



Genome analyses reveal two novel species of *Seiridium* from *Acacia mearnsii*

Janneke Aylward^{1,2} · Cobus M. Visagie¹ · Francois Roets² · Brenda D. Wingfield¹ · Michael J. Wingfield¹

Received: 9 September 2025 / Revised: 5 January 2026 / Accepted: 7 January 2026
© The Author(s) 2026

Abstract

Seiridium is a genus of *Sordariomycetes* (*Amphisphaeriales*, *Sporocadaceae*), primarily known for species that cause cypress canker on *Cupressaceae* trees. However, most species in this genus have been reported from angiosperms, including many species in the native range of their hosts. Several unidentified *Seiridium* strains, collected > 20 years ago from *Acacia mearnsii* trees in eastern South Africa and southeastern Australia, were recently recovered from a collection of preserved cultures. We considered the phylogenetic position of these *Seiridium* strains and assessed their pathogenicity on *A. mearnsii*. Maximum likelihood analysis of three concatenated gene regions revealed four well-supported clades. Two closely related clades corresponded to Australian and South African origins, whereas two other clades representing South African isolates formed a monophyletic group with *S. kartense*, a species known from *Eucalyptus cladocalyx* on Kangaroo Island in Australia. Genome-wide average pairwise nucleotide identity and genetic differentiation analysis supported three species, including isolates considered conspecific with *S. kartense* and two that we consider to represent new species. These are described and named here as *Seiridium mearnsii* sp. nov. and *Seiridium rouxiae* sp. nov. Inoculations with South African isolates failed to produce evidence of pathogenicity. *Seiridium* species from *A. mearnsii* have likely been introduced into South Africa from Australia, along with the planting material used to establish *A. mearnsii* forestry.

Keywords 2 new taxa · Angiosperm · *Cupressaceae* · Pathogenicity · SNPs

Introduction

Seiridium species are plant-associated fungi best known for the seven species that cause cypress canker disease on *Cupressaceae* trees (Bonthond et al. 2018; Danti et al. 2013). These cypress pathogens typically enter their hosts through wounds or cracks in the bark and cause resinous cankers that eventually kill branches by girdling. Although cypress canker has been a problem in plantations (Jones 1953; Van der Werff 1988), the disease most commonly affects

Cupressaceae that have been widely planted as ornamentals in many parts of the world (Graniti 1998).

Despite the attention given to the *Cupressaceae* pathogens, only 10 of the more than 50 described *Seiridium* species are associated with conifers (Sutton 1975; Bonthond et al. 2018; Crous et al. 2014), the others being mostly described from flowering plants (*Angiospermae*). Of these, Rosid taxa, such as species in the orders *Malpighiales*, *Myrtales* and *Rosales*, have either been particularly well-studied or are common hosts of *Seiridium* species (Sutton 1975; Li et al. 2022; Nag Raj 1993; Crous et al. 2012; Yuan and Mohammed 1997). Other angiosperm groups include Asterids, Magnoliids and early diverging Eudicots (Samarakoon et al. 2022; Crous et al. 2017; Marincowitz et al. 2008; Chen et al. 2003). Most *Seiridium* species are known only from plants native to their areas of origin. In contrast, *Seiridium* pathogens from the single plant family *Cupressaceae* are abundant outside the native ranges of their host plants due to their widespread use as ornamentals (Graniti 1998).

Seiridium species associated with angiosperms are not typically considered pathogenic, being isolated only from

Section Editor: Roland Kirschner

✉ Janneke Aylward
janneke.aylward@fab.i.up.ac.za

¹ Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

² Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1 Matieland, Stellenbosch 7602, South Africa

minor leaf lesions (e.g. *S. camelliae* and *S. kartense*; Bonthond et al. 2018; Maharachchikumbura et al. 2015). There are exceptions, such as three angiosperm associates from China, namely *S. guangyuanum*, *S. oleae* and *S. vernicola* that are pathogens on *Olea europaea* (Li et al. 2022). However, of these, only *S. oleae* has been isolated from olive trees, whereas the others are associated with disease symptoms on a *Euphorbiaceae* host. Pathogenicity studies have not been conducted with other species, such as *S. papillatum* and *S. phylicae* isolated from angiosperms (Yuan and Mohammed 1997; Ryan et al. 2014).

During a recent survey of *Seiridium* canker pathogens in South Africa (Aylward et al. 2025), several *Seiridium* collected from *Acacia mearnsii* and stored in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa, were recovered. These strains were collected from stem cankers and wood of *A. mearnsii*, and those from South Africa were reported as part of a doctoral thesis (Roux 1998). The pathogenicity of selected South African isolates was demonstrated on *A. mearnsii* and *Hesperocyparis lusitanica*, leading to the conclusion that they should be regarded as conspecific with the cypress canker pathogen *S. cardinale*.

Advances in DNA sequencing techniques and phylogenetic analyses since 1998 and the recent taxonomic revision of *Seiridium* (Bonthond et al. 2018) have made it possible to reconsider the isolates from *A. mearnsii*. This interest was due to their occurrence on a Rosid taxon that is not currently recognised as a *Seiridium* host and particularly because *A. mearnsii* is an important forestry species in South Africa (Chan et al. 2015). The aim of this study was thus to consider the phylogenetic position of the *Seiridium* isolates from *A. mearnsii* and to assess their pathogenicity on this tree species.

Materials and methods

Fungal strains

Eleven *Seiridium* strains isolated from *A. mearnsii* were recovered from the CMW culture collection (Table 1). These included nine isolates collected by Roux (1998) from two plantations in eastern South Africa before 2001. Two additional isolates were collected in 2000 from trees in a natural forest near Canberra, Australia. The South African culture deposits were accompanied by notes referring to isolations from “mottle lesions” or “basal cankers” on *A. mearnsii*, whereas the Australian isolates were associated with stem cankers.

None of the *Seiridium* strains considered in this study (Table 1) produced spores in culture after revival. Therefore, isolates were grown on four agar media supplemented with autoclaved bark and wood of *A. mearnsii* in an attempt to induce sporulation. These media included 2% malt extract agar (35 g/L MEA, JLW Supplies, South Africa), ½ strength potato dextrose agar (19.5 g/L PDA, JLW Supplies, South Africa), nitrogen-deficient media (Ndef; Havenga et al. 2020) and synthetic nutrient-deficient agar (SNA; Leslie and Summerell 2006). Plates were incubated at room temperature, in natural light, and monitored for 8 weeks, after which they were stored at 4 °C.

For growth studies, 5-mm plugs of actively growing mycelium were placed on 2% MEA, PDA, SNA and Oatmeal Agar (OA; Crous et al. 2009) in triplicate. Plates were incubated in the dark at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C, and radial growth measurements were taken in two perpendicular planes at 7 and 14 days. Colony morphology was assessed and colours described using the

Table 1 *Seiridium* strains isolated from *Acacia mearnsii*

Species	CMW ^a	CMW-IA ^a	Clade ^b	Origin	Year	Genome accession
<i>S. kartense</i>	4148	5382	IV	South Africa: Mpumalanga	1994	JBQXFR01
	*4150	5349	III	(collector J Roux)	1993	JBQXFS01
	4152	5393	IV		1994	JBQWNR01
	4153	5327	III		1994	JBQXFT01
	4158	5417	IV		1994	JBQWNS01
<i>S. mearnsii</i> sp. nov.	6768	3621	I	Australia: ACT	2000	JBQWNT01
	6769 ^T	3624	I	(collector MJ Wingfield)	2000	JBQWNU01
<i>S. rouxiae</i> sp. nov.	*4151 ^T	5345	II	South Africa: KwaZulu-Natal	1995	JBQWNU01
	*4159		II	(collector J Roux)	1994	–
	*4154	5351	II	South Africa: Mpumalanga	2001	JBQXFV01
	4155		II	(collector J Roux)	2001	–

^aStrain numbers as maintained in the microbial culture collections of the Forestry and Agricultural Biotechnology Institute (FABI) (CMW) and Innovation Africa (CMW-IA) at the University of Pretoria, South Africa. Those marked with an asterisk (*) were used in the pathogenicity trial

^bPhylogenetic clades according to Fig. 1

Methuen Handbook of Colour (Kornerup and Wanscher 1978).

Taxonomic analyses

The genomes of nine *Seiridium* strains from *A. mearnsii* were sequenced in a collaborative project with Hexagon Bio (Aylward et al. 2024; Supplementary File 1) and have been made available under NCBI BioProject PRJNA1005571. The beta-tubulin (*TUB*), translation elongation factor 1-alpha (*EF1α*) and the RNA polymerase II core subunit (*RPB2*) genes were extracted from the genomes using local BLASTn in CLC Genomics Workbench 24.0.1 (QIAGEN, Aarhus, Denmark). The *RPB2* region of isolates CMW 4155 and CMW 4159, for which genome sequences were not available, was sequenced as described in Aylward et al. (2025).

The *EF1α*, *RPB2* and *TUB* regions of all *Seiridium* species included in the recent study of Razaghi et al. (2024) were obtained from GenBank (Table S1). Sequences of each region were aligned using MAFFT v7.505 (Katoh and Standley 2013), and alignment edges were trimmed manually. A concatenated maximum likelihood (ML) phylogenetic tree was computed with IQ-TREE v3.0.1 (Wong et al. 2025), applying the best-fit model for each partition based on AIC statistics calculated in ModelFinder (Kalyaanamoorthy et al. 2017). Support in nodes was calculated with a non-parametric bootstrap analysis with 1000 replicates.

A phylogenomic tree of the *Seiridium* strains from *A. mearnsii* and three cypress canker pathogens for which genomes are publicly available was determined (Table S1). This was done by identifying BUSCO proteins in all genomes using BUSCO v5.7.1 (Waterhouse et al. 2017) and the fungi_odb10 lineage. The BUSCO_phylogenomics.py script (McGowan 2022) was used to extract shared, single-copy orthologs and perform alignments with MUSCLE 5.3 (Edgar 2004) and trimAl v1.5 (Capella-Gutiérrez et al. 2009). Model testing and tree inference were performed with IQ-TREE, estimating branch support for the concatenated tree with 1000 Ultrafast Bootstrap (Hoang et al. 2017) and SH-aLRT branch test replicates (Guindon et al. 2010). Gene and site concordance factors (gCF and sCF) were calculated in IQ-TREE by comparing single-locus trees to the concatenated tree (Minh et al. 2020). The relatedness among genome pairs was also explored by calculating genome-wide average nucleotide identity (ANI) values with OrthoANIu v1.2 (Yoon et al. 2017).

Single nucleotide polymorphisms (SNPs) were identified in the nine genomes to further assess taxonomic relationships. The raw reads of all nine strains were mapped to the CMW 4151 genome using the BWA-MEM algorithm of the Burrows-Wheeler Alignment tool (BWA) v0.7.18 (Li and Durbin 2009). SNPs were identified in each alignment using

the GATK v4.6.0 HaplotypeCaller (GATK, Broad Institute, <https://software.broadinstitute.org/gatk/>). After combining GVCf output files, the density distribution of quality values was viewed using R v4.4.0 (R Core Team 2024), and the distribution of these values across the CMW 4151 genome was viewed with functions in the *vcfR* v1.15.0 (Knaus and Grünwald 2016) and *poppr* v2.9.6 (Kamvar et al. 2014) packages. SNPs were subsequently filtered based on the read depth (DP) metric, maintaining SNPs with a read depth within the 20–80% quantile range of each sample. SNP loci with > 50% missing data were subsequently discarded.

The filtered SNP dataset was used to calculate genetic differentiation among the four phylogenetic clades of *A. mearnsii* isolates using functions in the *poppr* package. Nei's corrected heterozygosity metric (*H*; Hedrick 2005) was calculated for each clade, and a principal components analysis (PCA) was performed. The PCA plot was produced using the *ggplot2* v3.5.1 package (Wickam 2016).

Pathogenicity tests

The pathogenicity of four South African *Seiridium* strains was assessed in an invasive stand of *A. mearnsii* on a private farm in the Western Cape Province, South Africa, from December 2023 to February 2024. The *A. mearnsii* strains (CMW 4149 and CMW 4152) used by Roux (1998) to test pathogenicity were not viable and could not be included in the trial. The four available strains (CMW 4150, 4151, 4154 and 4159) represented *S. rouxiae* (Clade II) and *S. kartense* (Clade III) strains (Fig. 1). Two of these originated from the Mpumalanga province, and two from the KwaZulu-Natal province (Table 1). Clade IV strains did not grow sufficiently to be included, and the *S. mearnsii* (Clade I) strains could not be used due to quarantine considerations. Each of the four strains was grown on PDA for 1 week. Subsequently, five branches on 20 different *A. mearnsii* trees were inoculated with each of the four strains as well as an agar-only control, each on a different branch. This was done by removing the bark with a 5-mm cork borer to expose the cambium and placing a similarly sized agar plug into the wound, with mycelial growth facing the exposed host tissue. The inoculation sites were covered with plastic film to retain moisture and avoid contamination.

After 9 weeks, the bark was removed to the level of the inoculation site and total lesion lengths were measured. Small pieces of bark were cut from the edges of the lesions and plated onto PDA. Using the *nortest* v1.0–4 package (Gross and Ligges 2015) in R, a Shapiro–Wilk test was used to test whether the lesion length data deviated from normality (Royston 1982). A Kruskal–Wallis rank sum test was performed to evaluate whether lesion length differed significantly ($P \leq 0.05$) among two or more of the five groups (four treatments and one control; Hollander and Wolfe 1973).

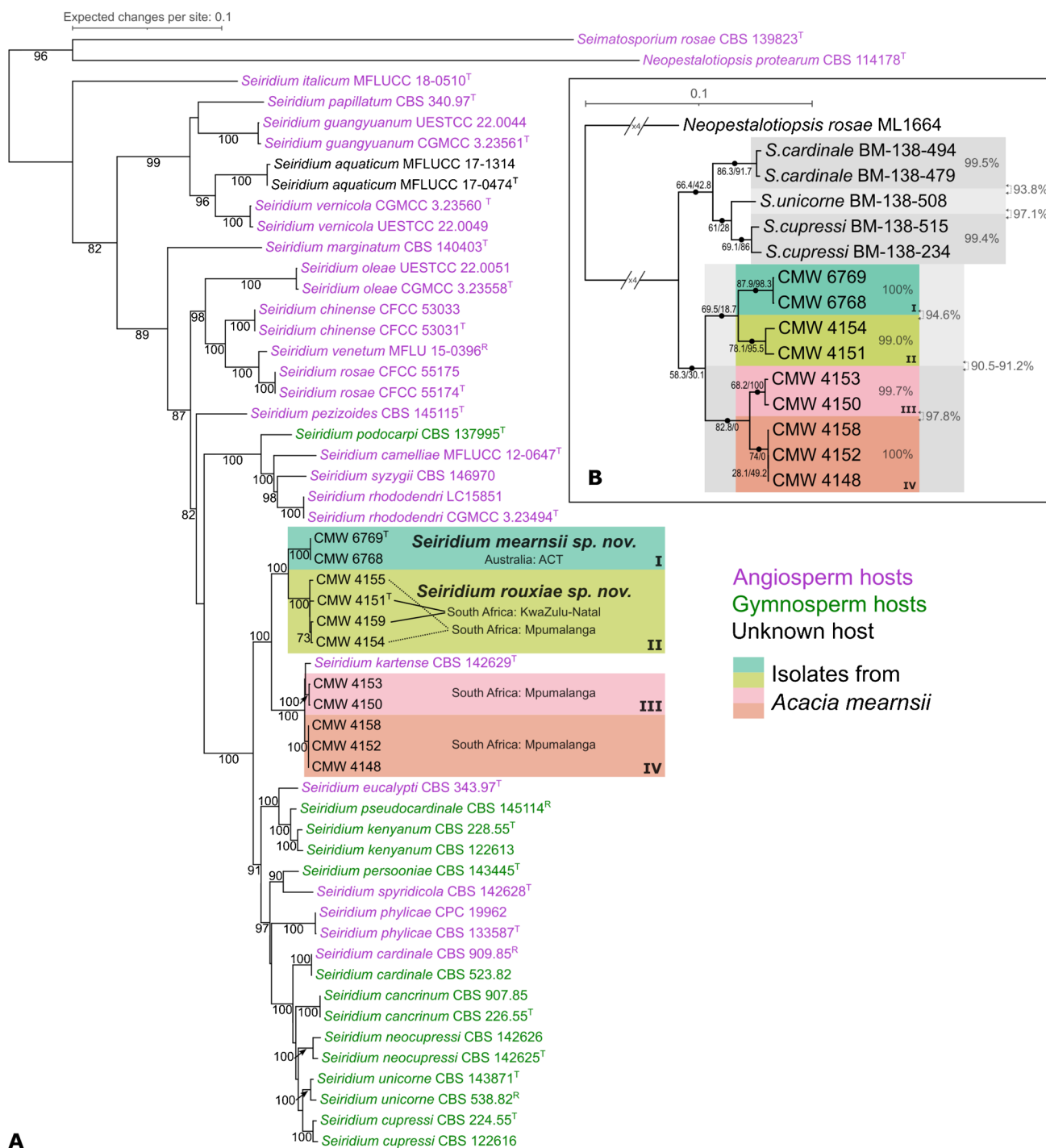


Fig. 1 Phylogenetic position of *Seiridium* isolates from *Acacia mearnsii*. **A** Maximum likelihood phylogeny of the concatenated *EF1a*, *TUB* and *RPB2* gene regions. Isolates from *A. mearnsii* are highlighted and form a monophyletic group with *S. kartense*. **B** Phylogenomic tree of 708 single-copy, shared fungal BUSCO proteins. Black circles represent 100% support values (UFboot and SH-aLRT).

Values above or below branches indicate the gene and site concordance factors (gCF and sCF), respectively. The average nucleotide identity (ANI) values within each clade and between adjacent clades are shown in grey on the right. See Table S1 for GenBank accession numbers and Supplementary Files 2–4 for alignments

Boxplots were generated using the *gplots* package (Warnes et al. 2024).

Results

Strain identification

The *EF1 α -RPB2-TUB* ML phylogeny of the *Seiridium* species (Fig. 1A) resolved the strains from *A. mearnsii* in a fully supported (100%) monophyletic group with *S. kartense*. Furthermore, the *A. mearnsii* isolates formed four fully supported subclades. The first two were sister clades (I and II) that included the two Australian strains (*S. mearnsii* sp. nov.) and four of the South African strains (*S. rouxiae* sp. nov.), respectively. Two strains of *S. rouxiae* were from the Mpumalanga province, whereas the remaining two were from the KwaZulu-Natal province. All isolates in Clades III and IV were collected from trees in the Mpumalanga province.

Phylogenomic analysis (Fig. 1B) of 708 single-copy BUSCO proteins that were present in all genomes (Tables 1 and S1) resolved the same four clades emerging from the ML phylogeny (Fig. 1A). The gene concordance factor (gCF) for the branch leading to all strains from *A. mearnsii* was 58.3%, and the individual clades (I–IV) were supported by > 74% gCF, with Clades I (87.9%) and II (78.1%) displaying high gCF (> 78%) and sCF (> 95%)

values. Genome-wide ANI values within each clade were 99–100%, whereas values between the sister Clades I and II, and III and IV were 94.6% and 97.8%, respectively. For comparison, ANI between the sister species *S. cupressi* and *S. unicorni* was 97.1% (Fig. 1B). Given the close phylogenetic relationship of strains from Clades III and IV and the fact that they were obtained from the same host and locality, we hypothesise that they represent one species, which would include the *S. kartense* type strain (CBS 142629). A genome sequence is not currently available for CBS 142629 and it, therefore, could not be included in the genomic analyses.

A total of 3,573,553 SNP loci were identified in the nine genomes. Due to the large number of loci, stringent filtering was applied as described above, reducing the number to 153,857 (File 1). Nei's corrected heterozygosity values (Hedrick 2005) were congruent with the ANI comparisons (Fig. 1B), reflecting no diversity ($H=0.00$) within Clade I or IV, some diversity ($H=0.008$) within Clade III, and the highest diversity ($H=0.020$) in Clade II. The total heterozygosity of the dataset was 0.413. The PCA plot (Fig. 2) confirmed the close relationship of Clade III and Clade IV isolates (identified as *S. kartense*), supporting the hypothesis that these clades represent a single phylogenetic species. Clade I and II isolates, however, were clearly differentiated in the PCA and are considered to represent different species, described below as *S. mearnsii* sp. nov. and *S. rouxiae* sp. nov.

Fig. 2 Principal components analysis (PCA) plot, depicting the genetic differentiation among the nine *Seiridium* isolates from *Acacia mearnsii*. Isolates (dots) are coloured according to their phylogenetic clade (Fig. 1)

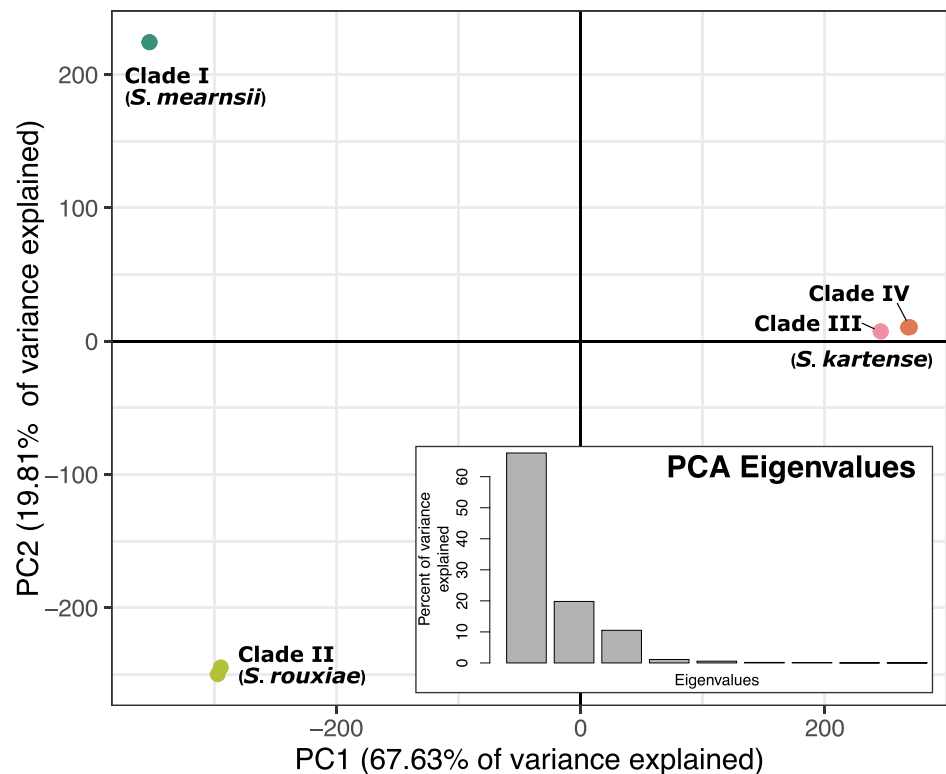




Fig. 3 Examples of inoculation sites and superficial streaking in branches inoculated with **A** the negative control and strains **B** *Seiridium kartense* CMW 4150, **C** *S. rouxiae* CMW 4151, **D** *S. rouxiae* CMW 4154, and **E** *S. rouxiae* CMW 4159

Pathogenicity tests

During the 9-week pathogenicity test, the *A. mearnsii* trees exuded copious amounts of gum at the sites of inoculation, for both the treatments and controls. Fungal structures were not observed on the tissues associated with the inoculation wounds. Removal of the bark to the level of the cambium showed no evidence of obvious lesion development (Figs. 3 and 4). Superficial “streaked” discolouration of the wood associated with the wounds was present on all the inoculated branches. This streaking was especially pronounced for two strains (CMW 4150 and CMW 4151), but streaking was also associated with several of the controls (Fig. 4). Re-isolation

from tissues associated with the wounds failed to yield isolates of the inoculated fungi.

Taxonomy

Seiridium mearnsii Aylward, Visagie & M.J. Wingf., sp. nov. (Figs. 5, 6A).

Mycobank: MB861561.

Etymology. Latin, *mearnsii*, referring to the host tree, *Acacia mearnsii*.

Type. Australia: Australian Capital Territory (ACT), Unarra Forest. Isolated from *Acacia mearnsii* stem cankers. November 2000. *M.J. Wingfield*. (Holotype PRU(M) 4640,

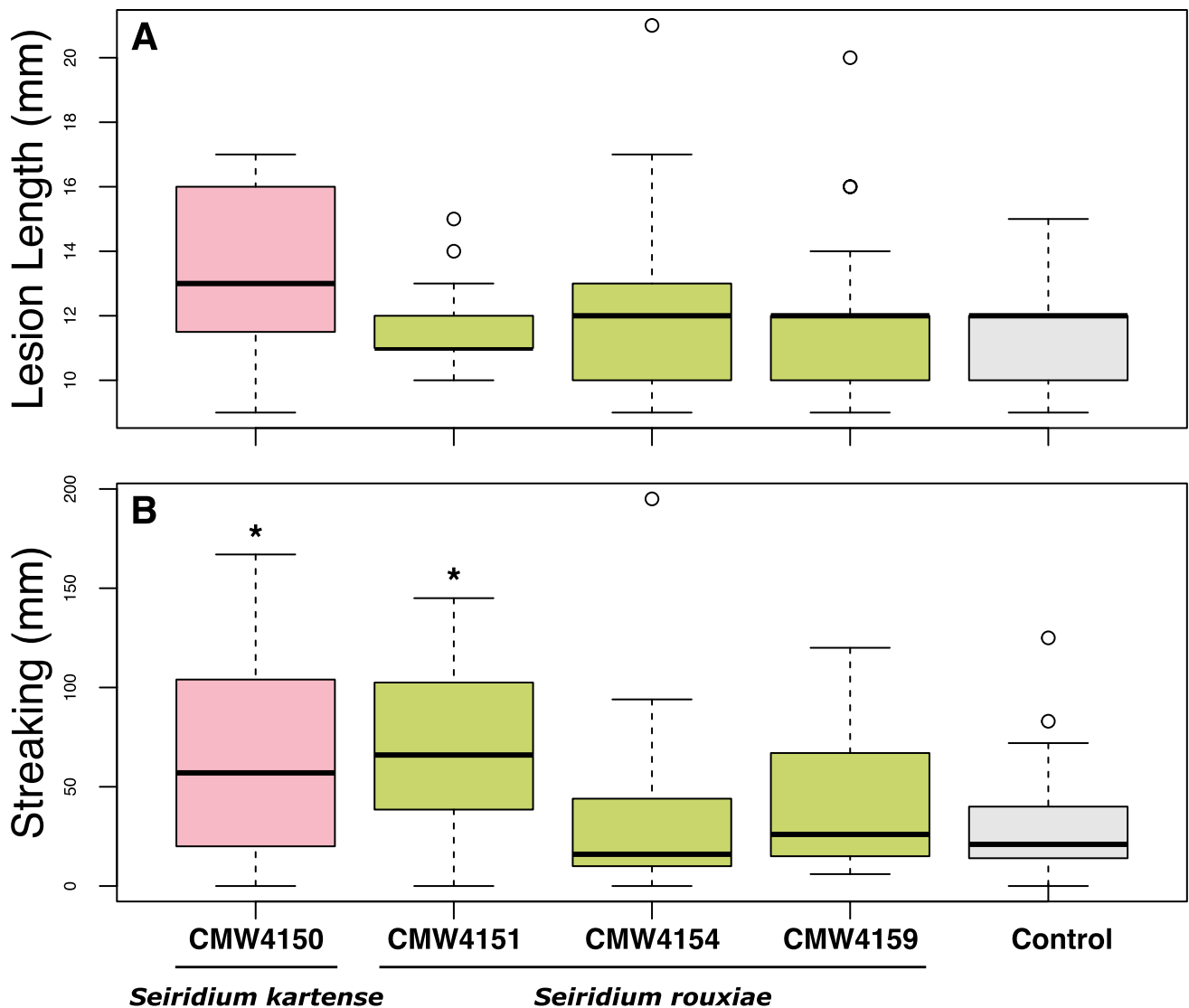


Fig. 4 Boxplots depicting the length of **A** lesions and **B** superficial streaking observed in treatments and controls. The asterisks in plot B indicate two samples with significantly ($P < 0.05$) longer streaking

than the control. Colours on the boxplots refer to the phylogenetic clades to which the isolates belong (pink, Clade III; green, Clade II; grey, control; Fig. 1)

preserved as a dried specimen, in a metabolically inactive state; ex-holotype culture CMW-IA 3624 = CMW 6769). GenBank: PX248600 (ITS); PX260321 (*TUB*); PX260310 (*RPB2*); PX260319 (*EF1 α*); JBQXFU01 (WGS).

Description. *Conidiomata* erumpent sporodochia on host material in vitro, globose, aggregated, producing black spore masses. *Conidiophores* compact, hyaline, septate, cylindrical, smooth- and thin-walled, (18)24–48(52) μm long ($n = 16$). *Conidiogenous cells* discrete, hyaline, cylindrical to globose, smooth- and thin-walled, (10)14–18(22) $\times$ (3)4–6 μm ($n = 10$). *Conidia* lunate to falcate, straight to curved, 5-septate, not striate, bearing two appendages, euseptate with visible pores, (30)34–38(44) $\times$ 6–9(10) μm ($n = 30$); basal cell obconic

with a truncate base, hyaline, walls smooth, 4–6(7) μm long; four median cells, smooth, cylindrical to doliiform, dark brown, (22)24–28(31) μm long; apical cell conical, hyaline, smooth, (3)4–6(7) μm long; appendages cylindrical, unbranched; apical appendage single, centric, (6)9–13(16) μm long; basal appendage, single, centric, (6)7–9(11) μm long.

Culture characteristics. Optimum growth at 20 °C. Colonies on MEA in the dark with an average radial growth rate of 2–4 mm/day, reaching 30–53 mm diam after 14 days at 20 °C; surface white at 10 °C, at 15 and 20 °C orange grey (5B2) at centre, white margin, at 25 °C orange white (6A2) to pale orange (6A3), umbonate, cottony, spreading out with sparse vegetative hyphae at the margin, margin

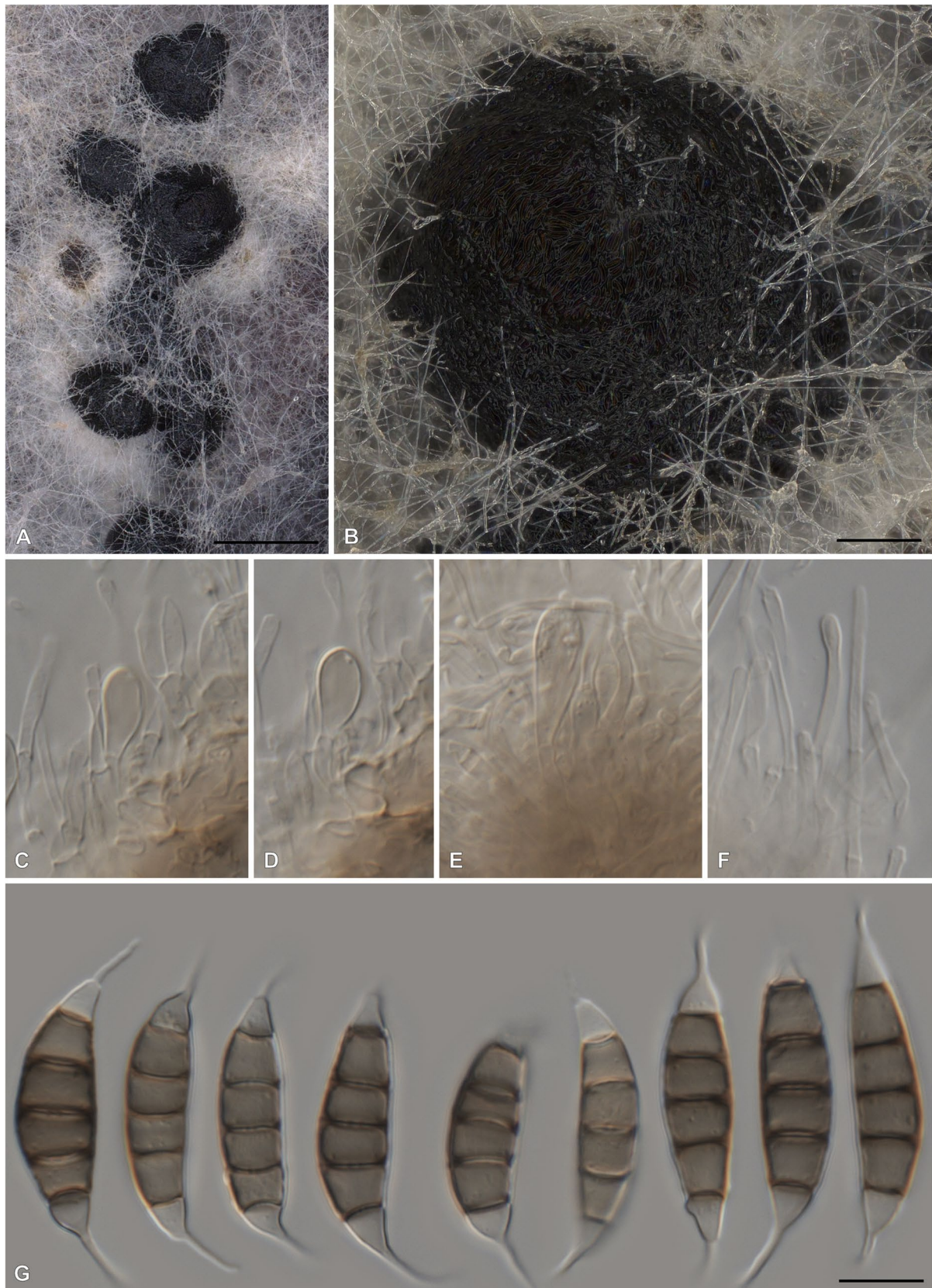


Fig. 5 *Seiridium mearnsii* sp. nov. (CMW-IA 3624, CMW 6769, culture ex-holotype). **A, B.** Sporodochia on *Acacia mearnsii* material in SNA. **C–F** Conidiophores and conidiogenous cells. **G** Conidia. Scale bars: A = 500 μ m; B = 100 μ m; **G** (applies to C–G) = 10 μ m

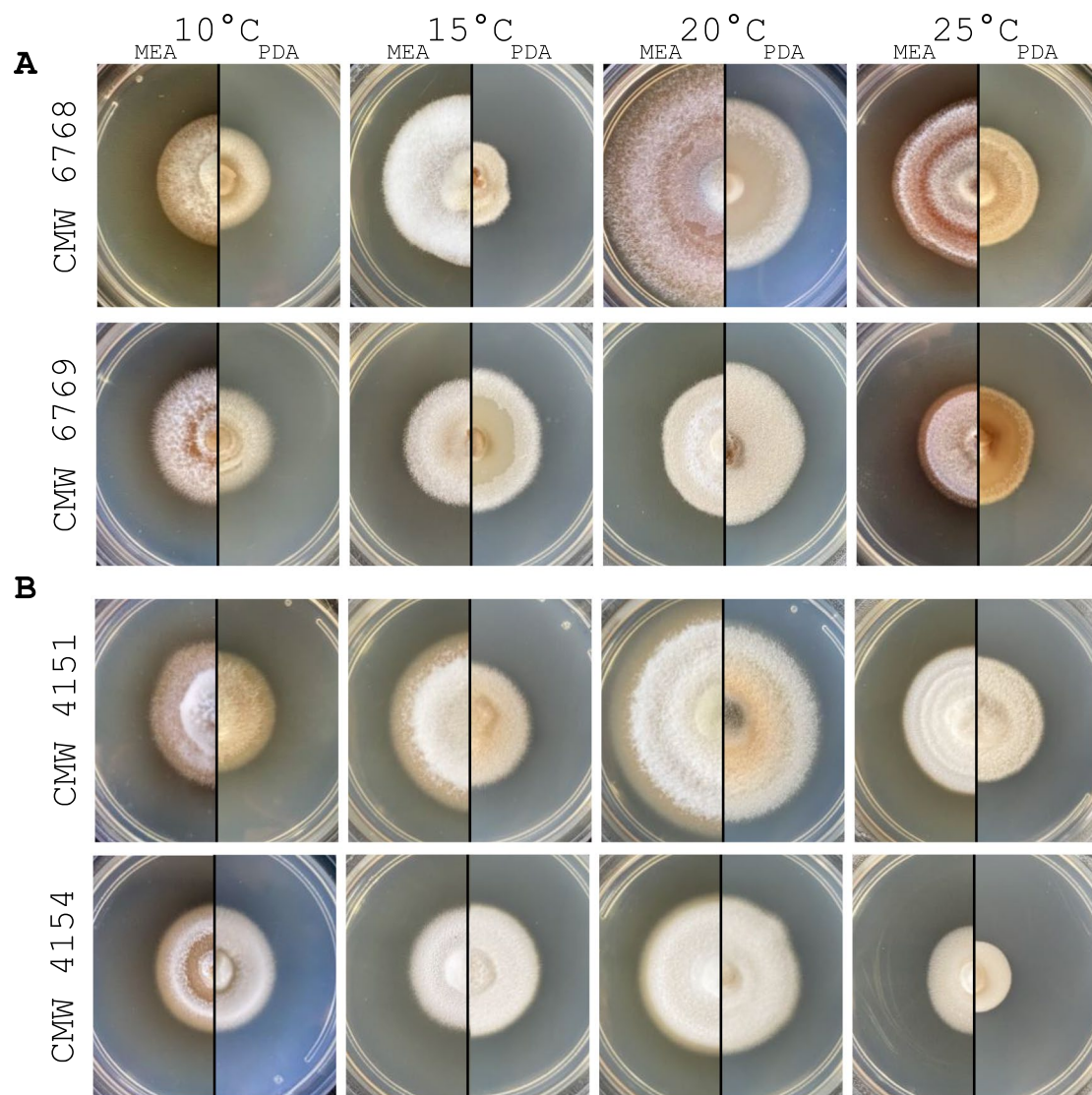


Fig. 6 Cultures of **A** *Seiridium mearnsii* and **B** *S. rouxiae* grown in 65-mm petri dishes containing malt extract agar (MEA, left) and potato dextrose agar (PDA, right) for 14 days in the dark at various temperatures. Neither species grew at 30 °C

entire. Reverse at 10 °C greyish orange (5A2) to orange grey (5B2) or pale orange (6A3) to light orange (6A4–5), at 15 and 20 °C brownish orange (5C5) or light brown (6D6) centre to greyish orange (5B3) or orange white (6A2) at margin. On PDA in the dark with an average radial growth rate of 1.5–3.5 mm/day, reaching 30–39 mm diam after 14 days at 20 °C; surface white, sometimes orange white at 15 °C and orange grey (5B2) at 20 °C, thick cottony at 10 °C, becoming thinner with increasing temperature and glabrous at 25 °C with sparse aerial mycelia. Reverse white to orange white (5A2), centre becoming orange grey (5A2) and reddish, blond, brownish orange (5C3) at 15 and 20 °C. Additional colony diam (after 14 days, in mm): MEA 10 °C 23–24; MEA 15 °C 26–35; MEA 25 °C 21–33; PDA 10 °C 17.5–20; PDA 15 °C 19–30; PDA 25 °C 24–26; no growth

at 30 °C, cultures grew back after re-incubation at 20 °C. On OA in the dark 2–4 mm/day, reaching 47–51 mm diam after 14 days at 20 °C; surface flat, sparse to moderate aerial mycelia, margin entire. White, translucent. On SNA in the dark 1–3 mm/day, reaching 26–39 mm diam after 14 days at 20 °C; surface flat, moderate aerial mycelium at the margin, margin entire. White, translucent.

Host. *Acacia mearnsii*.

Known distribution. Australia.

Additional specimen examined. Australia: Australian Capital Territory (ACT), Unarra Forest. Isolated from *Acacia mearnsii* stem cankers. November 2000. *M.J. Wingfield*. (Culture CMW-IA 3621 = CMW 6768). GenBank: PX248599 (ITS); PX260322 (*TUB*); PX260313 (*RPB2*); PX260318 (*EF1α*); JBQWNT01 (WGS).

Notes: The two *S. mearnsii* strains produced conidiomata on *A. mearnsii*-supplemented agar plates after approximately 4 months of storage at 4 °C. CMW 6769 sporulated only on SNA supplemented with *A. mearnsii* material, whereas CMW 6768 sporulated on all four supplemented media types. The *S. mearnsii* conidia were longer (av. 36 mm vs. 29 mm) and with slightly longer appendages (11 and 8.5 mm vs. 8 and 6 mm, respectively) compared to the *S. rouxiae* dimensions reported by Roux (1998). *Seiridium mearnsii* formed a unique monophyletic clade supported by 100% of bootstrap replicates in the concatenated *EF1α*–*RPB2*–*TUB* ML phylogeny and 622 (87.9%) of genes in the phylogenomic tree (Clade I, Fig. 1). It differed from its sister species, *S. rouxiae*, by 24 bp (98.57% identity) across the full *EF1α*, including one nonsynonymous mutation, 33–35 bp differences (98.04–98.15% identity) across the full *TUB* and 75–79 bp differences (97.96–98.06% identity) across the full *RPB2*, including four nonsynonymous mutations. Furthermore, an ANI value of 94.6% distinguished the two species. *Seiridium mearnsii* was collected from canker symptoms on *A. mearnsii* in the Unarra Forest outside of Canberra (Australia), but its pathogenicity has not been investigated. Other *Seiridium* species that have been collected from an Australian native (*Eucalyptus delegatensis*) are *S. eucalypti* (Nag Raj 1993) and *S. papillatum* (Yuan and Mohammed 1997).

Seiridium rouxiae Aylward, Visagie & M.J. Wingf., sp. nov. (Fig. 6B).

Mycobank: MB861562.

Etymology. Latin, *rouxiae*, the name refers to Prof. Jolanda Roux who is recognised for collecting the South African *Seiridium* isolates during her Doctoral studies at the University of the Free State.

Type. South Africa: KwaZulu-Natal province, Pietermaritzburg. Isolated from *Acacia mearnsii* basal cankers. May 1995. *J. Roux*. (Holotype PRU(M) 4638, preserved as a dried specimen, in a metabolically inactive state; ex-type strain: CMW-IA 5345 = CMW 4151). GenBank: PX248597 (ITS); PX260324 (*TUB*); PX260311 (*RPB2*); PX260316 (*EF1α*); JBQWNU01 (WGS).

Description. Conidia photographed and measured by Roux (1998), lunate to falcate, curved, 5-septate, not striate, bearing two appendages, euseptate with visible pores, 24–38 × 4–9 (av. 29 × 7) μm (*n* = 50); basal cell obconic with a truncate base, hyaline; four median cells, smooth, cylindrical to doliiform, 17–24 (av. 20) μm long; apical cell conical, hyaline, smooth; appendages cylindrical, unbranched; apical appendage single, centric, 4–13 (av. 8) μm long; basal appendage, single, centric and excentric, 3–12 (av. 6) μm long.

Culture characteristics. Optimum growth at 20 °C. Colonies on MEA in the dark with an average radial growth rate of 2–4 mm/day, reaching 31–51 mm diam after 14 days at 20 °C; surface white at 10 °C, at 15 and 20 °C greenish

grey (1C2) centre becoming pinkish white (10A2) and white at the margin, at 25 °C pinkish white to white centre and white at margin, umbonate, cottony, with abundant aerial mycelium, margin glabrous and entire. Reverse brownish orange (5C5) to greyish orange (5B4) to light orange (5A4) at margin. On PDA in the dark with an average radial growth rate of 1–3 mm/day, reaching 28–43 mm diam after 14 days at 20 °C; surface white at 10 and 25 °C, at 15 and 20 °C olive grey (1E2) to reddish grey (8B2) at centre, umbonate, cottony, with abundant aerial mycelium, margin entire. Reverse greyish orange (5B3) to brownish orange (5C5) to light brown (5D4) at centre (15 and 20 °C), orange white (5A2) at margin. Additional colony diam (after 14 days, in mm): MEA 10 °C 19–24; MEA 15 °C 25–38; MEA 25 °C 17–34; PDA 10 °C 19–22; PDA 15 °C 26–31; PDA 25 °C 16–27; no growth at 30 °C, cultures grew back after re-incubation at 20 °C. On OA in the dark 2–4 mm/day, reaching 47–60 mm diam after 14 days at 20 °C; surface flat, glabrous, with sparse aerial mycelium at the margin, margin entire. White, translucent. On SNA in the dark 1–3 mm/day, reaching 24–35 mm diam after 14 days at 20 °C; surface flat, with moderate aerial mycelium, margin entire. White, translucent.

Host. *Acacia mearnsii*.

Known distribution. South Africa.

Additional specimen examined. South Africa: Mpumalanga province, Piet Retief. Isolated from *Acacia mearnsii* basal cankers. January 2001. *J. Roux*. (PRU(M) 4639, stored in a metabolically inactive state; culture CMW-IA 5351 = CMW 4154). GenBank: PX248598 (ITS); PX260323 (*TUB*); PX260312 (*RPB2*); PX260317 (*EF1α*); JBQXFV01 (WGS).

Notes. The *S. rouxiae* strains investigated here were sterile, but Roux (1998) photographed conidia with apical and basal appendages and recorded conidial dimensions for CMW 4155 and CMW 4159 (Fig. 1; Table S2), which overlap with those reported for *S. kartense* (Bonthond et al. 2018). These conidia were slightly shorter than those observed for *S. mearnsii* (see details in notes for *S. mearnsii*). Roux (1998) also reported poor to no growth at 30 °C, but noted growth optima at 25 °C, in contrast to the 20 °C observed in this study. *Seiridium rouxiae* also produced olive grey to reddish grey pigments on PDA that were not observed in *S. mearnsii*. In the concatenated *EF1α*–*RPB2*–*TUB* ML phylogeny, *S. rouxiae* formed a unique monophyletic clade with 100% bootstrap support and 553 (78.1%) concordant genes in the phylogenomic tree (Clade II, Fig. 1). It differs from *S. mearnsii* by 24, 33–35 and 75–79 bp across the full *EF1α*, *TUB* and *RPB2* genes, respectively, and with an ANI value of 94.6% (see *S. mearnsii* notes for additional detail). *Seiridium rouxiae* was collected from canker symptoms on *A. mearnsii* trees planted as an exotic forestry species, but its pathogenicity could not be demonstrated in this study. *Seiridium rouxiae* and the strains of *S. kartense* reported

in this study are the first *Seiridium* species known from *A. mearnsii* in South Africa.

Discussion

Three *Seiridium* species were identified from isolates collected from *A. mearnsii* more than 20 years ago and subsequently stored in culture. Two of these species, described here as *S. mearnsii* sp. nov. and *S. rouxiae* sp. nov., were associated with *A. mearnsii* from different countries and were phylogenetically clearly distinct. They also had low genome-wide nucleotide identity (<95% ANI) and significant genetic differentiation compared to each other and to strains of the third species, *S. kartense*. ANI is commonly calculated for bacteria, with interspecies comparisons typically yielding values above 95% (Jain et al. 2018). In contrast, the limited number of studies that have considered expected genome variation within and between fungal species found that ANI thresholds are genus-specific. For example, inter-specific ANI values in 12 yeast genera ranged from 92 to 98% (Cortimiglia et al. 2024). In *Neopestalotiopsis* (*Sporocadaceae*), 98% has been recommended as the species-level threshold (Razaghi et al. 2024). Here, 97–98% appears to be a sensible threshold, considering the 97.1% ANI observed between *S. cupressi* and *S. unicornae* and the 97.8% between the *S. kartense* lineages.

We considered the Clade III and IV strains from the Mpumalanga province (South Africa) to be conspecific with *S. kartense*, based on their monophyly and the close genetic relationship between them. Morphological characteristics for isolate CMW 4150 (Clade III), reported by Roux (1998), were similar to those of *S. kartense*, although the CMW 4150 conidia were > 1 µm wider than those reported by Bonthond et al. (2018). *Seiridium kartense* is known only from a single collection from *Eucalyptus cladocalyx* leaves on Kangaroo Island, Australia (Bonthond et al. 2018). The other *Seiridium* species known from *Eucalyptus* are *S. eucalypti* and *S. papillatum* (Nag Raj 1993; Yuan and Mohammed 1997), both associated with *E. delegatensis* and not closely related to *S. kartense* or to each other (Fig. 1; Bonthond et al. 2018). The close relationship among *Seiridium* strains in the present study and *S. kartense*, strains from two different native Australian trees, is intriguing and strongly suggests that the fungus has been introduced into South Africa.

The inoculation test conducted in this study provided no evidence that the *Seiridium* spp. from *A. mearnsii* in South Africa are pathogenic. However, they were originally isolated from disease symptoms, and two strains (CMW 4149 and CMW 4152, not available for the present study) were reported to produce lesions on *A. mearnsii* (Roux 1998). An absence of pathogenicity in the present study could be the result of the cultures having lost virulence due to

long-term storage in culture (Abd-Elsalam et al. 2010). It is also relevant that this study has revealed the presence of more than one species of *Seiridium* among cultures that were previously thought to represent a single species. Further investigation of the pathogenicity of phylogenetic lineages that were not investigated in this study, i.e. *S. mearnsii* and *S. kartense* Clade IV, would be interesting.

This study has added to the number of *Seiridium* species known from angiosperm hosts. It also provides another example of the value of culture collections in storing cultures that could not be identified at the time they were collected (Wingfield et al. 2024), and the hidden diversity that these collections may hold. The genomes of these strains are valuable additions to the *Seiridium* genome data that have recently begun to emerge (Scali et al. 2024c; 2024b; 2024a; Razaghi et al. 2024). These strains were collected from a tree native to Australia and showed a close relationship between Australian and South African isolates. This suggests that these *Seiridium* species have an Australian origin and have likely been introduced into South Africa along with the planting material used to establish *A. mearnsii* forestry.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11557-026-02121-7>.

Acknowledgements We thank Dr. Jolanda Roux who isolated the *Seiridium* strains from *Acacia mearnsii* in South Africa and deposited these in the CMW collection. We are grateful to Elise Roberts and Sydney Atkins for their assistance with the pathogenicity trial. Furthermore, we acknowledge J. Spraker and the team at Hexagon Bio (CA, USA), who sequenced the genomes used in this study.

Author contribution All authors contributed to study design and data collection. Janneke Aylward conceived the study, performed experiments, analysed the data and wrote the first draft of the manuscript. Cobus Visagie performed microscopy for species descriptions. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by University of Pretoria. This work was supported by the DSTI-NRF SARChI in Fungal genomics (Grant UID 0216114244) and the Tree Protection Cooperative Program (TPCP). The genome sequencing data used in this manuscript were contributed by Hexagon Bio (CA, USA).

Data availability Sequence data generated and used in this study have been deposited at GenBank under NCBI BioProject PRJNA1005571 and the accessions listed in Table S1. Alignments are provided as supplementary files.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests Cobus M. Visagie is a section editor of *Mycological Progress*.

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

- Abd-Elsalam KA, Yassin MA, Moslem MA, Bahkali AH, de Wit PJGM, McKenzie EHC, Stephenson SL, Cai L, Hyde KD (2010) Culture collections, the new herbaria for fungal pathogens. *Fungal Divers* 45(1):21–32. <https://doi.org/10.1007/s13225-010-0063-z>
- Aylward J, Wilson AM, Visagie CM, Spraker J, Barnes I, Buitendag C, Ceriani C, Del Mar Angel L, du Plessis D, Fuchs T, Gasser K, Krämer D, Li W, Munsamy K, Piso A, Price J-L, Sonnekus B, Thomas C, van der Nest A, van Dijk A, van Heerden A, van Vuuren N, Yilmaz N, Duong TA, van der Merwe NA, Wingfield MJ, Wingfield BD (2024) IMA genome – F19: a genome assembly and annotation guide to empower mycologists, including annotated draft genome sequences of *Ceratocystis pirilliformis*, *Diaporthe australafricana*, *Fusarium ophioides*, *Paecilomyces lecythidis*, and *Sporothrix stenoceras*. *IMA Fungus* 15(1):12. <https://doi.org/10.1186/s43008-024-00142-z>
- Aylward J, Atkins S, Roets F, Wingfield BD, Wingfield MJ (2025) Several *Seiridium* species (*Sporocadaceae*: *Xylariales*) cause cypress canker in South Africa. *Fungal Biol* 129(1):101528. <https://doi.org/10.1016/j.funbio.2024.101528>
- Bonthond G, Sandoval-Denis M, Groenewald JZ, Crous PW (2018) *Seiridium* (*Sporocadaceae*): an important genus of plant pathogenic fungi. *Persoonia* 40:96. <https://doi.org/10.3767/persoonia.2018.40.04>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) Trimal: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinform* 25(15):1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Chan JM, Day P, Feely J, Thompson R, Little KM, Norris CH (2015) *Acacia mearnsii* industry overview: current status, key research and development issues. *South for* 77(1):19–30. <https://doi.org/10.2989/20702620.2015.1006907>
- Chen Y, Wel G, Chen W, Wang Z, Lu Z (2003) Three new species of *Monochaetia* and *Seiridium* in China. *J Agricult Biologic Sci* 22(2):89–91
- Cortimiglia C, Alonso-Del-Real J, Belloso Daza MV, Querol A, Iacono G, Cocconcelli PS (2024) Evaluating the genome-based average nucleotide identity calculation for identification of twelve yeast species. *J Fungi* 10(9):646. <https://doi.org/10.3390/jof10090646>
- Crous PW, Shivas RG, Wingfield MJ, Summerell BA, Rossman AY, Alves JL, Adams GC, Barreto RW, Bell A, Coutinho ML, Flory SL, Gates G, Grice KR, Hardy GESJ, Kleczewski NM, Lombard L, Longa CMO, Louis-Seize G, Macedo F, Mahoney DP, Maresi G, Martin-Sanchez PM, Marvanová L, Minnis AM, Morgado LN, Noordeloos ME, Phillips AJL, Quaedyvlieg W, Ryan PG, Saiz-Jimenez C, Seifert KA, Swart WJ, Tan YP, Tanney JB, Thu PQ, Videira SIR, Walker DM, Groenewald JZ (2012) Fungal planet description sheets: 128–153. *Persoon - Molecul Phylog Evolut Fungi* 29(1):146–201. <https://doi.org/10.3767/003158512X661589>
- Crous PW, Shivas RG, Quaedyvlieg W, van der Bank M, Zhang Y, Summerell BA, Guarro J, Wingfield MJ, Wood AR, Alfenas AC, Braun U, Cano-Lira JF, García D, Marin-Felix Y, Alvarado P, Andrade JP, Armengol J, Assefa A, den Breejen A, Camele I, Cheewangkoon R, De Souza JT, Duong TA, Esteve-Raventós F, Fournier J, Frisullo S, García-Jiménez J, Gardienet A, Gené J, Hernández-Restrepo M, Hirooka Y, Hospenthal DR, King A, Lechat C, Lombard L, Mang SM, Marbach PAS, Marincowitz S, Marin-Felix Y, Montaña-Mata NJ, Moreno G, Perez CA, Pérez Sierra AM, Robertson JL, Roux J, Rubio E, Schumacher RK, Stchigel AM, Sutton DA, Tan YP, Thompson EH, Vanderlinde E, Walker AK, Walker DM, Wickes BL, Wong PTW, Groenewald JZ (2014) Fungal planet description sheets: 214–280. *Persoonia - Molecul Phylogen Evolut Fungi* 32(1):184–306. <https://doi.org/10.3767/003158514X682395>
- Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, Hardy GESJ, Smith D, Summerell BA, Cano-Lira JF, Guarro J, Houbraken J, Lombard L, Martín MP, Sandoval-Denis M, Alexandrova AV, Barnes CW, Baseia IG, Bezerra JDP, Guarnaccia V, May TW, Hernández-Restrepo M, Stchigel AM, Miller AN, Ordoñez ME, Abreu VP, Accioly T, Agnello C, Agustín Colmán A, Albuquerque CC, Alfredo DS, Alvarado P, Araújo-Magalhães GR, Arauzo S, Atkinson T, Barili A, Barreto RW, Bezerra JL, Cabral TS, Camello Rodríguez FC, Cruz RHSE, Daniëls PP, Da Silva BDB, De Almeida DAC, Giraldo A, Groenewald M, Kraak B, Meijer M, Silva GA, Smith MT, Wang XW, Groenewald JZ (2017) Fungal planet description sheets: 625–715. *Persoon - Molecul Phylogen Evolut Fungi* 39(December):270–467. <https://doi.org/10.3767/persoonia.2017.39.11>
- Crous PW, Verkley GJM, Groenewald JZ, Samson RA (eds) (2009) Fungal biodiversity. CBS Laboratory Manual Series 1. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands
- Danti R, Della Rocca G, Panconesi A (2013) Cypress canker. In: Gonthier P, Nicolotti G (eds) Infectious forest diseases. CAB International, pp 359–375. <https://doi.org/10.1079/9781780640402.0000>
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5(1):113. <https://doi.org/10.1186/1471-2105-5-113>
- Graniti A (1998) Cypress canker: a pandemic in progress. *Annu Rev Phytopathol* 36(1):91–114. <https://doi.org/10.1146/annurev.phyto.36.1.91>
- Gross J, Ligges U (2015) nortest: tests for normality. R package version 1.0–4. <https://CRAN.R-project.org/package=nortest>
- Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59(3):307–321. <https://doi.org/10.1093/sysbio/syq010>
- Havenga M, Wingfield BD, Wingfield MJ, Roets F, Dreyer LL, Tatham CT, Duong TA, Wilken PM, Chen S, Aylward J (2020) Mating strategy and mating type distribution in six global populations of the *Eucalyptus* foliar pathogen *Teratosphaeria destructans*. *Fungal Genet Biol* 137:103350. <https://doi.org/10.1016/j.fgb.2020.103350>
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59(8):1633–1638. <https://doi.org/10.1111/j.0014-3820.2005.tb01814.x>

- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFboot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 35(2):518–522. <https://doi.org/10.1093/molbev/msx281>
- Hollander M, Wolfe DA (1973) Nonparametric statistical methods. John Wiley & Sons, New York
- Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9(1):5114. <https://doi.org/10.1038/s41467-018-07641-9>
- Jones DR (1953) Studies on a canker disease of cypresses in East Africa, caused by *Monochaetia unicornis* (Cooke & Ellis) Sacc: i. observations on the pathology, spread and possible origins of the disease. *Ann Appl Biol* 40(2):323–343. <https://doi.org/10.1111/j.1744-7348.1953.tb01086.x>
- Kalyanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS (2017) Modelfinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14(6):587–589. <https://doi.org/10.1038/nmeth.4285>
- Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281. <https://doi.org/10.7717/peerj.281>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772–780. <https://doi.org/10.1093/molbev/mst010>
- Knaus BJ, Grünwald NJ (2016) VcfR: a package to manipulate and visualize VCF format data in R. *bioRxiv*:041277. <https://doi.org/10.1101/041277>
- Kornerup A, Wanscher JH (1978) *Metheun Hand Book of Colour*, 3rd edn. Eyre Methuen, London
- Leslie JF, Summerell BA (2006) *The Fusarium laboratory manual*, 1st edn. Blackwell Publishing, Ames, Iowa
- Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinform* 25(14):1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- Li W-L, Dissanayake AJ, Zhang T, Maharachchikumbura SSN, Liu J-K (2022) Identification and pathogenicity of pestalotioid fungi associated with woody oil plants in Sichuan Province, China. *J Fungi* 8(11):1175. <https://doi.org/10.3390/jof8111175>
- Maharachchikumbura SSN, Camporesi E, Liu Z-Y, Hyde KD (2015) *Seiridium venetum* redescribed, and *S. camelliae*, a new species from *Camellia reticulata* in China. *Mycol Progress* 14(10):85. <https://doi.org/10.1007/s11557-015-1110-7>
- Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ (2008) Microfungi occurring on *Proteaceae* in the fynbos. *CBS Biodiversity Series No. 7*. CBS Fungal Biodiversity Centre, Utrecht, Netherlands.
- McGowan J (2022) Jamiemcg/BUSCO_phylogenomics: BUSCO rewrite_November_2022. Zenodo. <https://doi.org/10.5281/zenodo.4320788>
- Minh BQ, Hahn MW, Lanfear R (2020) New methods to calculate concordance factors for phylogenomic datasets. *Mol Biol Evol* 37(9):2727–2733. <https://doi.org/10.1093/molbev/msaa106>
- Nag Raj TR (1993) *Coelomycetous anamorphs with appendage-bearing conidia*. Mycologue Publications,
- R Core Team (2024) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.
- Razaghi P, Raza M, Han S, Ma Z, Cai L, Zhao P, Chen Q, Phurbu D, Liu F (2024) *Sporocadaceae* revisited. *Stud Mycol* 109:155–272. <https://doi.org/10.3114/sim.2024.109.03>
- Roux J (1998) Diseases of *Acacia mearnsii* in South Africa, with particular reference to Ceratocystis wilt. University of the Orange Free State, South Africa
- Royston JP (1982) An extension of Shapiro and Wilk's W test for normality to large samples. *J Roy Stat Soc: Ser C (Appl Stat)* 31(2):115–124. <https://doi.org/10.2307/2347973>
- Ryan PG, Ortmann HE, Herian K (2014) Cascading effects of introduced scale insects on *Nesospiza* finches at the Tristan da Cunha archipelago. *Biol Conserv* 176:48–53. <https://doi.org/10.1016/j.biocon.2014.05.020>
- Samarakoon MC, Hyde KD, Maharachchikumbura SSN, Stadler M, Gareth Jones EB, Promputtha I, Suwannarach N, Camporesi E, Bulgakov TS, Liu J-K (2022) Taxonomy, phylogeny, molecular dating and ancestral state reconstruction of *Xylariomycetidae* (*Sordariomycetes*). *Fungal Divers* 112(1):1–88. <https://doi.org/10.1007/s13225-021-00495-5>
- Scali E, Rocca GD, Danti R, Garbelotto M, Barberini S, Baroncelli R, Emiliani G (2024a) Sequence and assembly of the genome of *Seiridium unicornis*, isolate CBS 538.82, causal agent of cypress canker disease. *J Plant Pathol* 106(3):1385–1388. <https://doi.org/10.1007/s42161-024-01633-z>
- Scali E, Rocca GD, Danti R, Garbelotto M, Barberini S, Emiliani G (2024b) Genome assembly of two California isolates of *Seiridium cardinale* (BM-138-000494 and BM-138-000479). *J Plant Pathol* 106(3):1389–1392. <https://doi.org/10.1007/s42161-024-01665-5>
- Scali E, Rocca GD, Danti R, Garbelotto M, Barberini S, Frascella A, Emiliani G (2024c) Genome sequence and assembly of the causal agent of cypress canker disease *Seiridium cupressi*, isolates BM-138-000234 and BM-138-000515. *J Plant Pathol* 106(1):267–270. <https://doi.org/10.1007/s42161-023-01545-4>
- Sutton BC (1975) *Coelomycetes. V. Coryneum*. Mycological Papers, vol 138. Commonwealth Mycological Institute, Kew, Surrey, England
- Van der Werff HS (1988) Cypress canker in New Zealand plantations. *NZ J Forest Sci* 18(1):101–108
- Warnes G, Bolker B, Bonebakker L, Gentleman R, Huber W, Liaw A, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, Venables B (2024) gplots: various R programming tools for plotting data. R package version 3.1.3.1. <https://CRAN.R-project.org/package=gplots>.
- Waterhouse RM, Seppey M, Simão FA, Manni M, Ioannidis P, Kliuchnikov G, Kriventseva EV, Zdobnov EM (2017) BUSCO applications from quality assessments to gene prediction and phylogenomics. *Mol Biol Evol* 35(3):543–548. <https://doi.org/10.1093/molbev/msx319>
- Wickham H (2016) *Ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York
- Wingfield MJ, Pham NQ, Marincowitz S, Duong TA, Wingfield BD, Wilson AM (2024) Blast from the past: a study of decades-old fungal cultures resolves a long-standing tree disease mystery. *J Plant Pathol* 106(2):377–384. <https://doi.org/10.1007/s42161-023-01502-1>
- Wong TKF, Ly-Trong N, Ren H, Baños H, Roger AJ, Susko E, Bielow C, De Maio N, Goldman N, Hahn MW (2025) IQ-TREE 3: phylogenomic inference software using complex evolutionary models. *EcoEvoRxiv*. <https://doi.org/10.32942/X2P62N>
- Yoon S-H, Ha S-m, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* 110(10):1281–1286. <https://doi.org/10.1007/s10482-017-0844-4>
- Yuan ZQ, Mohammed C (1997) *Seiridium papillatum*, a new species (mitosporic fungus) described on stems of eucalypts in Australia. *Aust Syst Bot* 10(1):69–75. <https://doi.org/10.1071/SB96008>

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