022). These plantations now span over 7 M hectares and are dominated by fast-growing, non-native Australian tree species (Harwood & Nambiar 2014). In Indonesia, the widespread decline of *Acacia mangium* (Tarigan *et al.* 2011) has led to the increasing establishment of *Eucalyptus* plantations. These primarily consist of *E. pellita*, *E. grandis* and *E. urophylla*, along with their interspecific hybrid genotypes (Harwood & Nambiar 2014). This large-scale conversion to *Eucalyptus* monocultures has introduced substantial new challenges, notably the emergence of pests and diseases that threaten the sustainability of the local forestry industry (Wingfield *et al.* 2008, 2015, Hurley *et al.* 2016).

The *Cryphonectriaceae* (*Diaporthales*, *Ascomycota*) includes numerous fungal taxa that are important pathogens of woody plants and shrubs globally (Gryzenhout *et al.* 2009, Wingfield *et al.* 2025). The most notable of these is *Cryphonectria parasitica*, the causal agent of chestnut blight, responsible for the near destruction of *Castanea dentata* (*Fagales*) in North America (Anagnostakis 1987, Hepting 1974, Rigling & Prospero 2018). While early research focused primarily on *Cryphonectriaceae*

species in the Northern Hemisphere associated with hosts in the *Fagales*, the discovery of *Cryphonectria* canker on *Eucalyptus* in Cuba (Bruner 1917) signalled a broader geographic and host range for these fungi. This finding prompted more intensive surveys in tropical and Southern Hemisphere regions, leading to the discovery of many novel species in new genera including *Chrysoporthe*, *Celoporthe*, *Holocryphia*, *Xanthoporthe* and *Cryptometrion*, many of which are associated with cankers on *Myrtales*, including *Eucalyptus* (Gryzenhout *et al.* 2004, 2009, Wingfield *et al.* 2025).

In Indonesia, several species of *Cryphonectriaceae* have been identified over the past two decades, some of which are of particular concern to plantation forestry. *Chrysoporthe deuterochubensis* has been frequently isolated from diseased *Eucalyptus* stems and from other shrubs belonging to the *Myrtaceae* and *Melastomataceae*, and it has been shown to cause disease on planted *Eucalyptus* trees in pathogenicity trials (Tarigan *et al.* 2024). In North Sumatra and Riau, two *Celoporthe* spp. have been isolated from native *Syzygium* species, namely *Cel. indonesiensis* (Chen *et al.* 2011), and *Cel. foliorum* (Chen *et al.* 2022), but their potential to cause disease on *Eucalyptus* has not been fully evaluated. Additionally, *Cryptometrion aestuescens*, a novel species described by Gryzenhout *et al.* (2010), was reported to cause severe stem cankers on *E. grandis* clonal hedges in North Sumatra.

Recent studies have shown that species of the *Cryphonectriaceae* can naturally exist as endophytes in healthy

tissues of trees and woody shrubs in the *Myrtales* (Gryzenhout et al. 2006d, Mause-Sitoe et al. 2016, Grannados et al. 2020, Roux et al. 2020, Chen et al. 2022, Wingfield et al. 2025). These fungi typically do not produce disease symptoms unless the host plant is subjected to environmental or biotic stress. Endophytic *Cryphonectriaceae* species can be found inhabiting various parts of trees, including stems (Beier et al. 2017), branches (Mause-Sitoe et al. 2016, Grannados et al. 2020), shoots (Bisseger et al. 1994), bark (Coelho et al. 2021) and sprouts (Guérin & Robin 2003). Their latent presence within asymptomatic plant tissue poses significant biosecurity risks, as they may escape detection and later emerge as aggressive pathogens under favourable host and environmental conditions (Mause-Sitoe et al. 2016, Grannados et al. 2020, Wingfield et al. 2025).

During 2018 surveys of the fungal community associated with *Eucalyptus* scab and shoot malformation caused by *Elsinoe necatrix* in North Sumatra, Indonesia (Pham et al. 2021), two isolates resembling members of the *Cryphonectriaceae* were isolated from asymptomatic *Eucalyptus* leaf tissues. The objectives of this study were to identify these isolates based on multi-locus DNA sequence data and morphological characteristics, to assess their relative pathogenicity on different *Eucalyptus* genotypes, and thus to determine whether they pose a potential risk to commercial forestry in Indonesia.

MATERIALS AND METHODS

Sample collection and fungal isolations

Fresh branchlets of a *E. grandis* × *urophylla* clone, bearing leaves with scab-like necrotic spots caused by *Elsinoe necatrix* (Pham et al. 2021) were collected in Tele, North Sumatra, placed in sealable plastic bags and transferred to a laboratory for fungal isolations. Leaf samples were surface disinfected with 70 % ethanol and then washed with sterile distilled water. Apparently healthy leaf tissue adjacent to the necrotic scabs was cut into 1 mm² pieces and placed onto 2 % malt extract agar (MEA; Biolab, Midrand, South Africa) supplemented with streptomycin sulphate (100 ppm) and incubated at 25 °C for 3–7 d. Emerged fungal colonies resembling the *Cryphonectriaceae* were transferred to fresh MEA plates. Primary isolations were incubated for 3–7 d at 25 °C for fungal growth. Single hyphal tips of fungal colonies were sub-cultured to new MEA plates to obtain pure isolates. The isolates were deposited in the research culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI) and the publicly accessible culture collection (CMW-IA) of Innovation Africa at the University of Pretoria, South Africa, and the culture collection (CBS) of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands. Dried cultures representing the holotype of the novel taxa were deposited in the H.G.W.L. Schweickhardt herbarium (PRU), University of Pretoria, South Africa.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was isolated from 7-d-old cultures grown on 2 % MEA at 25 °C using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA), fol-

lowing the manufacturer's instructions. Amplification of the nuclear internal transcribed spacer regions 1 and 2 (ITS), including the 5.8S rRNA gene, was performed using primers ITS1 and ITS4 (White et al. 1990), the nuclear large subunit (LSU) of rRNA using LR0R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994), two regions of β -tubulin gene (*TUB1* and *TUB2*) using Bt1a/Bt1b and Bt2a/Bt2b (Glass & Donaldson 1995), the second largest subunit of RNA polymerase II (*RPB2*) with RPB2-5F2 and fRPB2-7cR (Liu et al. 1999, Sung et al. 2007) and the translation elongation factor 1- α (*TEF1*) using EF1-728F and EF1-986R (Carbone & Kohn 1999). The PCR amplifications were conducted in 13 μ L reaction volumes containing 1 μ L of genomic DNA, 5× MyTaq buffer (Bioline, London, UK), 0.25 μ L of MyTaq DNA polymerase, 0.5 μ L of each primer (10 μ M), and sterile deionised water. Amplified products were cleaned using ExoSAP-IT™ (Thermo Fisher Scientific) and sequenced in both directions. Sequence assembly and editing were conducted in Geneious Prime v. 2025.1.2 (<https://www.geneious.com>).

Phylogenetics analyses

Sequences generated in this study were aligned with reference sequences of closely related *Cryphonectriaceae* taxa (Table S1) using MAFFT v. 7 (Katoh & Standley 2013; <http://mafft.cbrc.jp/alignment/server/>). The resulting alignments were manually checked, trimmed and concatenated using Geneious Prime. Maximum likelihood phylogenetic analyses were performed using W-IQ-TREE (Trifinopoulos et al. 2016). Partition-specific substitution models were chosen using ModelFinder (Kalyaanamoorthy et al. 2017), and branch support was evaluated using 1000 Ultrafast bootstrap replicates (UFBoot; Hoang et al. 2018). Additionally, the Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT) was conducted with 1000 replicates to further assess node support (Guindon et al. 2010). *Diaporthe eres* (LC3198) and *Cytospora ailanthicola* (CFCC 89970) were included as outgroup taxa. The resulting phylogenetic tree was visualised using FigTree v. 1.4.5 (<https://github.com/rambaut/figtree>).

Morphology

Fungal fruiting structures were induced in the laboratory by transferring a piece of fungal culture onto water agar (WA, 20 g Difco Bacto agar in 1 L water) with autoclaved *Eucalyptus* twigs and leaves placed on the agar surface. The plates containing the twigs and leaves were incubated at 25 °C in the dark for 4 weeks. When fruiting bodies developed on the plant tissue, they were dissected with a scalpel to expose the inner structures including spores. The structures were initially mounted on slides in water and later replaced with 85 % lactic acid, in which they were studied, measured, and images captured. Observations were made using a microscope (Nikon SMZ18, Eclipse Ni, Tokyo, Japan) mounted with a camera (Nikon DS-Ri2) and an imaging software (NIS-Elements BR 4.30.00). Up to fifty measurements were made depending on availability. Dimensions were presented as minimum–maximum (average $\pm$ standard deviation) (n = number of observations). The pieces of twig and leaf containing fungal fruiting structures were sectioned vertically using a cryomicrotome (Leica Biosystems CM1520, Germa-

ny) to study their position in the substrate tissue. They were cut to 10–12 µm thickness, and the sections were mounted in 85 % lactic acid for observation.

A growth study was carried out on 2 % MEA in 90 mm Petri dishes. Five replicate plates were incubated at seven temperatures ranging from 5–35°C, at 5 °C intervals. Plugs of mycelium (5 mm) taken from 7-d-old cultures were placed at the centres of Petri dishes with mycelial side down, and the plates were maintained in incubators at the various growth temperatures in the dark. The study was terminated when the mycelial growth of the isolates at the optimum temperature reached the edges of the plates. Two diameter measurements perpendicular to each other were taken for each culture, and the averages for the replicate plates were computed. Culture characteristics were noted when the isolates were 14-d-old, and culture colours were described following the ISCC-NBS system.

Pathogenicity tests

Two isolates (CMW 55025 and CMW 55026) were selected for a pathogenicity trial to evaluate their relative aggressiveness on *Eucalyptus*. Inoculations were performed on 16-wk-old plants of a *E. grandis* × *pellita* (GP) and a *E. pellita* (EP) genotype, grown in 25-cm-diam. plastic planting bags and maintained in a greenhouse at approximately 28 °C. For each clone, 20 plants were inoculated per isolate, and an equal number of trees were included as negative controls.

A sterile scalpel was used to excise a 1 cm bark flap approximately 10–15 cm above the soil line to expose the cambial tissue. A 5-mm-diam. mycelial plug taken from the actively growing margin of a 7-d-old culture on potato dextrose agar (PDA; BD Difco, NJ, USA) was inserted into each wound with the mycelium facing the cambium. A sterile PDA disc was used for the control inoculations that were conducted in the same manner. Inoculation sites were sealed with Parafilm®M (Amcor, Zürich, Switzerland) to prevent desiccation and contamination. Lesion development was assessed after 6 wk by removing the bark at each wound site and measuring the length of the discoloured cambial tissue. Re-isolations were made from fruiting structures that formed on the bark and cambium after incubation in a moist chamber for seven days so as to verify the presence of the inoculated fungi.

Lesion length data were analysed using GraphPad Prism v. 10.4.1. Outliers were detected using the robust regression and outlier removal (ROUT) method with a coefficient value (Q) of 10 % (Motulsky & Brown 2006). The normality of the data was assessed using the Shapiro-Wilk test (Shapiro & Wilk 1965), and homogeneity of variances was tested using the Brown-Forsythe test (Brown & Forsythe 1974). A Kruskal-Wallis test was then applied, followed by Dunn's post-hoc test for pairwise comparisons at a 5 % significance level (Dunn 1964).

RESULTS

Fungal isolates, DNA sequencing and phylogenetic analysis

Isolations from apparently healthy *Eucalyptus* leaf tissue associated with *Elsinoe necatrix* symptoms resulted in a collec-

tion of approximately 150 fungal cultures (data not shown). Among these, two isolates resembling *Cryphonectriaceae* species were obtained. Amplicons of approximately 600 bp for the ITS, 850 bp for LSU, 500 bp for *TUB1*, 500 bp for *TUB2*, 650 bp for *TEF1*, and 900 bp for *RPB2* were generated for all the isolates. The combined sequence dataset for the six regions used for phylogenetic analysis included 100 ingroup taxa and contained 4256 characters, including alignment gaps. Based on the results of ModelFinder, a TIM2e+I+G4 model was selected for ITS and LSU, the HKY+F+I+G4 for *TUB1* and *TUB2*, the TPM2+F+I+G4 for *TEF1*, and the TN+F+G4 for *RPB2*, and these models were applied to individual partitions in the concatenated dataset for the ML analyses. The ML tree with UFBoot values and SH-aLRT branch supports is presented in Fig. 1. The isolate CMW 55025 grouped together with species of *Celoportha*, but formed a lineage clearly distinct from other species in this genus. The isolate CMW 55026 formed a unique lineage, representing a sister lineage to *Pseudocryphonectria* and is introduced here as a novel genus in the *Cryphonectriaceae*.

Taxonomy

Celoportha endophylla N.Q. Pham, Marinc., M.J. Wingf., *sp. nov.* MB 862245. Fig. 2.

Etymology: Name refers to its apparently endophytic lifestyle.

Diagnosis: Morphologically distinguishable by the absence of paraphyses and long attenuated apices of its conidiogenous cells.

Typus: **Indonesia**, North Sumatra, Tele, leaf of *Eucalyptus* sp. infected with *Elsinoe necatrix*, Apr. 2018, M.J. Wingfield & N.Q. Pham [**holotype** PRU(M) 4641, dried culture and twigs with artificially induced conidiomata; ex-holotype cultures CMW-IA 7269, CMW 55025, CBS 147167]. GenBank: PX727429 (ITS); PX727433 (LSU); PX733386 (*TUB1*); PX733388 (*TUB2*); PX733390 (*TEF1*); PX733392 (*RPB2*).

Description: On *Eucalyptus* twigs and leaves, where conidiomata were artificially induced. *Sexual morph* not observed. *Conidiomata* immersed, becoming erumpent, stromatic, uni- or multi-locular, convoluted, 85–404 (228.9 ± 86.61) µm long (n = 29), 87–407 (185.7 ± 73.09) µm wide (n = 29). *Conidiomatal walls* pseudoparenchymatous, textura angularis, 6–36 (19.2 ± 6.99) µm thick (n = 32), consisting of a few layers or thick-walled, brown cells in outer stratum, becoming thinner, paler, more flattened towards inner stratum. *Conidiophores* hyaline, borne along inner walls of locules, cylindrical, simple or branched, septate or aseptate, 4–13 (7.6 ± 2.91) µm long (n = 25), 1–3 (2 ± 0.34) µm wide (n = 25). *Paraphyses* not observed. *Conidiogenous cells* hyaline, enteroblastic, determinate, discrete or integrated, flask-shaped with attenuated apices, occasionally as aperture borne just below septum, 5–9 × 2–3 (7.0 ± 1.11 × 2.0 ± 0.27) µm (n = 25). *Conidia* hyaline, aseptate, fusiform to allantoid with pointed base, straight or slightly curved, exuding in cirrhous, yellowish in mass, 3–5 × 2 (4.1 ± 0.43 × 1.8 ± 0.15) µm (n = 50).

ITS+LSU+TUB1+TUB2+TEF1+RPB2

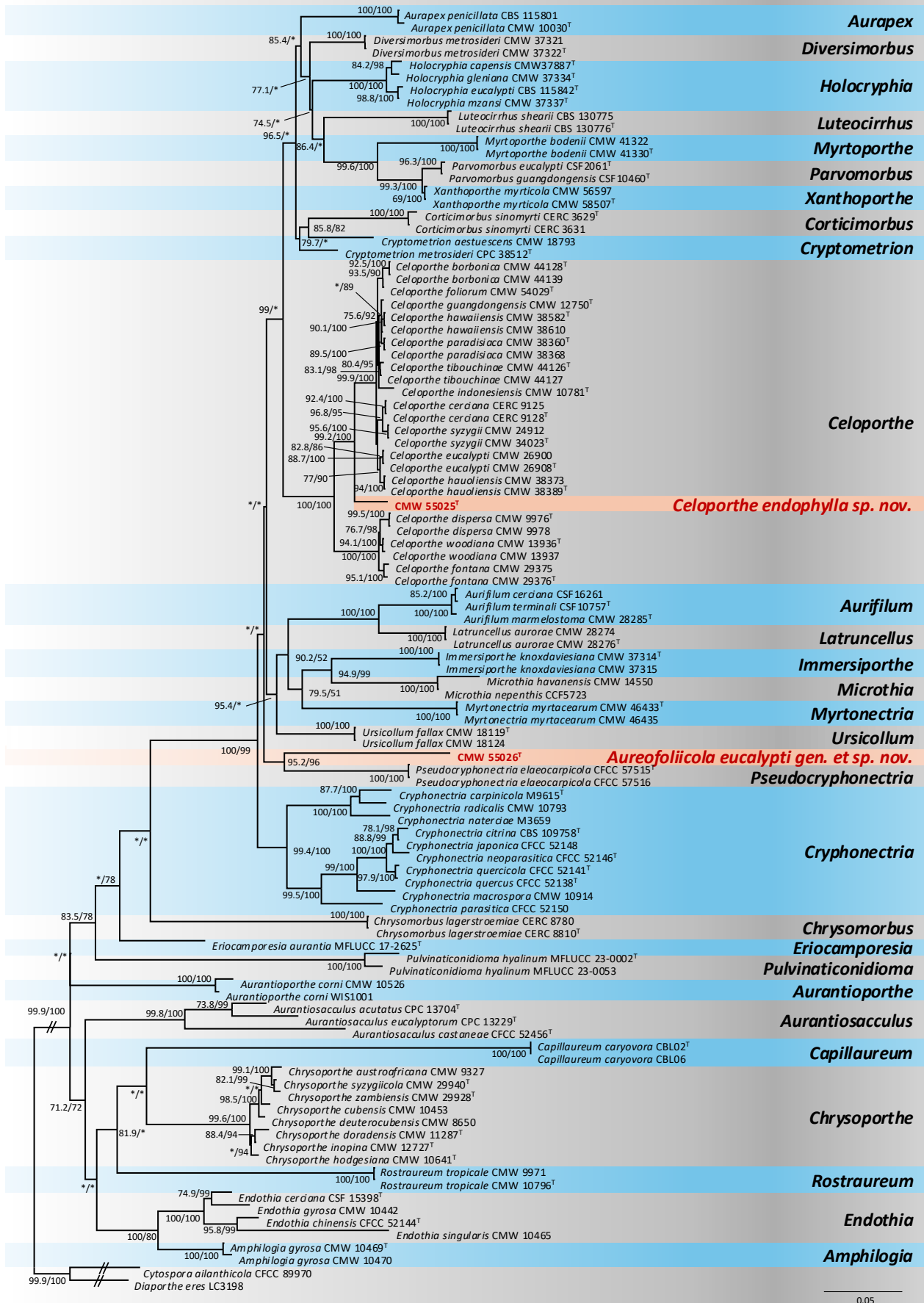


Fig. 1. Phylogenetic tree based on a Maximum Likelihood (ML) analysis of a combined DNA data set of ITS, LSU, *TUB1*, *TUB2*, *TEF1* and *RPB2* sequences for *Cryphonectriaceae* species. Isolates sequenced in this study are presented in red and boldface. SH-aLRT branch support and UFBoot values $\geq 70\%$ for ML analyses are indicated at the nodes as SH-aLRT/UFBoot. Branch support values $< 70\%$ are marked with “*”. Isolates representing ex-type cultures are marked with a “T”. *Diaporthe eres* (LC3198) and *Cytospora allanthicola* (CFCC 89970) represent the outgroup taxa.

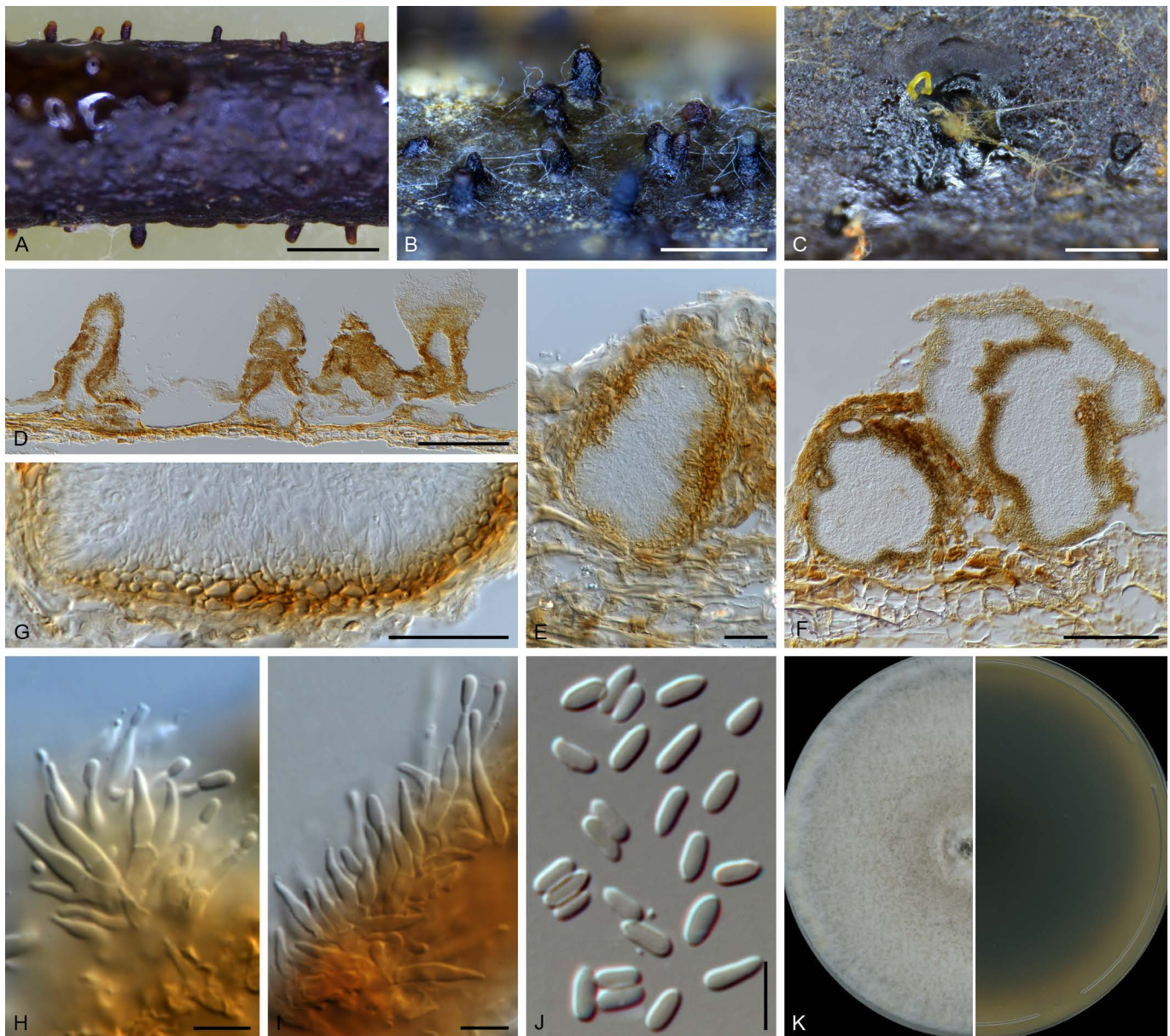


Fig. 2. Micrograph of *Celoportha endophylla* sp. nov. [Holotype PRU(M) 4641; ex-holotype culture CMW-IA 7269, CMW 55025, CBS 147167]. **A, B.** Conidiomata formed on *Eucalyptus* twig in the laboratory. **C.** Conidia oozing in a cirrus. **D–F.** Configuration of conidioma in twig (D, F) and leaf (E). **G.** Conidiomatal wall and inner layers producing conidiophores. **H, I.** Conidiophores and conidiogenous cells. **J.** Conidia. **K.** Colony at optimum temperature (25 °C) on 2 % MEA in the dark for 14 d (left: upper, right: under). Scale bars: A = 1 mm; B, C = 500 µm; D, G = 250 µm; F = 100 µm; E = 25 µm; H–J = 5 µm.

Culture characteristics: Growth on 2 % MEA in the dark, optimal growth at 25 °C, reaching 71 mm diam. in 7 d, followed by 20 °C (52 mm), 30 °C (38 mm), 15 °C (29 mm), and 10 °C (9.8 mm). No growth at 5 °C and 35 °C. **Culture morphology** on 2 % MEA in 14 d in the dark, showing circular growth, even edge; *mycelia* fluffy texture, medium dense; *colours* at 10 °C very dark red becoming paler towards edges, covered with white aerial mycelia, reverse dark brown; 15 °C on black background, covered with light yellowish brown to deep orange yellow aerial hyphae, reverse black; 20 °C on black background with greyish yellow to dark greyish yellow, reverse black; 25 °C on black background with light greyish yellowish brown, paler towards edges, pigmented water droplets or exudates present, reverse black; 30 °C on black background with moderate yellowish brown centre becoming paler towards edges, edges often white to yellowish white, raised, reverse black.

Notes: *Celoportha endophylla* is the 15th species of the most species-rich genus in the *Cryphonectriaceae*. It was isolated from an asymptomatic *Eucalyptus* leaf tissue, in contrast to *Cel. foliorum*, another foliicolous species in the genus, isolated from a leaf spot on a *Syzygium* sp. (*Myrtaceae*) (Chen *et al.* 2022). Both species occurring on leaf tissues lack paraphyses, which separate them from other *Celoportha* species, which are corticolous and have paraphyses. *Celoportha endophylla* has a larger conidial range (3–5 × 2 µm, av. 4.1 × 1.8 µm) than *Cel. foliorum* (3–4 × 1–2 µm, av. 3.6 × 1.5 µm). Conidiogenous cells (phialides) of *Cel. endophylla* have attenuated apices that are more elongated than those of *Cel. foliorum*.

Aureofoliicola N.Q. Pham, Marinc., M.J. Wingf., **gen. nov.** MB 862249.

Etymology: Name refers to its yellow spore masses and foliicolous lifestyle.

Type species: *Aureofoliicola eucalypti* N.Q. Pham, Marinc., M.J. Wingf.

Description: *Conidiomata* stromatic, unilocular. *Conidiophores* filiform, septate. *Conidiogenous cells* enteroblastic, determinate, integrated. *Conidia* hyaline, fusiform to cylindrical.

Aureofoliicola eucalypti N.Q. Pham, Marinc. M.J. Wingf., **sp. nov.** MB 862253. Fig. 3.

Etymology: Name refers to its host plant, *Eucalyptus* sp.

Diagnosis: Morphologically identifiable by its filiform conidiophores and lack of paraphyses.

Typus: **Indonesia**, North Sumatra, Tele, leaf of *Eucalyptus* sp. infected with *Elsinoe necatrix*, Apr. 2018, M.J. Wingfield

& N.Q. Pham [**holotype** PRU(M) 4642, dried culture and twigs with artificially induced conidiomata; ex-holotype culture CMW-IA 7270, CMW 55026, CBS 147168]. GenBank: PX727430 (ITS); PX727434 (LSU); PX733387 (*TUB1*); PX733389 (*TUB2*); PX733391 (*TEF1*); PX733393 (*RPB2*).

Description: On *Eucalyptus* twigs and leaves, where conidiomata were artificially induced. *Sexual morph* not observed. *Conidiomata* stromatic, unilocular, convoluted, 182–587 (372.0 ± 100.48) μm long ($n = 25$), 153–478 (315.0 ± 82.98) μm wide ($n = 25$). *Conidiomatal wall* pseudoparenchymatous, 16–39 (25.8 ± 6.62) μm thick ($n = 25$), composed of three strata, outer stratum consisting of a few layers of thick-walled, brown cells, often mixed with plant tissue, mid-stratum consisting of thick-walled, sub-hyaline, compressed cells, inner stratum consisting of a few thin-walled, hyaline and highly compressed cells. *Conidiophores* hyaline, filiform, septate, simple or scarcely branched near

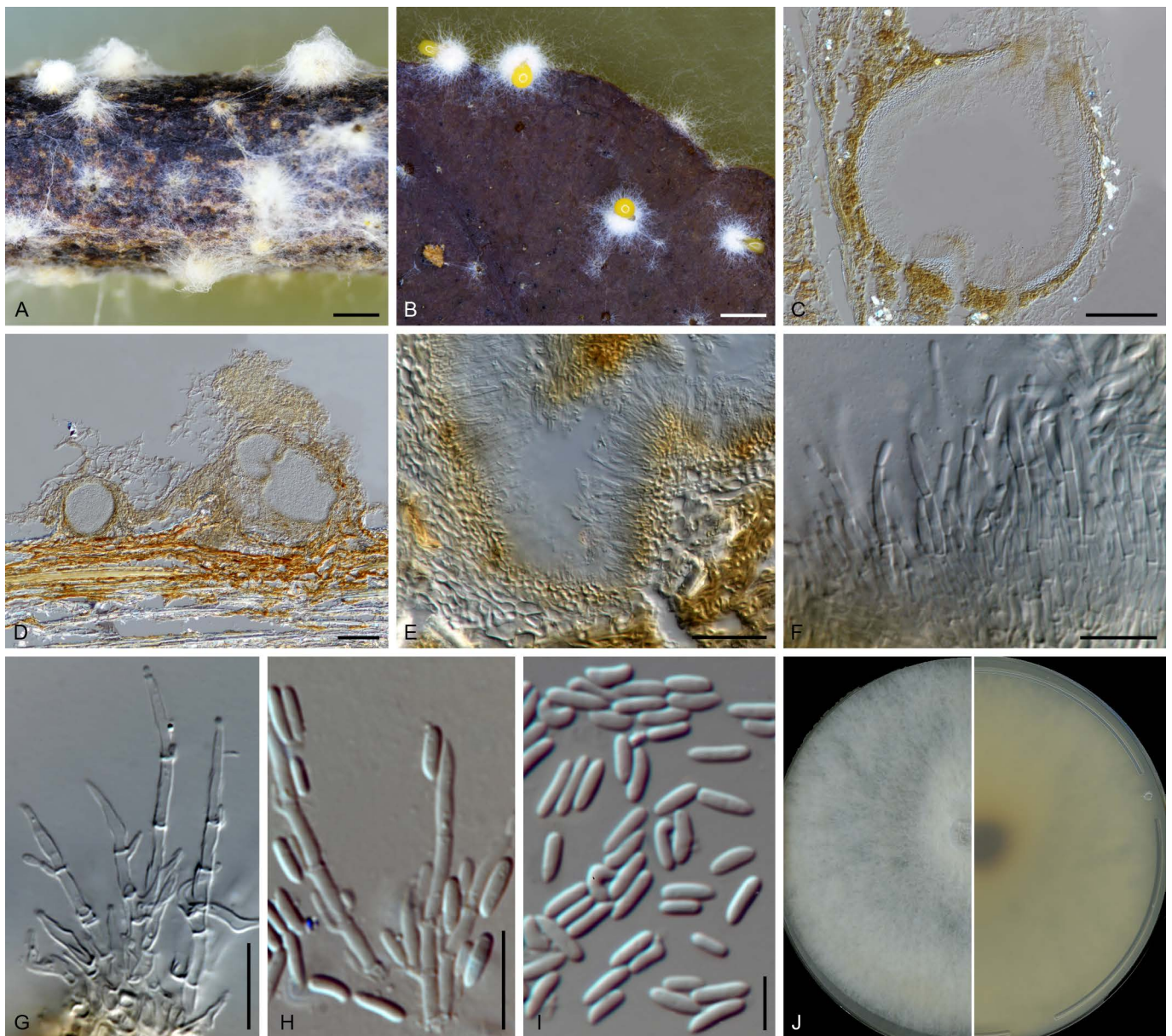


Fig. 3. Micrograph of *Aureofoliicola eucalypti* gen. et. sp. nov. [Holotype PRU(M) 4642; ex-holotype culture CMW-IA 7270, CMW 55026, CBS 147168]. **A, B.** Conidiomata formed on *Eucalyptus* twig (A) and leaf (B) in the laboratory. **C, D.** Vertical section of conidioma on leaf (C) and twig (D). **E.** Conidiomatal wall and conidiophores borne inside locule. **F.** Close-up of conidiophores born on the inner wall. **G, H.** Filiform conidiophores and integrated conidiogenous cells, apical or lateral beneath the septum. **I.** Conidia. **J.** Colony at optimum temperature (25 °C) on 2 % MEA in the dark for 14 d (left: upper, right: under). Scale bars: A, B = 1 mm; C, D = 100 μm ; E = 25 μm ; F–H = 10 μm ; I = 5 μm .

base, borne along inner layers of locules, 18–45 (27.4 ± 7.05) μm long ($n = 25$). *Conidiogenous cells* enteroblastic, apical or lateral beneath septum, integrated, determinate, hyaline, cylindrical, tapered to apex when terminal, 8–13 (10.6 ± 1.49) μm long ($n = 25$) when apical, 5–8 (6.9 ± 0.86) μm long ($n = 25$) when lateral, 1–2 (1.6 ± 0.13) μm wide ($n = 25$). *Conidia* hyaline, fusiform to cylindrical, aseptate, straight or slightly curved, apex round, base pointed, exuding in cirrhous, yellowish in mass, $4\text{--}6 \times 1\text{--}2$ ($5.0 \pm 0.58 \times 1.6 \pm 0.16$) μm ($n = 50$).

Culture characteristics: Growth on 2 % MEA, optimal growth at 25 °C reaching 71 mm in 7 d, followed by 20 °C (54 mm), 15 °C (30 mm), 30 °C (23 mm), 10 °C (11 mm), and no growth at 5 °C and 35 °C. **Culture morphology** on 2 % MEA in 14 d in dark, showing circular growth, even edge; *mycelia* texture fluffy texture, medium sparse density; *colours* at 10 °C yellowish white, sparse dense becoming less dense towards edges, reverse pale yellow with dark olive brown limited to centre; 15 °C centre yellowish grey aerial hyphae towards edges yellowish white, reverse pale yellow background with dark red brown limited to centre; 20 °C yellowish white, yellowish grey aerial hyphae over colony with dark olive brown dotted structures, reverse pale yellow background with dark red limited to centre; 25 °C mixture of aerial hyphae of medium grey, moderate olive brown, dark greyish yellow in concentric zones, reverse pale yellow background with dark reddish brown limited to centre or as random patches; 30 °C dark olive brown to deep yellowish brown becoming paler towards edges, reverse the same.

Notes: *Aureofollicola eucalypti* is phylogenetically closely related to the monospecific *Pseudocryphonectria*. *Pseudocryphonectria elaeocarpicola* causes a stem blight on *Elaeocarpus* sp. (*Elaeocarpaceae*, *Oxalidales*) (Huang *et al.* 2022). They are, however, morphologically distinct from each other. *Pseudocryphonectria* has both obclavate and cylindrical conidia, and conidiophores that are often reduced to ampulliform conidiogenous cells. In contrast, *Aureofollicola* has conidia that are all fusiform and septate filiform conidiophores, which distinguishes it from other genera in the *Cryphonectriaceae*.

Pathogenicity tests

Six weeks after inoculation, *Cel. endophylla* (CMW 55025) produced brown to dark brown lesions around the inoculation points on the tested plants (Fig. 4). The lesion lengths associated with each treatment showed heterogeneity of variance ($F_{(3,76)} = 77.59$, $p < 0.0001$) and did not follow a normal distribution ($p < 0.05$). No outlier was detected using the ROUT method. A significant difference in lesion length among different treatments was seen in the Kruskal-Wallis test ($H_{(3)} = 76.24$; $p < 0.0001$). Post-hoc Dunn's tests revealed significant differences between lesion length caused by CMW 55025 on clone EP ($\bar{x} = 343.00$ mm) compared to clone GP ($\bar{x} = 36.05$ mm), and these were longer than those in the controls ($p < 0.05$). *Celoportha endophylla* was re-isolated from lesions on the inoculated trees but never from the controls. In contrast to *Cel. endophylla*, no lesions developed in association with inoculations using *A. eucalypti* (CMW 55026).

DISCUSSION

Two novel taxa in the *Cryphonectriaceae*, a family that includes numerous important canker pathogens of woody plants, were discovered in this study. Multi-locus DNA sequence comparisons for six regions, as well as morphological observations, showed that the isolates represented a new species of *Celoportha*, described here as *Celoportha endophylla* sp. nov., and a new genus and species, *Aureofollicola eucalypti* gen. et sp. nov. The discovery of these two taxa highlights the hidden diversity of *Cryphonectriaceae* on *Myrtales* hosts and suggests that others remain to be discovered in Southeast Asia.

To date, six foliicolous species, including two novel species in this study, are reported in the family *Cryphonectriaceae*, where the remaining 70 species are corticolous, and most of them cause stem canker diseases (Wingfield *et al.* 2025). Among the foliicolous species, three were isolated from leaf spots: *Aurantiosacculus* (Aur.) *acutatus*, *Aur. eucalyptorum* (Crous *et al.* 2012) and *Cel. foliorum* (Chen *et al.* 2022), and the remaining species, *Microthia nepenthis* (Koukol *et al.* 2018), *Cel. endophylla* and *A. eucalypti*, were from

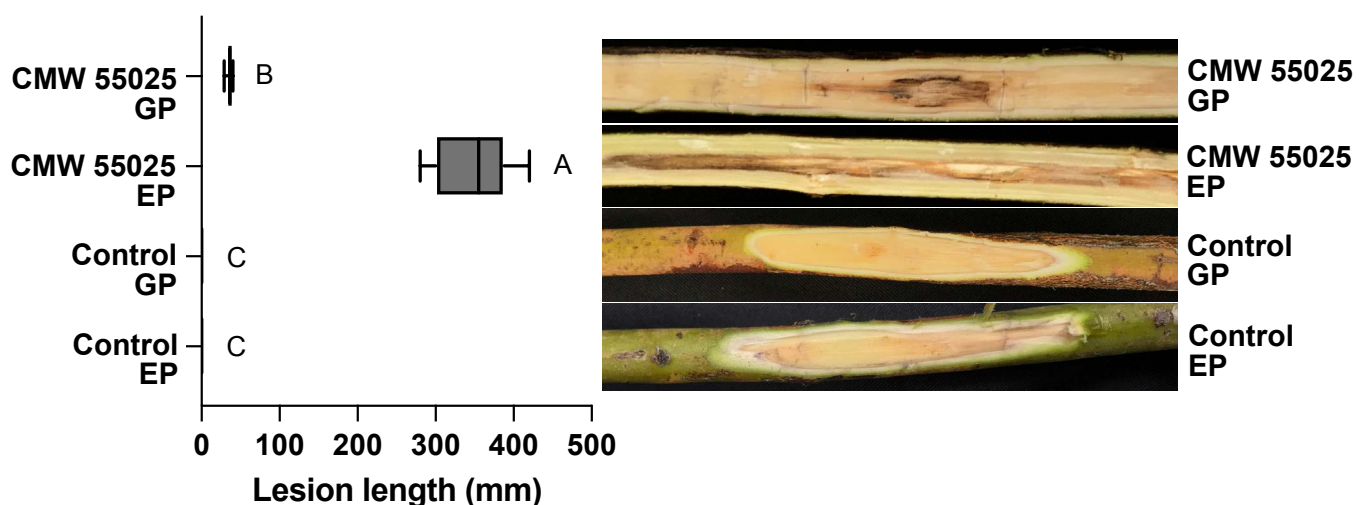


Fig. 4. Lesion length (mm) of *Celoportha endophylla* (CMW 55025) on a *E. pellita* (EP) and a *E. grandis* × *pellita* (GP) genotype. Different letters indicate significant differences in pathogenicity based on the Dunn's post-hoc test ($p < 0.05$). Whiskers indicate min–max values. *Aureofollicola eucalypti* (CMW 55026) was not included in the graph as it did not produce lesions on inoculated stems and was not different from the controls.

necrotised or asymptomatic leaves. Follicolous species of the *Cryphonectriaceae* are relatively rare, occupy an unusual ecological niche, which is poorly understood, and not typically associated with disease symptoms. They could, however, be pathogens and provide inoculum for stem infections; a question that was considered in this study.

The genus *Celoportha* was originally established by Nakabonge *et al.* (2006) to accommodate fungi closely related to *Chrysosporthe*, but distinguishable on the basis of DNA sequence data and morphology (Gryzenhout *et al.* 2009, Chen *et al.* 2022). Five *Celoportha* spp. are known from Africa (Nakabonge *et al.* 2006, Vermeulen *et al.* 2013, Ali *et al.* 2018), three from the Pacific region (Roux *et al.* 2020), and six from Southeast Asia and South China (Wang *et al.* 2018, Chen *et al.* 2011, 2022). The description of *Cel. endophylla* brings the number of species in *Celoportha* to 15, all of which occur on trees and shrubs in the *Myrtales* (Chen *et al.* 2022). Notably, *Cel. endophylla* described here, and one other species *Cel. foliorum*, are the only follicolous species in the genus and both occur in Indonesia.

Aureofoliicola represents the 29th genus recognised in *Cryphonectriaceae*. It is clearly morphologically distinct from other genera, producing only a single conidial type and septate, filiform conidiophores, rather than the dimorphic conidia and reduced conidiophores characteristic of the sister genus, *Pseudocryphonectria*. Unlike the majority of *Cryphonectriaceae*, which are typically bark-inhabiting fungi associated with stem cankers, *Aureofoliicola* was isolated from *Eucalyptus* leaves.

Both *Cel. endophylla* and *A. eucalypti* were described here from single isolates. While descriptions based on limited material require cautious interpretation, this is a common situation in fungal systematics, especially for endophytes and other rare fungi that occur sporadically or at low abundance in host tissues. In such cases, the integration of multi-locus phylogenetic data with morphological evidence from culture provides sufficient resolution to justify the recognition of novel taxa. The distinct phylogenetic positions of both isolates, coupled with consistent diagnostic features, support their formal description despite the restricted sample size. Moreover, deposition of the ex-type cultures in international collections ensures accessibility for further study, enabling future work to verify and refine their taxonomic placement as additional isolates are discovered.

Pathogenicity trials conducted on young *Eucalyptus* seedlings revealed a marked difference between *Cel. endophylla* and *A. eucalypti*. Only the former species showed evidence of pathogenicity on stems, unlike the tissues from which it was isolated. Whether it is a pathogen under natural conditions was not determined. Yet it remains interesting that it might have the capacity to cause disease, as is known for many other apparently endophytic fungi (Tanney *et al.* 2025).

The international movement of infected *Eucalyptus* planting material is rising (Wingfield *et al.* 2001, 2008, 2015, Burgess & Wingfield 2017). Many fungi, including members of the *Cryphonectriaceae*, are now known to persist asymptotically as endophytes, allowing them to evade detection during routine phytosanitary inspections (Kemler *et al.* 2013, Tanney *et al.* 2025, Wingfield *et al.* 2025). Historical introductions of *Cryphonectriaceae* species into new environments illustrate how such hidden infections can give rise to serious canker diseases on *Eucalyptus* utilised

in plantation forestry (Burgess & Wingfield 2017, Wingfield *et al.* 2025). In this context, the isolation of viable cultures was particularly valuable, as it made a pathogenicity test possible and thus offered some evidence of whether these fungi represent a potential threat to the commercial forestry industry.

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- Supplementary Material:** <http://fuse-journal.org/>
- Table S1.** Collection details and GenBank accessions of isolates included in the phylogenetic analyses.