



Host range of *Colletotrichum pyricola*: an aggressive pathogen of eucalypts

Aindreeya Alcova¹ · Peter K. Ades¹ · Jacqueline Edwards² · Yu Pei Tan³ · Niloofar Vaghefi¹ · Paul W.J. Taylor¹

Received: 9 May 2025 / Accepted: 12 October 2025
© The Author(s) 2026

Abstract

Colletotrichum is a fungal genus, comprising many plant pathogens that pose a significant threat to agriculture globally, however, their diversity and host range on Australian native plants remain poorly understood. *Colletotrichum pyricola*, first described as a fruit rot pathogen of *Pyrus communis* (common pear) in New Zealand, has also been reported in Australia, but its broader host range and potential biosecurity risk remain unknown. In this study, pathogenicity bioassays revealed that *Co. pyricola* can infect a range of Australian natives, including *Banksia burdettii*, *Clematis aristata*, *Eucalyptus caesia*, and *Leptospermum grandifolium*, as well as fruit trees, *Malus domestica* (apple) and *Persea americana* (avocado). Detached-leaf pathogenicity bioassays using both wound and non-wound inoculation showed *Co. pyricola* was also able to cause disease symptoms on a range of eucalypt species (*Angophora*, *Corymbia*, and *Eucalyptus*). Additionally, the re-isolation of *Co. pyricola* from asymptomatic, non-wounded tissues and microscopic observation of appressoria support a latent or hemibiotrophic lifestyle. Therefore, these asymptomatic infections may allow the fungus to persist undetected across hosts and environments, facilitating long-term dispersal. These findings expand the known host range and potential geographic distribution of *Co. pyricola* in Australia, highlighting the importance of understanding fungal pathogens in native plant communities.

Keywords *Colletotrichum pyricola* · Myrtaceae · Eucalypts · Host range · Native Australian flora · Pathogenicity · Fruit trees

Introduction

The ‘eucalypts’ are a group of closely related genera (*Angophora*, *Corymbia*, and *Eucalyptus*) that belong to the Myrtaceae family. With over 800 recorded species, eucalypts are predominantly endemic to Australia, with an almost continuous distribution across the continent, acting as a keystone species that defines much of the landscape (Fensham et al. 2020). *Eucalyptus*, the largest genus of the eucalypts, is widely recognised as an iconic Australian plant

(Nicolle et al. 2025), collectively comprising 77% of Australia’s native forested land (ABARES 2023). This genus is also an important global commodity, being one of the most extensively grown genera for hardwood timber (Brockerhoff et al. 2013). Furthermore, eucalypts have been reported to harbour a diversity of fungi, including a wide range of plant pathogenic fungi (Crous et al. 2019).

Plant pathogens in natural populations have coevolved with their host and environment, typically existing in low levels as endemic diseases without causing detrimental damage to their host. However, environmental changes and the movement of plants (and their pathogens) due to globalisation may unbalance the plant-pathogen-environment interaction, leading to a shift from endemic to epidemic disease or new host exposures (Anderson et al. 2004; Stenlid and Oliva 2016; Burgess et al. 2022). Host shifts may occur between closely related plant hosts, while host jumps may occur due to the geographical proximity of hosts, irrespective of the hosts’ genetic relatedness (Roy 2001; Bufford et al. 2016). Given that many crops are adjacent to or in the

✉ Paul W.J. Taylor
paulwjt@unimelb.edu.au

¹ School of Agriculture, Food and Ecosystem Sciences, The University of Melbourne, Melbourne, Australia

² School of Applied Systems Biology, La Trobe University, Melbourne, Australia

³ Queensland Plant Pathology Herbarium, Department of Primary Industries, Brisbane, Australia

vicinity of native plants, such as forests or other remnant vegetation, these natural pathogen populations have the potential to cause disease on agricultural and horticultural crops (Dentika et al. 2023). In Australia, minimal research has been conducted into the potential for natural populations to act as sources of infectious propagules, which may fuel disease in surrounding cultivated systems, or vice versa.

Colletotrichum is a globally significant genus of fungi, primarily comprising plant pathogens. These plant pathogens cause anthracnose on a diverse range of plant hosts, with most crops being susceptible to one or more species of *Colletotrichum* (Dean et al. 2012). This genus currently accommodates over 300 species, which are divided into at least 16 species complexes (Talhinhas and Baroncelli 2023). While *Colletotrichum* spp. associated with crops are well studied, very little is known about their diversity and pathogenicity on Australian native plants. Relatively few species of *Colletotrichum* have been identified on Australian native plants and verified using molecular phylogenetics (e.g., Golzar 2009; Liu et al. 2013; Shivas et al. 2016; Prasannath et al. 2020). Several species of *Colletotrichum* have been identified on *Eucalyptus* spp. globally; however, none have been formally reported from Australia (Crous et al. 2019).

This study provides the first report of *Colletotrichum pyricola* as a pathogen of fruit trees and native plants in Australia. Pathogenicity bioassays were utilised to explore the pathogenicity and host range of *Co. pyricola* acquired from culture collections and new field collections in Victoria. Preliminary pathogenicity bioassays revealed that *Co. pyricola* can infect *Banksia burdettii* and *Eucalyptus caesia*. This study, therefore, aims to: (i) taxonomically characterise isolates of *Co. pyricola* from fruit trees and native plant species in Australia, (ii) evaluate the pathogenicity of

each isolate on its host plant, and (iii) assess the host range and aggressiveness of *Co. pyricola* on various *Banksia* and eucalypt species.

Materials and methods

Isolates and morphological analysis

Leaves and twigs presenting symptoms of *Colletotrichum* spp., including diffuse, irregular, red-purple lesions (Fig. 1), were collected from national and state parks in Victoria and Queensland, Australia. Collections were limited to plants within arm's reach along hiking trails. Samples were placed in paper bags, transported back to the laboratory and stored at 4 °C until processed. Tissue from the margin of lesions was surface sterilised with ethanol (80% v/v) for 30 s and sodium hypochlorite (~1% v/v) for 2 min and then washed three times with sterile water. Sterile tissue was cultured (~1 cm²) on water agar (Ajax Finechem) for 5 days and then sub-cultured onto potato dextrose agar (PDA; Amyl Media) or cornmeal agar (CMA; Oxoid) to promote sporulation. Pure cultures were obtained by single-spore isolation as described by Choi et al. (1999). All cultures were maintained at 25 °C with a 12 h photoperiod. Culture morphology was observed on 10-day-old PDA cultures, and isolates were tentatively identified as *Colletotrichum* species based on morphology (Damm et al. 2012). Six isolates of *Colletotrichum* were obtained from the Victorian Plant Pathology Herbarium (VPRI), and 44 from the Queensland Plant Pathology Herbarium (BRIP), including one isolate previously identified as *Co. pyricola* from *Malus domestica* ('Jonathan' apple; Table 1).



Fig. 1 Examples of disease symptoms (indicated by black arrows) associated with *Colletotrichum pyricola*: (a) *Clematis aristata* stem; and (b) *Leptospermum grandifolium* leaves

Table 1 Collection details of *Colletotrichum pyricola* isolates used in this study

Isolate	Host	Common name	Tissue	Locality	Collection date
VPRI 10714	<i>Eucalyptus caesia</i>	Silver Princess	Unknown	Croydon, VIC	Apr. 1979
VPRI 15937	<i>Banksia burdettii</i>	Burdett's Banksia	Unknown	Monbulk, VIC	07 Jun. 1988
VPRI 43730	<i>Persea americana</i>	'Hass' avocado	Fruit	Shoreham, VIC	26 May 2020
BRIP 54037	<i>Malus domestica</i>	'Jonathan' apple	Unknown	Cottonvale, QLD	30 Mar. 1972
BRIP 76704*	<i>Clematis aristata</i>	Old man's beard	Stem	Taggerty, VIC	Sep. 2022
BRIP 76705*	<i>Leptospermum grandifolium</i>	Mountain tea-tree	Leaf	Taggerty, VIC	Sep. 2022

*Isolates collected during fieldwork

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from mycelium using a DNeasy Plant Mini Kit (Qiagen), as per the manufacturer's instructions. DNA quantity and quality were assessed with a NanoDrop™ 2000/2000c Spectrophotometer (Thermo Fisher Scientific). DNA was then diluted to 2 ng/μL with autoclaved MilliQ water and stored at -20 °C.

Six loci were used for species identification: actin (*act*), chitin synthase 1 (*chs-1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), histone 3 (*his3*), the internal transcribed spacers of the nuclear ribosomal DNA and the intervening 5.8 S region (ITS), and beta-tubulin (*tub2*). The primer pairs used for amplification and sequencing were ACT-512F/ACT-783R and CHS-354R/CHS-79F for *act* and *chs-1*, respectively (Carbone and Kohn 1999); GDF1/GDR1 for *gapdh* (Guerber et al. 2003); CYLH3F/CYLH3R for *his3* (Crous et al. 2004); ITS1F/ITS4 for ITS (Gardes and Bruns 1993; White et al. 1990); and Btub2Fd/Btub4Rd for *tub2* (Woudenberg et al. 2009). Each PCR reaction mixture was made to a total volume of 25 μL comprising 1 x Hot Start Taq Master Mix (New England Biolabs), 0.5 mM of MgCl₂, 0.4 μM of forward and reverse primer, and 6ng of DNA template. PCR amplification was performed in a BioRad iCycler Thermal Cycler. The PCR conditions were 30 s at 95 °C, then 30 cycles of 30 s at 95 °C, 30 s at the annealing temperature (55 °C for ITS and *gapdh* and 58 °C for *act*, *tub2*, and *chs-1*; Weir et al. 2012), 60 s at 68 °C, and then 5 min at 68 °C. The purification of PCR products was performed using the QIAquick PCR Purification Kit (QIAGEN), following the manufacturer's instructions. Purified PCR products were submitted to the Australian Genome Research Facility (AGRF, Melbourne) or Macrogen (Seoul, South Korea) for forward and reverse Sanger sequencing.

Phylogenetic analyses

Forward and reverse DNA sequences were quality-trimmed and aligned to produce consensus sequences using the *de novo* assembly and manually edited in Geneious Prime

2024.0.3 (Biomatters Inc.). Five isolates of *Co. pyricola* (Table 1) were putatively identified by the Basic Local Alignment Search Tool (BLAST) search of *gapdh* gene sequences. All sequences, including the additional isolate already identified as *Co. pyricola*, were deposited in GenBank (Supplementary Table 1). Reference sequences from the *Colletotrichum acutatum* species complex were obtained from GenBank (Supplementary Table 1). *Colletotrichum orchidophilum* (ex-type strain CBS 632.80) was used as the outgroup. Reference sequences were aligned with the consensus sequences for each locus with the MAFFT plugin version 7.490 (Katoh and Standley 2013). Maximum likelihood (ML) trees were generated with the RAxML v. 4.0 (Stamatakis 2014) plugin. For the ML analysis, the best-fit nucleotide substitution model was determined using MrModelTest 2.4 (Nylander 2004). Single-locus ML trees were built to visually assess congruency across the six loci. Given the congruency, all loci were concatenated into a single alignment, which was used to build a multi-locus ML tree based on 1000 bootstrap replicates. Multi-loci Bayesian inference (BI) was performed with the MrBayes 3.2.6 (Ronquist et al. 2012) plugin in Geneious. MrModelTest 2.4 was used to determine the best-fit nucleotide substitution model for each locus. Posterior probabilities were determined with Markov Chain Monte Carlo (MCMC) sampling. Eight independent MCMC chains were run for 10⁶ generations, with tree sampling every 100 generations. The analysis was terminated upon reaching a standard deviation of split frequencies of less than 0.01, with the heating parameter set to 0.2. The burn-in phase was set to discard the first 25% of trees, with the remaining trees used to determine the posterior probabilities (de Silva et al. 2019).

Fungal inoculum preparation and inoculations

Conidial suspensions were made by pouring 10 ml of sterilised water onto 7 to 10-day-old PDA (full or ½ strength) or CMA cultures, and the mycelium was scraped with a sterile blade. The liquid was then filtered through two layers of sterile paper towels. Inocula concentration was measured with a haemocytometer and adjusted to 1 × 10⁶ conidia/mL.

Due to the lack of sporulation of BRIP 54037 and the waxy cuticle of all eucalypt species tested, which made adhesion of the spore suspension difficult, mycelial agar plugs (5 mm diameter) from 7- to 10-day-old cultures were used for isolates BRIP 54037 and VPRI 10714. Mycelial plugs were also used for stem inoculations.

Wound and non-wound treatments were used in all experiments. For the non-wound treatment, inoculum was applied as a 6 µL droplet of spore suspension or mycelial plug to the leaf, stem or fruit. For the wound treatment, a sterile needle was used to penetrate the cuticle and epidermis of the leaf, stem, or fruit skin before applying the spore suspension or mycelial plug. Stem bioassays were inoculated with a mycelial plug and then wrapped in parafilm. Control fruits, leaves, and stems were inoculated with 6 µL of sterile water or a sterile agar plug (CMA or ½ strength PDA). This inoculation method was used in all experiments.

Pathogenicity bioassays

Bioassays were conducted on detached leaves, stems, and *in planta* to establish the pathogenicity of each isolate on its original host (Table 1). Although *Co. pyricola* was first isolated from the fruit rot of *Pyrus communis*, its pathogenicity on pear has yet to be confirmed. Therefore, bioassays were conducted with VPRI 43730 on *Py. communis* fruit. Each experiment included four replicates and one control per treatment and was repeated three times.

Plant and fruit material

Detached leaves of *Malus domestica* ('Jonathan' apple) and *Persea americana* ('Hass' avocado) fruit trees were obtained from plants growing in the glasshouse. Plant bioassays were conducted using potted plants of *Banksia burdettii*, *Clematis aristata*, *Eucalyptus caesia*, and *Leptospermum grandifolium* purchased from local nurseries in Melbourne, Australia. Detached stems and leaves of *E. caesia* were also collected from established plants on the University of Melbourne, Parkville campus. For the fruit bioassays, *Malus domestica* ('Jonathan' and 'Granny Smith' apple) and *Py. communis* ('Packham's Triumph' pear) were purchased from local supermarkets.

Detached leaf bioassays

Stems with approximately 10 to 20 cm of new growth were detached from plants, and surface sterilised with 1% sodium hypochlorite for 30–60 s, depending on the fragility of the leaves, and then washed three times for 1 min in sterile water. The base of the stems were trimmed to remove dried

and damaged tissue and then placed in Falcon tubes filled with tap water. Leaves were alternately assigned to either the wound or non-wound treatment, with one to four inoculations per leaf depending on leaf size. Falcon tubes containing the stems were then placed in clear plastic containers lined with moist paper towels. The experiment was conducted in a growth cabinet (26 °C, 12 h light/dark cycle) for 10 days. To maintain leaf turgor, stems were trimmed to remove dead tissue, and the water was replaced every 1–2 days.

Fruit bioassay

Fruit was surface sterilised with ethanol (80% v/v) for 1 min and sodium hypochlorite (~1% v/v) for 5 min and then washed three times for 1 min in sterile water. Fruits were placed atop sterile Petri dishes inside a clear plastic container lined with moist paper towels. Each fruit had one wound and non-wound treatment, on opposite sides of the fruit. Inoculated fruits were placed in a growth cabinet (26 °C, 12 h light/dark cycle) for 10 days.

In planta bioassays

For *in planta* bioassays, inoculated potted plants were maintained in a growth cabinet or glasshouse for up to 30 days or until lesion development at the sites of inoculation.

Re-isolation

After the duration of each experiment, margins of lesions or inoculation sites of asymptomatic tissue were surface sterilised with ethanol (80% v/v) for 30 s and 1% sodium hypochlorite for 2 min, washed three times with sterile water and then cultured on PDA. Cultures were examined after 7 days for the growth of *Co. pyricola* based on culture morphology.

Cross-host pathogenicity bioassay

Cross-host bioassays were conducted on detached leaves, as previously described, to assess the pathogenicity of VPRI 15937 on *Banksia* spp. and VPRI 10714 on eucalypt species. Each experiment included four replicates and one control per treatment for each host species and was repeated three times. Inoculated leaves were placed in a growth cabinet (26 °C, 12 h light/dark cycle) for 10 days. After each experiment, re-isolation was performed as previously described.

Plant material

Samples of twelve *Eucalyptus* spp. (*E. cinerea*, *E. dives*, *E. erythrocorys*, *E. globulus* subsp. *bicostata*, *E. lacrimans*, *E.*

leucoxydon, *E. macrocarpa*, *E. macrorhyncha*, *E. pauciflora* subsp. *niphophila*, *E. rubida*, *E. viminalis*, and *E. websteriana*), one species each of *Angophora* (*A. costata*) and *Corymbia* (*Corymbia citriodora*), and five *Banksia* spp. (*B. epica*, *B. integrifolia*, *B. marginata*, *B. spinulosa*, and *B. victoriae*) were acquired from established trees at the University of Melbourne, Parkville campus, or seedlings and potted plants purchased from local nurseries in Melbourne, Australia.

Disease assessment and statistical analyses

For eucalypt species, the leaves were imaged against a known-distance reference. ImageJ software (Schneider et al. 2012) was used to measure lesion area. The variance in data was heterogeneous; therefore, Welch's two-sample t-test was performed using the `t.test()` function. P values of <0.05 were considered significant. Data was plotted in R v. 4.3.2 (R Core Team 2023) using bar (`geom_bar`) plots with the `ggplot2` package (Wickham 2016).

Appressoria microscopy

Light microscopy of appressoria, germ tubes, and internal light spots was used to confirm infection at 5 days post-inoculation (dpi). Asymptomatic tissue was cut into 1 cm² pieces and transferred to a clearing solution of 100% ethanol and acetic acid (1:2, v/v) for 8–24 h, depending on leaf thickness. Once cleared, the tissue was transferred to clean water for at least 1 min before staining with Trypan Blue for 1–2 h. The tissue was de-stained in ethanol (80% v/v) for 5 s before mounting on a slide with lactic acid. Appressoria and other infection structures were imaged with a Leica DM6000 LED compound microscope, with bright field optics and photographed with a Leica DMC2900 camera and Leica Application Suite X (LASX) software.

Results

Phylogenetic analyses

Six isolates were tentatively identified as *Colletotrichum pyricola* from the *Colletotrichum acutatum* species complex, based on the closest matches obtained from a BLAST search of *gapdh* gene sequences. The concatenated alignment used for phylogenetic analyses was a total of 2,170 characters (*act*: 1–219; *chs-1*: 220–488; *gapdh*: 489–749; *his3*: 750–1,131; ITS: 1,132–1,673; *tub2*: 1,674–2,170), comprising 495 variable characters (*act*: 75; *chs-1*: 36; *gapdh*: 129; *his3*: 90; ITS: 49; *tub2*: 116) and 1675 invariable

characters (*act*: 144; *chs-1*: 233; *gapdh*: 132; *his3*: 292; ITS: 493; *tub2*: 381). For ML analysis, MrModelTest determined the best-fit model for the concatenated alignment to be the general time reversible with gamma distribution and a proportion of invariable sites (GTR+G+I). For BI, the best-fit nucleotide substitution model for each locus based on MrModelTest was GTR+I for *act* and ITS, HKY+I for *chs-1*, and HKY+G for *gapdh*, *his3*, and *tub2*. The tree topology of RAxML analysis of individual loci was congruent with the multi-locus RAxML and BI analyses. Therefore, only the ML tree using RAxML analysis is shown, with bootstrap support values from ML analysis and posterior probabilities from BI, shown at the nodes (Fig. 2). All isolates tentatively identified as *Co. pyricola* formed a monophyletic clade with maximum bootstrap and posterior probability values with the type isolate of *Co. pyricola* (CBS 128531).

Pathogenicity bioassays

Koch's postulates were fulfilled for all isolates on the original host, except VPRI 43730 on *Pe. americana*. All isolates were able to cause a lesion on their original host for the wound inoculation treatment (Table 2; Fig. 3), except for VPRI 43730 on *Pe. americana* leaves (Fig. 3f). For the non-wound inoculation, only *E. caesia* (Fig. 3a) and *L. grandifolium* (Fig. 3e.) developed disease symptoms by 10 dpi. However, despite no lesion formation for the non-wound treatment, *Co. pyricola* was re-isolated from the asymptomatic tissue of *B. burdettii*, *Cl. aristata*, *Pe. americana*, and *M. domestica* (Fig. 3.). Light microscopy of chlorophyll-cleared asymptomatic leaf tissue from the non-wound inoculation treatments revealed appressoria formation, as well as internal light spots (ILS) within the appressoria for *Pe. americana* and *Cl. aristata* infected leaves (Fig. 4c; Table 2). Fruit bioassays of *Pe. americana* inoculated with VPRI 43730 were unsuccessful due to the abundance of other *Colletotrichum* spp. on the fruit, irrespective of the inoculation site across three replicates. *Pyrus communis* fruit inoculated with VPRI 43730 produced severe symptoms at the wound site (Fig. 3h). At 10 dpi, *Py. communis* fruit exhibited large brown, sunken lesions with concentric rings of acervuli producing masses of orange conidiospores towards the centre of the lesion. For the non-wound treatment, small lesions began to develop around 16 dpi as the fruit ripened.

Host range on eucalypt species

Colletotrichum pyricola was able to cause infection for both the wound and non-wound inoculation treatments of most of the species tested, as shown in Table 3; Fig. 5. However, there was substantial variability in disease response and symptoms

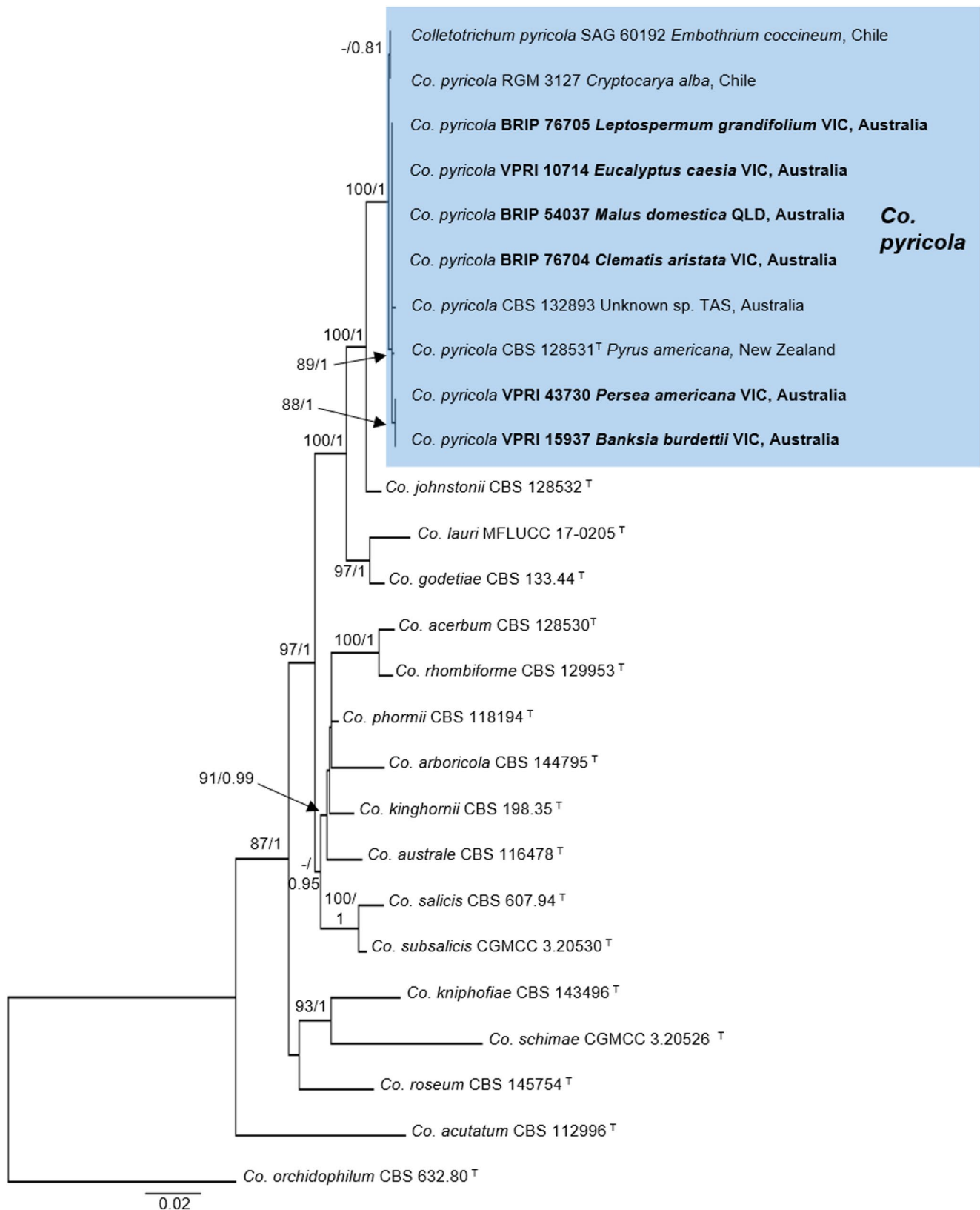


Fig. 2 Maximum likelihood phylogeny based on a concatenated alignment of the *act*, *chs-1*, *gapdh*, *his3*, ITS and *tub2* sequences. Maximum likelihood (ML) bootstrap support values (ML>75%) and Bayesian posterior probabilities (PP; PP>90%) are shown at the nodes (ML/PP). Tree is rooted to *Colletotrichum orchidophilum* (CBS 632.80). Scale

bar indicates 0.02 substitutions per site. Species name is followed by isolate number, host, state, and country for *Co. pyricola* isolates. Type specimens are indicated by ^T. Blue blocks highlight the monophyletic *Co. pyricola* clade, including the type specimen (CBS 128531)

Table 2 Qualitative assessment of lesion development following inoculation with *Colletotrichum pyricola* isolates on the original host

Isolate	Host plant	Plant organ	Lesion development ^a		Non-wound	
			Wound	Non-wound	Re-isolated ^b	Appressoria ^c
VPRI 43730	<i>Persea americana</i> ‘Hass’	Leaves*	+	-	+	AP+ILS
	<i>Pyrus communis</i> ‘Packham’	Fruit	+	+	+	/
BRIP 54037	<i>Malus domestica</i> ‘Jonathan’	Leaves*	+	-	+	AP
	<i>Malus domestica</i> ‘Granny Smith’	Fruit	+	-	+	/
BRIP 76704	<i>Clematis aristata</i>	Leaves*	+	-	+	AP+ILS
		Stem*	+	-	+	/
BRIP 76705	<i>Leptospermum grandifolium</i>	Leaves*	+	+	+	/
VPRI 15937	<i>Banksia burdettii</i>	Leaves*	+	-	+	AP
VPRI 10714	<i>Eucalyptus caesia</i> subsp. <i>magna</i>	Leaves*	+	+	+	/

*Indicates experiments conducted on both detached and *in planta* tissue

^a Presence (+) or absence (-) of lesion development

^b Presence (+) or absence (-) of *Co. pyricola* re-isolated from non-wound tissue

^c Presence of appressoria (AP) and internal light spot (ILS) in asymptomatic non-wounded tissue. Symptomatic species not tested are indicated by (/)

exhibited by the different eucalypt species (Fig. 5). All eucalypt species tested developed lesions for the wound treatment, except *E. erythrocorys*. For the non-wound treatment, *E. cinerea*, *E. erythrocorys*, *E. globulus* subsp. *bicostata*, and *E. viminalis* did not produce any lesions. However, *Co. pyricola* was re-isolated from all eucalypt species, including from asymptomatic tissue, for both wound and non-wound treatments. Light microscopy of chlorophyll-cleared asymptomatic tissue from the non-wound treatment revealed appressoria formation for all asymptomatic eucalypt species (Table 3; Fig. 4). Furthermore, appressoria of *E. erythrocorys* (Fig. 4a), *E. globulus* subsp. *bicostata*, and *E. viminalis* presented ILS (Table 3).

To determine the severity of *Co. pyricola* on different eucalypt species, the lesion area was measured for seven *Eucalyptus* species that produced measurable lesions. *Eucalyptus lacrimans* produced the largest mean lesion area for the wound treatment, however, there was no significant difference compared with *E. caesia* ($P > 0.05$; Fig. 6). This can be attributed to the considerable variability in lesion area of *E. lacrimans*, as shown by the large standard error of $\pm 0.39 \text{ cm}^2$. For the non-wound treatment, *E. caesia* produced the largest mean lesion area, which was significantly different from all other hosts ($P < 0.01$). Wound inoculation treatment resulted in significantly ($P < 0.05$) greater disease response when compared with the non-wound inoculation treatments across all hosts. There was a significant difference in lesion size for all hosts of the wound treatment ($P < 0.05$), except *E. caesia* – *E. lacrimans*, *E. pauciflora* – *E. viminalis*, *E. leucoxylon* – *E. pauciflora*, and *E. leucoxylon* – *E. viminalis* (Fig. 6) (Table 3).

Host range on *Banksia* species

Disease symptoms did not develop on any *Banksia* spp. for both the wound and non-wound treatments, except for *B.*

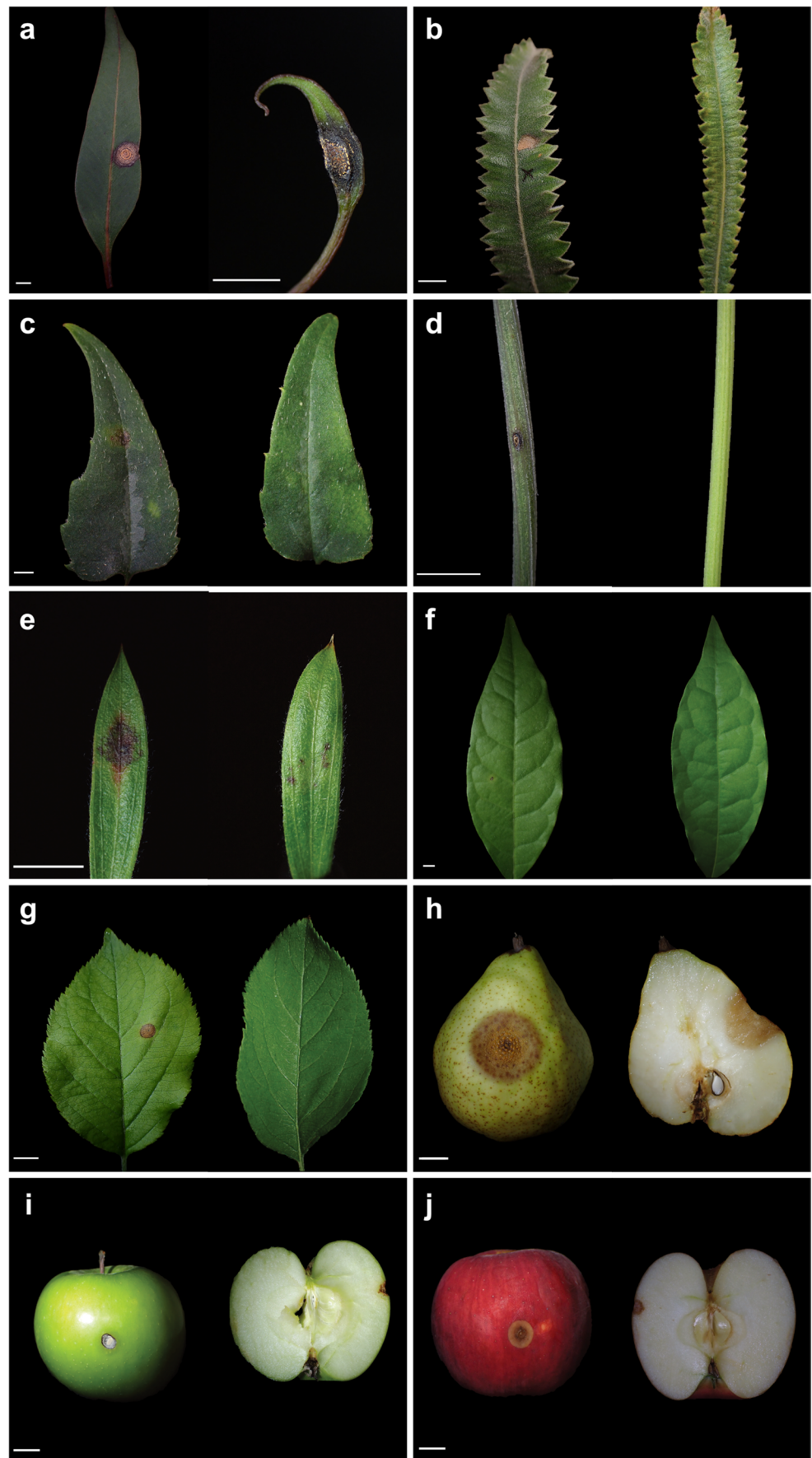
epica when wounded (Fig. 7). *Colletotrichum pyricola* was re-isolated from asymptomatic, non-wounded leaves of *B. marginata*, *B. integrifolia*, and *B. victoriae* but not *B. spinulosa*. Microscopy of the chlorophyll-cleared asymptomatic tissue revealed appressoria development; however, further infection structures were absent (Table 4).

Discussion

Colletotrichum species are well-known threats to agriculture, yet their impact on Australian native flora remains largely unknown. *Colletotrichum pyricola* was originally reported from fruit rot of *Py. communis* (common pear) in New Zealand and has since been identified in Australia on *D. odora* (Shivas et al. 2016) and an unidentified host (Zapata et al. 2024). In this study, pathogenicity bioassays reveal that *Co. pyricola* infects a range of Australian native plants, including *B. burdettii* and several eucalypt species, as well as fruit tree hosts *M. domestica* and *Pe. americana*. These findings highlight its broad host range and potential threat to both native and agricultural systems.

Colletotrichum pyricola was re-isolated from both wounded and non-wounded, asymptomatic tissue in the original host and cross-host pathogenicity bioassays, in all experiments except for *B. spinulosa*. This suggests the fungus established infection within the plant tissue, which was confirmed by visualisation of appressoria on the leaf surface at the inoculation site. These findings may be explained by the potential hemibiotrophic lifestyle of *Co. pyricola*, where biotrophic infection occurs without triggering symptoms (Prusky et al. 2013). This is supported by the ability to cause symptoms in wound-inoculated tissue, ruling out an exclusively endophytic lifestyle. Alternatively, the fungus may enter a latency

Fig. 3 Disease symptoms caused by *Colletotrichum pyricola* at wound (left) and non-wound (right) inoculation sites, 10 days post-inoculation: **(a)** VPRI 10714 on *Eucalyptus caesia* leaves; **(b)** VPRI 15937 on *Banksia burdettii* leaves; **(c-d)** BRIP 76704 on *Clematis aristata* leaves and stems; **(e)** BRIP 76705 on *Leptospermum grandifolium* leaves; **(f)** VPRI 43730 on *Persea americana* leaves; **(g)** BRIP 54037 on *Malus domestica* leaves; **(h)** VPRI 43730 on *Pyrus communis* fruit (left) and cross-section (right); and **(i-j)** BRIP 54037 on *M. domestica* ('Granny smith' and 'Jonathan' apple) fruit (left = surface, right = cross-section). Scale bars (a - g) = 5 mm and (h - j) = 10 mm



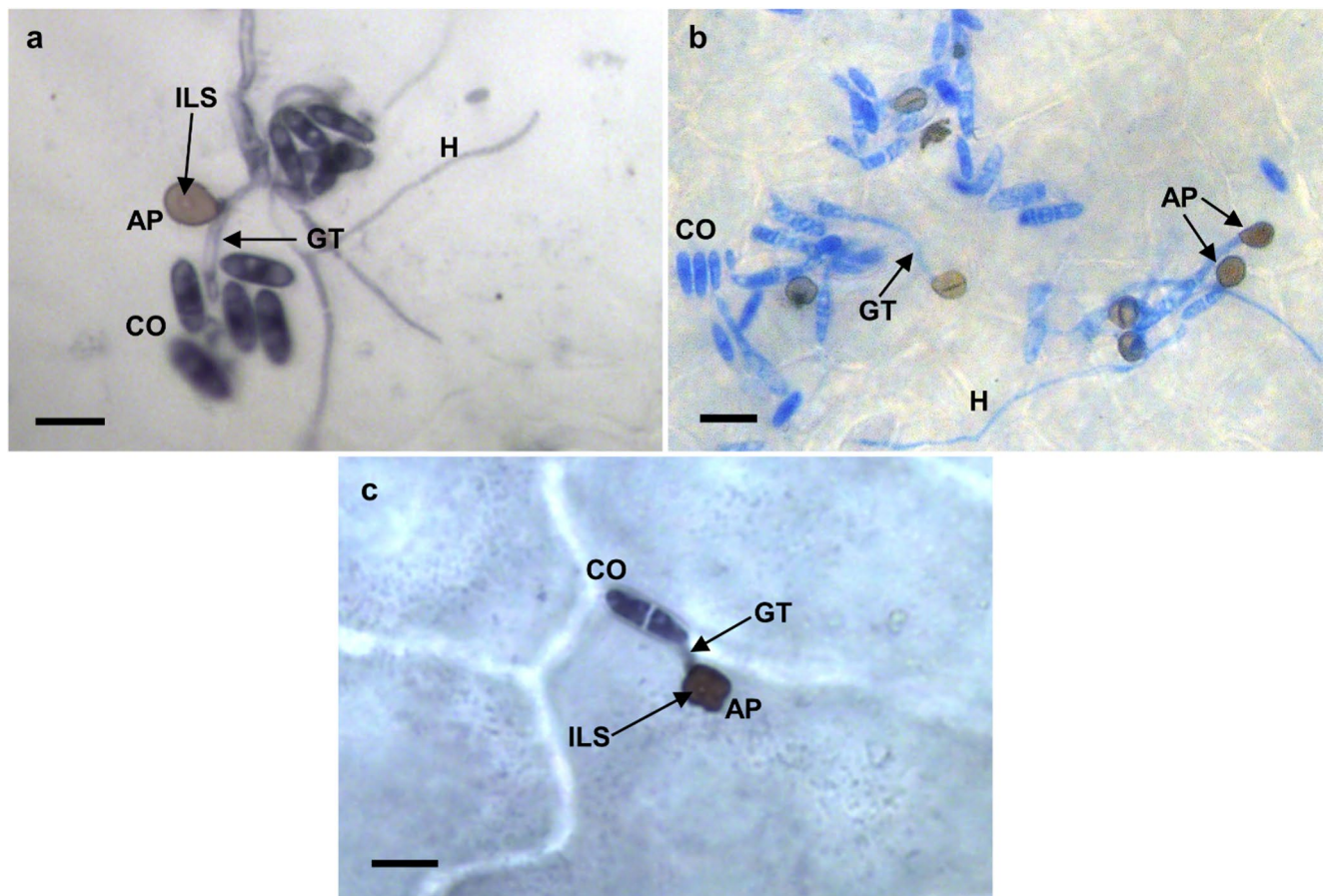


Fig. 4 Light microscopy of *Colletotrichum pyricola* spore germination and appressorium development 5 days post-inoculation: **(a)** *Eucalyptus erythrocorys*; **(b)** *E. leucoxydon*; and **(c)** *Clematis aristata*. Appres-

soria (AP); Internal light spot (ILS); conidia (CO); germ tube (GT); hypha (H). Scale bars = 10 μ m

Table 3 Qualitative assessment of lesion development following cross-host inoculation of *Colletotrichum pyricola* (VPRI 15937) on eucalypt species

Host plant	Presence/ absence of infection ^a		Non-wound site	
	Wound	Non-wound	Re-isolated ^b	Appressoria ^c
<i>Angophora costata</i>	+	+	+	/
<i>Corymbia citriodora</i>	+	+	+	/
<i>Eucalyptus cinerea</i>	+	-	+	AP
<i>E. dives</i>	+	+	+	/
<i>E. erythrocorys</i>	-	-	+	AP+ILS
<i>E. globulus</i> subsp. <i>bicostata</i>	+	-	+	AP+ILS
<i>E. lacrimans</i>	+	+	+	/
<i>E. leucoxydon</i>	+	+	+	/
<i>E. macrocarpa</i>	+	+	+	/
<i>E. macrorhyncha</i>	+	+	+	/
<i>E. pauciflora</i>	+	+	+	/
<i>E. rubida</i>	+	+	+	/
<i>E. viminalis</i>	+	-	+	AP+ILS
<i>E. websteriana</i>	+	+	+	/

^a Presence (+) or absence (-) of lesion development

^b Presence (+) or absence (-) of *Co. pyricola* re-isolated from non-wound tissue

^c Presence of appressoria (AP) and internal light spot (ILS) in asymptomatic non-wounded tissue. Symptomatic species not tested are indicated by (/)

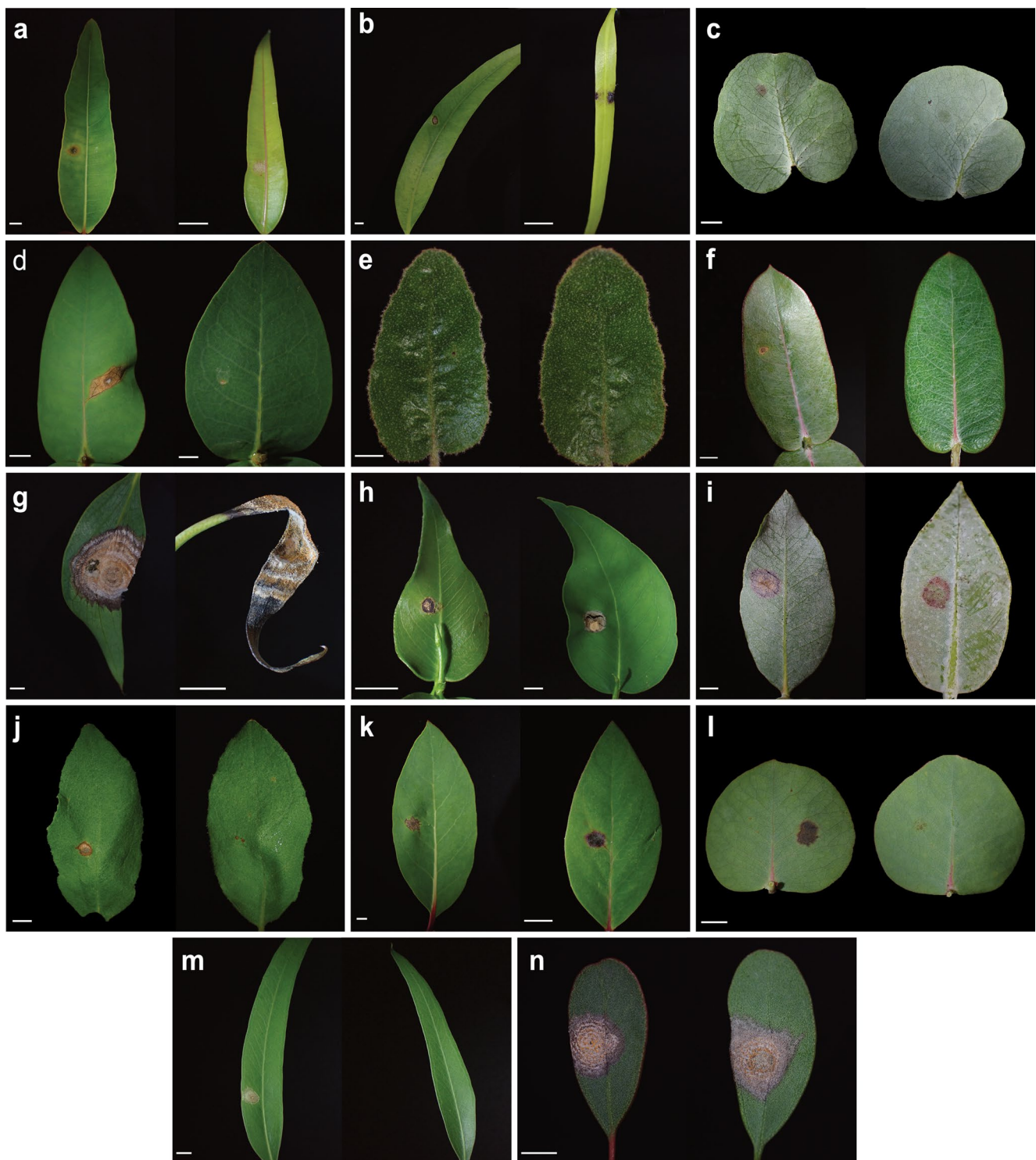


Fig. 5 Disease symptoms caused by *Colletotrichum pyricola* (VPRI 10714) on detached leaves at wound (left) and non-wound (right) inoculation sites, 10 days post-inoculation: **(a)** *Angophora costata*; **(b)** *Corymbia citriodora*; **(c)** *Eucalyptus cinerea*; **(d)** *E. dives*; **(e)** *E. eryth-*

rocorys; **(f)** *E. globulus* subsp. *bicostata*; **(g)** *E. lacrimans*; **(h)** *E. leucoxylon*; **(i)** *E. macrocarpa*; **(j)** *E. macrorhyncha*; **(k)** *E. pauciflora*; **(l)** *E. rubida*; **(m)** *E. viminalis*; and **(n)** *E. websteriana*. Scale bars = 5 mm

phase, remaining asymptomatic until favourable host or environmental conditions trigger a shift to necrotrophy and symptom development (Talhinhas et al.

2011). Asymptomatic, or cryptic, infections may serve as inoculum reservoirs, enabling pathogen persistence and increasing the potential for new host infections.

Table 4 Qualitative assessment of lesion development following cross-host inoculation of *Colletotrichum pyricola* (VPRI 15937) on *Banksia* species

Host plant	Presence/ absence of infection ^a		Non-wound	
	Wound	Non-wound	Re-isolated ^b	Appressoria ^c
<i>Banksia marginata</i>	-	-	+	AP
<i>B. integrifolia</i>	-	-	+	AP
<i>B. epica</i>	+	-	+	AP
<i>B. victoriae</i>	-	-	+	AP
<i>B. spinulosa</i>	-	-	-	AP

^a Presence (+) or absence (-) of lesion development^b Presence (+) or absence (-) of *Co. pyricola* re-isolated from non-wound tissue^c Presence of appressoria (AP) and internal light spot (ILS) in asymptomatic non-wounded tissue. Symptomatic species not tested are indicated by (/)

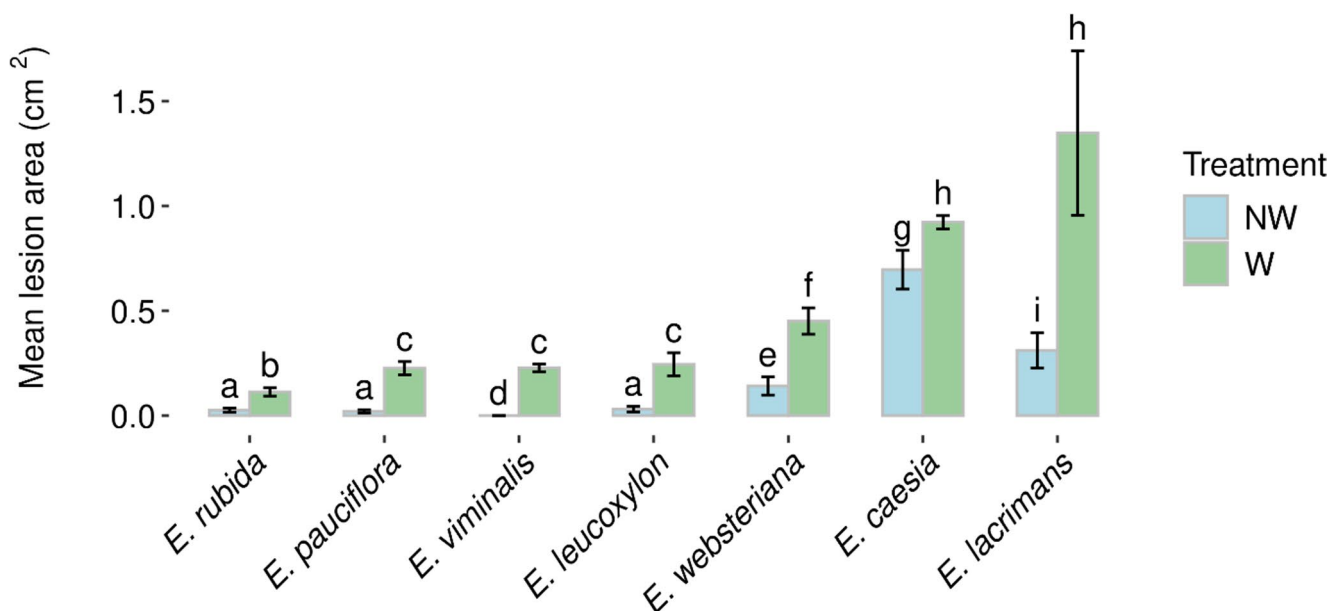
By persisting undetected across host species and within both natural and cultivated systems, these infections can facilitate long-term survival and dispersal (Freeman et al. 2001; Stukenbrock and McDonald 2008; Stergiopoulos and Gordon 2014). As a result, extended asymptomatic infection in *Colletotrichum* species may pose significant biosecurity risks, particularly at the interface of native and commercial systems.

To confirm *Co. pyricola* as a pathogen of *Py. communis*, fruit bioassays were conducted. Wounded fruit developed large lesions, while non-wounded fruit developed lesions only after ripening. Infection likely

occurred in unripe fruit, remaining latent until ripening, when the pathogen transitioned to a necrotrophic lifestyle, causing postharvest disease. Wounding can disrupt this latent phase, triggering necrotrophic infection and necrosis regardless of ripeness (Jiang et al. 2014). Therefore, *Co. pyricola* may present a biosecurity risk to Australia's pear and apple industries.

In cross-host bioassays, the eucalypt species tested showed considerable morphological variation, which may influence host susceptibility. Many eucalypts develop a waxy or glaucous layer that reduces water adhesion to the leaf surface and has been shown to reduce spore germination of *Phaeoseptoria eucalypti* (Heather 1967). However, infection without wounding suggests that *Co. pyricola* can penetrate this protective barrier and infect leaves directly. Since foliar characteristics, such as leaf morphology and surface waxes, change across seedling, juvenile and adult stages (Wirthensohn and Sedgley 1996; England and Attwill 2006; Vlasveld et al. 2018), further pathogenicity bioassays on eucalypts at different life stages would provide insight into how susceptibility varies over time.

The wide host range of *Co. pyricola* also increases its potential geographic range. The limited number of eucalypt species tested are collectively distributed across much of mainland Australia, excluding the Northern Territory. Moreover, many of these species, such as *B. burdettii* and *E. caesia*, are commonly grown as ornamentals (Brooker et al. 2015), which may further facilitate the dispersal and host range expansion of *Co. pyricola*. Notably, isolates

**Fig. 6** Bar graph showing mean lesion area (cm²) measured 10 days post-inoculation on detached leaves of *Eucalyptus* spp. (*E.* = *Eucalyptus*) inoculated with *Colletotrichum pyricola* under wound (W) and non-wound (NW) treatments. Error bars represent the standard error of

the mean. Significant differences among treatments were determined using Welch's two-sample t-test ($P < 0.05$) and are indicated by different letters above bars



Fig. 7 Disease symptoms caused by *Colletotrichum pyricola* (VPRI 15937) on a detached *Banksia epica* leaf after wound inoculation, 10 days post-inoculation. Scale bar = 5 mm

from *B. burdettii* and *E. caesia* were collected from ornamental plants growing outside their native range in Western Australia (Western Australian Herbarium 1998).

Despite its apparent wide host range and geographic distribution, *Co. pyricola* has not been reported on any eucalypt species since its initial isolation from *E. caesia* in 1979. Over 50 *Colletotrichum* isolates were collected during field collections of national and state parks in Queensland and Victoria (*unpublished data*). Only two *Co. pyricola* isolates were identified from *Clematis* (BRIP 76704) and *Leptospermum* (BRIP 76705) in Cathedral Ranges State Park in Victoria. Interestingly, both isolates were isolated from plants within two meters of each other. This finding raises questions about the distribution and host range of *Co. pyricola* in Australia's natural ecosystems. However, the frequency of *Co. pyricola* from field collections likely reflects the limitations of the collecting method, not actual occurrence. Collections were restricted to plants easily accessible along hiking tracks, meaning eucalypts were often not accessible. Extensive field surveys are needed to better understand the host range and

distribution of *Co. pyricola*, particularly in eucalypts. Given the susceptibility of eucalypts from three genera to *Co. pyricola*, many other eucalypt species may also be susceptible. As *Co. pyricola* is pathogenic to species from the *Angophora*, *Corymbia*, *Eucalyptus* and *Leptospermum* genera in the Myrtaceae family, it may also be a serious pathogen of this family. However, further investigation is necessary to define its host range.

Since the report of *Co. pyricola* from four native plant species in Australia, the question of *Co. pyricola* endemism remains unclear. Zapata et al. (2024) identified eight isolates of *Co. pyricola* from native woody plants in south-central Chile, including *Cryptocarya alba*, *Nothofagus obliqua*, *Peumus boldus* and one isolate from *Embothrium coccineum* (Zapata and Opazo 2017). Zapata et al. (2024) hypothesised that *Co. pyricola* was endemic to Chile; however, this is not supported by the findings in the present study. Additionally, one isolate of *Co. pyricola* was identified from an unknown plant host in Tasmania, Australia (Zapata et al. 2024). The known distribution of *Co. pyricola* in Australia, New Zealand and South America supports the notion that its distribution may be of Gondwanan origin (McCarthy et al. 2007; Zapata et al. 2024). However, further work is necessary to understand the biogeographic distribution and origin of this species.

These results expand the known host range of *Co. pyricola* to include temperate and sub-tropical fruit trees, as well as Australian native plants, suggesting a potential overlap in pathogen presence across native, agricultural, and horticultural systems. Native plants may act as a source of inoculum capable of infecting cultivated crops, and vice versa (Crouch et al. 2009; Dentika et al. 2023). For example, MacKenzie et al. (2007) reported that *Co. gloeosporioides*, isolated from native oak (*Quercus* spp.) and grape (*Vitis* spp.) in Florida, was more pathogenic to cultivated strawberry (*Fragaria × ananassa*) when native plant populations were located closer to strawberry fields. This suggests that selection pressures imposed by the proximity to cultivated strawberries allowed populations of *Co. gloeosporioides* to infect the cultivated hosts. Many eucalypt species persist as remnant vegetation adjacent to agricultural or forestry systems, increasing the likelihood of pathogen transmission due to geographical proximity (Roy 2001).

Colletotrichum pyricola is pathogenic to a wide host range of subtropical and temperate fruit trees and Australian native plants, including a range of eucalypts. However, the biological niche *Co. pyricola* occupies within the native ecosystems of these eucalypts is unknown. Further sampling of native eucalypts or native plants broadly is necessary to understand the distribution and host range of *Co. pyricola*, highlighting the importance of surveying surrounding native and remnant vegetation for plant pathogens of agricultural,

horticultural and forestry systems. Furthermore, disease outbreaks in commercial systems should account for potential impacts on surrounding native flora. Understanding the diversity and overlap of fungal pathogens in native and agricultural systems is critical for effective disease management and conservation of Australia's unique diversity of flora.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13313-026-01109-x>.

Acknowledgements Field collections for this study were completed under permits granted through the Queensland Department of Environment and Science (P-PTUKI-100174002), the Victorian Department of Environment, Land, Water and Planning (10010198), and Parks Victoria (AA-0001700). Costs associated with field collections in Victoria were supported by the Bill Borthwick Student Scholarships from the Victorian Environmental Assessment Council (VEAC).

Author contributions A.A. conducted experiments and wrote the manuscript. A.A., P.W.J.T. and N.V. designed and optimised experiments. P.K.A. assisted with data analysis. P.W.J.T. provided principal supervision, and N.V. and J.E. assisted in supervision. A.A., P.W.J.T., N.V., P.K.A., J.E., and Y.P.T. edited the manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. The first author was funded by a Melbourne Research Scholarship awarded by the University of Melbourne.

Data availability All data generated during this study are included in this article and as supplementary data. Sequences of isolates used in the phylogenetic analysis are available in GenBank. Further data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Not Applicable.

Competing Interests No competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol Evol* 19:535–544. <https://doi.org/10.1016/j.tree.2004.07.021>

- Brockerhoff EG, Jactel H, Parrotta JA, Ferraz SFB (2013) Role of Eucalypt and other planted forests in biodiversity conservation and the provision of biodiversity-related ecosystem services. *Ecol Manage* 301:43–50. <https://doi.org/10.1016/j.foreco.2012.09.018>
- Brooker MIH, Slee AV, Connors JR, Duffy S, West J (2015) EUCLID: eucalypts of Australia, 4th edn. CSIRO, Canberra, Australia
- Bufford JL, Hulme PE, Sikes BA, Cooper JA, Johnston PR, Duncan RP (2016) Taxonomic similarity, more than contact opportunity, explains novel plant-pathogen associations between native and alien taxa. *New Phytol* 212:657–667. <https://doi.org/10.1111/np.h.14077>
- Burgess TI, Oliva J, Sapsford SJ, Sakalidis ML, Balocchi F, Paap T (2022) Anthropogenic disturbances and the emergence of native diseases. a threat to forest health. *Curr. For Rep* 8: 111–123. <https://doi.org/10.1007/s40725-022-00163-0>
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91:553–556. <https://doi.org/10.1080/00275514.1999.12061051>
- Choi YW, Hyde KD, Host WWH (1999) Single spore isolation of fungi. *Fungal Divers* 3:29–38
- Crouch JA, Tredway LP, Clarke BB, Hillman BI (2009) Phylogenetic and population genetic divergence correspond with habitat for the pathogen *Colletotrichum cereale* and allied taxa across diverse grass communities. *Mol Ecol* 18:123–135. <https://doi.org/10.1111/j.1365-294X.2008.04008.x>
- Crous PW, Groenewald JZ, Risède JM, Simoneau P, Hywel-Jones NL (2004) *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Stud Mycol* 50:415–430.
- Crous PW, Wingfield MJ, Cheewangkoon R, Carnegie AJ, Burgess TI, Summerell BA, Edwards J, Taylor PWJ, Groenewald JZ (2019) Foliar pathogens of eucalypts. *Stud Mycol* 94:125–298. <https://doi.org/10.1016/j.simyco.2019.08.001>
- Damm U, Cannon PF, Woudenberg JHC, Crous PW (2012) The *Colletotrichum acutatum* species complex. *Stud Mycol* 73:37–113. <https://doi.org/10.3114/sim0010>
- de Silva DD, Groenewald JZ, Crous PW, Ades PK, Nasruddin A, Mongkolporn O, Taylor PWJ (2019) Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. *IMA Fungus* 10:8. <https://doi.org/10.1186/s43008-019-0001-y>
- Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol* 13:414–430. <https://doi.org/10.1111/j.1364-3703.2011.00783.x>
- Dentika P, Gumbau M, Ozier-Lafontaine H, Penet L (2023) Natural flora is indiscriminately hosting high loads of generalist fungal pathogen *Colletotrichum gloeosporioides* complex over forest Niche, vegetation strata and elevation gradient. *J Fungi* 9(3):296. <https://doi.org/10.3390/jof9030296>
- England JR, Attiwill PM (2006) Changes in leaf morphology and anatomy with tree age and height in the broadleaved evergreen species, *Eucalyptus regnans* F. Muell. *Trees* 20:79–90. <https://doi.org/10.1007/s00468-005-0015-5>
- Fensham RJ, Laffineur B, Collingwood TD, Beech E, Bell S, Hopper SD, Phillips G, Rivers MC, Walsh N, White M (2020) Rarity or decline: key concepts for the red list of Australian eucalypts. *Biol Conserv* 243:108455. <https://doi.org/10.1016/j.biocon.2020.108455>
- Freeman S, Horowitz S, Sharon A (2001) Pathogenic and nonpathogenic lifestyles in *Colletotrichum acutatum* from strawberry and other plants. *Phytopathology* 91:986–992. <https://doi.org/10.1094/PHYTO.2001.91.10.986>
- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol Ecol* 2:113–118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>

- Golzar H (2009) First report of anthracnose of *Acacia* in Australia. Australas. Plant Dis Notes 4:70–71. <https://doi.org/10.1071/DN09030>
- Guerber JC, Liu B, Correll JC, Johnston PR (2003) Characterization of diversity in *Colletotrichum acutatum* sensu Lato by sequence analysis of two gene introns, MtDNA and intron RFLPs, and mating compatibility. Mycologia 95:872–895. <https://doi.org/10.1080/15572536.2004.11833047>
- Heather WA (1967) Leaf characteristics of *Eucalyptus bicostata* Maiden seedlings affecting the deposition and germination of spores of *Pheoseptoria eucalypti* (Hansf.). Walker. Aust. J. Biol. Sci. 20: 1155–1160. <https://doi.org/10.1071/B19671155>
- Jiang J, Zhai H, Li H, Wang Z, Chen Y, Hong N, Wang G, Chofong GN, Xu W (2014) Identification and characterization of *Colletotrichum fruticola* causing black spots on young fruits related to bitter rot of Pear (*Pyrus bretschneideri* Rehd.) in China. Crop Prot 58:41–48. <https://doi.org/10.1016/j.cropro.2014.01.003>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. <https://doi.org/10.1093/molbev/mst010>
- Liu F, Damm U, Cai L, Crous PW (2013) Species of the *Colletotrichum gloeosporioides* complex associated with anthracnose diseases of *Proteaceae*. Fungal Divers 61:89–105. <https://doi.org/10.1007/s13225-013-0249-2>
- MacKenzie SJ, Seijo TE, Legard DE, Timmer LW, Peres NA (2007) Selection for pathogenicity to strawberry in populations of *Colletotrichum gloeosporioides* from native plants. Phytopathology 97:1130–1140. <https://doi.org/10.1094/PHYTO-97-9-1130>
- McCarthy D, Ebach MC, Morrone JJ, Parenti LR (2007) An alternative gondwana: biota links South America, new Zealand and Australia. Biogeografia 2:1–12
- Montreal Process Implementation Group for Australia (MIG) and National Forest Inventory Steering Committee (NFISC) (2023) Indicator 1.1a.i Forest area by type. In: Australia's State of the Forests Report. Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES), Canberra. <https://www.agriculture.gov.au/abares/forestsaustralia/sofr/criterion-1/indicator-1.1a.i-forest-area-by-type>. Accessed 18 Sep 2024
- Nicoll D, Ritter MK, Jones RC, Phillips GP, French ME, Cumming R, Bell SAJ (2025) The genus problem – *Eucalyptus* as a model system for minimising taxonomic disruption. Taxon 74:495–506. <https://doi.org/10.1002/tax.13240>
- Nylander JAA (2004) MrModelTest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University
- Prasannath K, Galea VJ, Akinsanmi OA (2020) Characterisation of leaf spots caused by *Neopestalotiopsis clavispora* and *Colletotrichum Siamense* in macadamia in Australia. Eur J Plant Pathol 156:1219–1225. <https://doi.org/10.1007/s10658-020-01962-6>
- Prusky D, Alkan N, Mengiste T, Fluhr R (2013) Quiescent and necrotrophic lifestyle choice during postharvest disease development. Annu Rev Phytopathol 51:155–176. <https://doi.org/10.1146/annurev-phyto-082712-102349>
- R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Roy BA (2001) Patterns of association between crucifers and their flower-mimic pathogens: host jumps are more common than Coevolution or cospeciation. Evolution 55:41–53. <https://doi.org/10.1111/j.0014-3820.2001.tb01271.x>
- Schneider C, Rasband W, Eliceiri K (2012) NIH image to imageJ: 25 years of image analysis. Nat Methods 9:671–675. <https://doi.org/10.1038/nmeth.2089>
- Shivas RG, Tan YP, Edwards J, Dinh Q, Maxwell A, Andjic V, Liberato JR, Anderson C, Beasley DR, Bransgrove K, Coates LM, Cowan K, Daniel R, Dean JR, Lomavatu MF, Mercado-Escueta D, Mitchell RW, Thangavel R, Tran-Nguyen LTT, Weir BS (2016) *Colletotrichum* species in Australia. Australas Plant Pathol 45:447–464. <https://doi.org/10.1007/s13313-016-0443-2>
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stenlid J, Oliva J (2016) Phenotypic interactions between tree hosts and invasive forest pathogens in the light of globalization and climate change. Phil Trans R Soc B 371:20150455. <https://doi.org/10.1098/rstb.2015.0455>
- Stergiopoulos I, Gordon TR (2014) Cryptic fungal infections: the hidden agenda of plant pathogens. Front Plant Sci 5:506. <https://doi.org/10.3389/fpls.2014.00506>
- Stukenbrock EH, McDonald BA (2008) The origins of plant pathogens in agro-ecosystems. Annu Rev Phytopathol 46:75–100. <https://doi.org/10.1146/annurev.phyto.010708.154114>
- Talhinhas P, Baroncelli R (2023) Hosts of *Colletotrichum*. Mycosphere 14:158–261. <https://doi.org/10.5943/mycosphere/14/si2/4>
- Talhinhas P, Mota-Capitão C, Martins S, Ramos AP, Neves-Martins J, Guerra-Guimarães L, Várzea V, Silva MC, Sreenivasaprasad S, Oliveira H (2011) Epidemiology, histopathology and aetiology of Olive anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. Plant Pathol 60:483–495. <https://doi.org/10.1111/j.1365-3059.2010.02397.x>
- Vlasveld C, O'Leary B, Udovicic F, Burd M (2018) Leaf heteroblasty in eucalypts: biogeographic evidence of ecological function. Aust J Bot 66:191–201. <https://doi.org/10.1071/BT17134>
- Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. Stud Mycol 73:115–180. <https://doi.org/10.3114/sim0011>
- Western Australian Herbarium (1998) - Florabase - the Western Australian Flora. Department of biodiversity, conservation and attractions. <https://florabase.dbca.wa.gov.au/>. Accessed 18 Sep 2024
- White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: A guide to methods and applications. Academic, San Diego, pp 315–322
- Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer, New York. <https://ggplot2.tidyverse.org>
- Wirthensohn MG, Sedgley M (1996) Epicuticular wax structure and regeneration on developing juvenile *Eucalyptus* leaves. Aust J Bot 44:691–704. <https://doi.org/10.1071/BT9960691>
- Woudenberg JH, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW (2009) Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia 22:56–62. <https://doi.org/10.3767/003158509X427808>
- Zapata M, Opazo A (2017) Detection of *Colletotrichum pyricola* on urban trees of *Embothrium coccineum* in Chile. Bosque 38:195–201. <https://doi.org/10.4067/S0717-92002017000100019>
- Zapata M, Rodríguez-Serrano E, Castro JF, Santelices C, Carrasco-Fernández J, Damm U, Palfner G (2024) Novel species and records of *Colletotrichum* associated with native Woody plants in south-central Chile. Mycol Prog 23:18. <https://doi.org/10.1007/s11557-024-01956-2>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.