FUSE

Fungal Systematics and Evolution

VOLUME 16
DECEMBER 2025
PAGES 71–80

doi.org/10.3114/fuse.2025.16.5

Microfungi associated with dying quiver trees (*Aloidendron dichotomum*) in South Africa

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Key words:
Aloe
Botryosphaeriaceae
new taxa
Thermoascaceae
Togniniaceae

Abstract: Quiver trees *(Aloidendron dichotomum)* are large iconic succulent plants found in arid areas of southern Africa. These trees have been observed suffering from die-back symptoms for many years. Various environmental and abiotic factors have been investigated as possible causes of the symptoms. However, biotic causes, especially microfungi that commonly cause die-backs in trees, have never been considered. During a routine disease survey, symptomatic stems and roots of the dying trees were collected in the Cape Province, South Africa. Isolations were made from tissues at the leading edges of the lesions on symptomatic stems and roots, and the resulting fungi were identified using morphological characteristics and DNA sequence data of four loci (LSU, SSU, ITS and β-tubulin). Five species were identified: *Paecilomyces formosus*, *Phaeoacremonium* (*Pm.*) *parasiticum*, *Pm. luteum*, *Xylogone sphaerospora*, and the newly described *Coniophoma aloidendri gen. et sp. nov*. Three species, *Pm. parasiticum* and *C. aloidendri* from this study and *Alanphillipsia* (*Ala.*) *aloes* were tested for their pathogenicity on *A. dichotomum* plants in a greenhouse trial. All three species gave rise to lesions significantly different in size from the controls. The *Pm. parasiticum* strains showed larger necrotic lesions than *C. aloidendri* and *Ala. aloes*. However, none of the isolated fungi were aggressive or are known as primary pathogens, and the cause of the die-back on symptomatic trees remains to be determined.

Citation: Marincowitz S, Pham NQ, Wingfield BD, Wingfield MJ (2025). Microfungi associated with dying quiver trees (*Aloidendron dichotomum*) in South Africa. *Fungal Systematics and Evolution* **16**: 71–80. doi: 10.3114/fuse.2025.16.5.
Received: 13 January 2025; **Accepted:** 26 March 2025; **Effectively published online:** 21 May 2025
Corresponding editor: P.W. Crous

INTRODUCTION

Aloidendron dichotomum, also known as the quiver tree, is one of five indigenous *Aloidendron* spp. found in southern Africa. *Aloidendron* (*Asphodelaceae*) was segregated from the larger genus *Aloe* to accommodate the tree aloe species (Grace *et al.* 2013, Smith *et al.* 2019). Quiver trees are commonly featured in iconic scenes of southern Africa as giant succulent trees against the backdrop of rocky and arid landscapes (Fig. 1). They are mainly found in the Northern Cape region of South Africa and parts of southern Namibia (Smith & Van Wyk 2008) (Fig. 2). They grow rapidly for the first 50 years, reaching up to nine meters in height under optimal conditions, then mature for another 150 years without a considerable increase in volume, followed by 50 years of senescence (Kaleme 2003). A high moisture content in their stems and leaves can support various mammals, birds and insects (Midgley *et al.* 1997). *Aloidendron dichotomum* is currently listed as 'vulnerable' in the IUCN Red List of Threatened Species (Foden *et al.* 2022).

The decline of quiver trees has been observed in their natural habitat for decades, and various investigations have been conducted to determine its cause. Global warming, geographical effects of climate change, and various emission scenarios suggest climate change as the most probable explanation for the decline (Foden 2002, Foden *et al.* 2007, 2022, Guo *et al.* 2011, Van der Merwe & Geldenhuys 2017), although there is some debate regarding this explanation (Jack *et al.* 2016). The possible role of fungal pathogens in the mortality of the *A. dichotomum* population has been considered, but without any clear result (Foden *et al.* 2007).

Roux *et al.* (2009) surveyed more than 300 symptomatic *A. dichotomum* trees in Goegap Nature Reserve (Northern Cape Province), specifically considering the possibility of a fungal disease. They found high levels of insect infestation and animal damage but no evidence of biotic pathogens. Various microfungi including *Alanphillipsia aloes*, *Dothiora aloidendri*, *Fusarium pharetrum*, *Hantamomyces aloidendri*, *Lapidomyces aloidendricola*, *Neophaeococcomyces aloes*, *Neoscytalidium dimidiatum*, *Phoma aloes* and *Staurosphaeria aloe* have been isolated from dying quiver trees in taxonomic studies (Crous *et al.* 2006, 2013, 2015, 2020, 2021, Lombard *et al.* 2019), but these have not been tested for their pathogenicity. During a routine disease survey in the areas where *A. dichotomum* grows naturally, many dying trees were found with lesions that might be attributed to the disease. The aim of this study was thus to isolate fungi from these lesions and to conduct pathogenicity tests with those most commonly isolated.

MATERIALS AND METHODS

Fungal isolation from diseased tissues

Stems and roots with necrotic lesions were collected from six dying *A. dichotomum* trees near Nieuwoudtville, Cape Province, South Africa, in 2020 (Fig. 2). The samples were retained in paper bags under refrigerated conditions and transferred to the laboratory for further investigation. Microscopes (Nikon SMZ 18 or Nikon Eclipse N*i*, Tokyo, Japan) were used to examine the samples for visible fungal structures on the symptomatic tissues. Small pieces of tissue (less than 3 mm in diam.) were cut from the interface between the lesions and healthy tissues and placed on 2 % malt extract agar (MEA: 20 g Biolab malt extract, 20 g Difco agar, 1 L water) supplemented with streptomycin (100 mg/L). The isolates were incubated at room temperature until fungal growth developed.

Fungal hyphae growing from the diseased tissue pieces were transferred onto fresh 2 % MEA to obtain pure cultures. These cultures were then incubated at 23 °C in the dark until they could be grouped based on colony characteristics. One or two isolates representing each fungal morphotype were selected for identification based on DNA sequence data.

For morphological identification of the putative new taxon, isolates were grown on water agar (WA: 20 g Difco agar, 1 L water) with sterilised toothpicks placed on the agar surface to induce the production of fruiting structures. Fruiting structures were mounted on microscope slides, and up to fifty measurements were made for characteristic structures. Dimensions were recorded as minimum–maximum (average ± standard deviation). Images were captured using Nikon DS-Ri2 cameras mounted on the microscopes. All the measurements and image-taking were done using an imaging program (NIS Elements, Tokyo, Japan).

The optimum temperature for growth and culture characteristics for the putative new taxon were studied on 2 % MEA. Plugs (5 mm diam.) of actively growing mycelium were placed at the centres of 90-mm-diam. Petri dishes containing MEA. The Petri dishes were incubated at six temperatures, 10–35 °C at 5° intervals, with five replicates at each temperature. Two colony diameters perpendicular to each other were recorded after 15 d, and the averages were used to calculate the growth rate. Colours were named after the ISCC-NBS system. Cultures were deposited in the culture collection (CMW) at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The ex-type culture of the new taxon was deposited

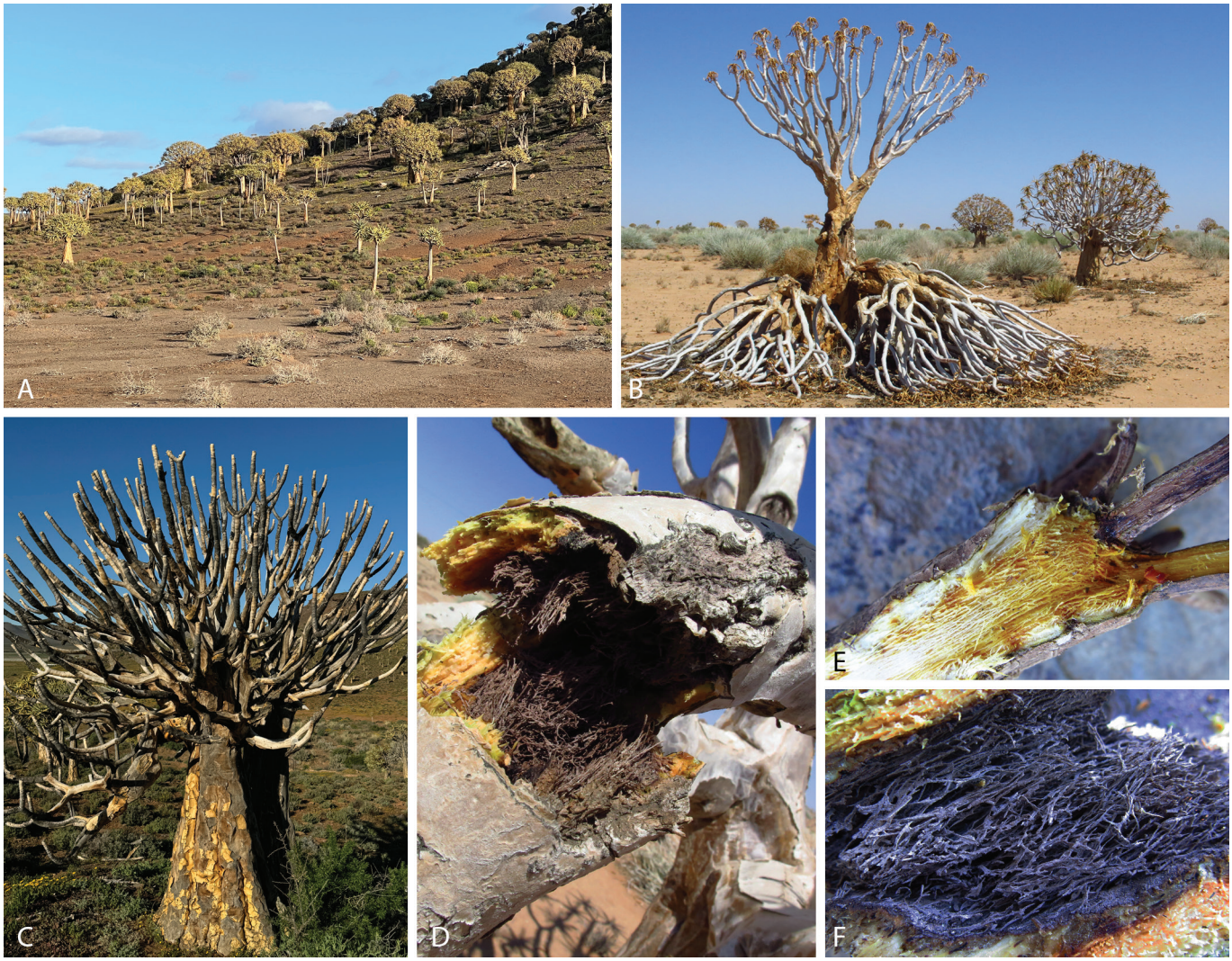

Fig. 1. Quiver trees, *Aloidendron dichotomum*, in the field. **A.** Healthy trees and trees showing die-back symptoms dispersed in a natural habitat. **B, C.** Trees with die-back symptoms. **D–F.** Inside symptomatic stems.

in the culture collection of Innovation Africa (CMW-IA) based at the University of Pretoria (https://fabi.up.ac.za), and herbarium specimens were deposited in the H.G.W.J. Schweickerdt herbarium (PRU), University of Pretoria, South Africa.

Sequencing and phylogenetic analyses

Fungal strains were grown on 2 % MEA for 7 d at 25 °C and subsequently used for DNA extraction. Mycelium was gently removed from the medium surface using sterile needles and transferred to 1.5 mL Eppendorf tubes. DNA extraction was performed using the Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommended protocols. The internal transcribed spacer (ITS) 1 and 2, including the 5.8S rRNA region, was amplified using primers ITS1F and ITS4 (White *et al.* 1990, Gardes & Bruns 1993); the nuclear large subunit (LSU) of rRNA with primers LR0R and LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994); and the nuclear small subunit (SSU) of rRNA with primers NS1 and NS4 (White *et al.* 1990). Part of the β-tubulin (*TUB2*) gene region was amplified using primers BT2a and BT2b (Glass & Donaldson 1995) as an additional barcoding gene. PCR reactions were conducted utilising an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The amplified fragments were cleaned using the ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions using an ABI PRISM™ 3100 DNA sequencer (Applied Biosystems, USA) at the sequencing facility of the Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa.

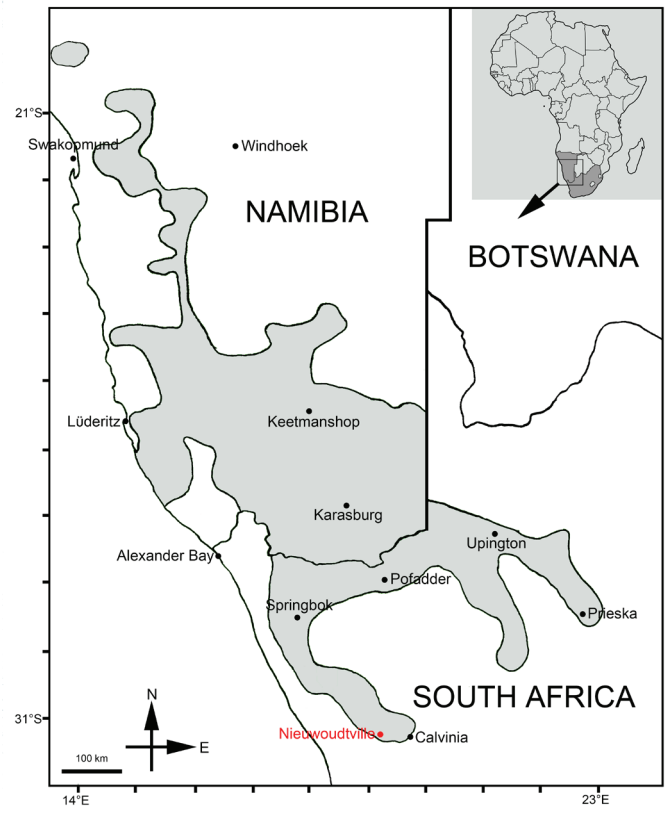

Fig. 2. Distribution and natural habitats of *Aloidendron dichotomum* (shaded) and the location where the samples were collected (Nieuwoudtville, red dot) (map adapted from Jack *et al.* 2016).

Geneious Prime v. 2022.1.1 (https://www.geneious.com) was utilised to assemble and edit the raw sequences. All the sequences generated in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov) (Table S1).

Preliminary identification was conducted using ITS sequences to perform a nucleotide BLAST search against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov). This information was used to develop datasets for further phylogenetic analysis. Sequences of species related to this study were retrieved from GenBank (Table S1). The sequences were aligned using MAFFT v. 7 (Katoh & Standley 2013) and manually inspected using MEGA v. 7 (Kumar *et al.* 2016). The appropriate model was determined using jModeltest v. 1.2.5 (Posada 2008). Bayesian inference (BI) analysis was performed using MrBayes v. 3.2.6 (Ronquist *et al.* 2012) on the CIPRES Science Gateway v. 3.3. Four Markov chain Monte Carlo (MCMC) chains were initiated from a random starting tree for five million generations, and trees were sampled every 100th generation. The initial 25 % of sampled trees were eliminated as burn-in, and the remaining trees were used to determine the posterior probabilities. Maximum-likelihood (ML) analysis was conducted using RAxML v. 8.2.4 (Stamatakis 2014) on the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010), with the default GTR substitution matrix and 1000 rapid bootstraps. The final consensus trees were viewed utilising MEGA v. 7 (Kumar *et al.* 2016).

Pathogenicity trials

Three species, each including two strains, were selected for a pathogenicity test on *A. dichotomum* plants: Two of the species, *Phaeoacremonium parasiticum* (CMW 56378, 56380) and the new taxon (CMW 56381, 56376), were from the present study, and the third species, *Alanphillipsia aloe* (CMW 56344, 56346), previously isolated from dying *Euphorbia mauritanica* in 2020 (Marincowitz *et al.* 2023), was retrieved from the culture collection (CMW).

Toothpicks were submerged in 2 % malt broth and autoclaved twice at a two-day interval. They were then placed on the surface of half-strength potato dextrose agar (PDA: 19.5 g Difco Potato Dextrose Agar, 1 L water). Discs of the fungal isolates to serve as inoculum were placed alongside the toothpicks and allowed to grow and penetrate the wood tissue.

Well-established young *A. dichotomum* plants with approximately five vertical rows of leaves were maintained in a greenhouse at 25 °C for 3 mo before inoculation. Six isolates, three of each test species and a negative control, were inoculated on the stems of 15 plants. Colonised or sterile (control) toothpicks were inserted into the stems of the plants to reach approximately the mid-point of the stem tissue. The inoculated plants were watered regularly and monitored for the development of disease symptoms.

Eight weeks after inoculation, the treated plants were split in half to expose the entire length of the toothpick inoculum. The toothpicks were removed, and the areas of discoloured tissue were traced with a marker using a transparent plastic sheet. The trial was conducted only once, as it was difficult to obtain plants that are not commonly propagated commercially. The traces of the infected areas were scanned using a scanner (Epson Perfection V700 photo, China) and measured using Adobe Photoshop 2021 (Adobe

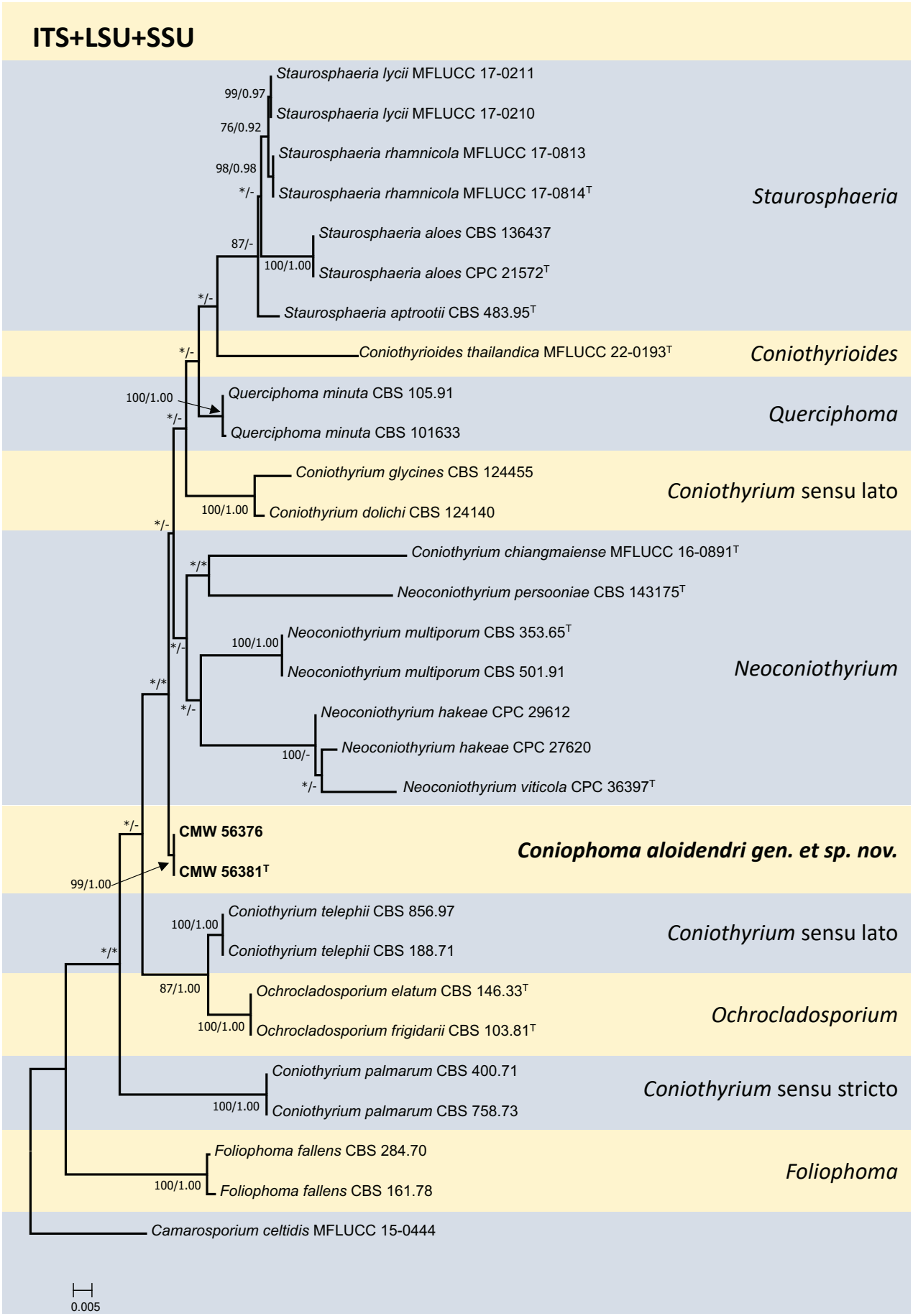

Fig. 3. Phylogenetic tree based on a maximum likelihood (ML) analysis of a combined DNA data set of ITS, LSU and SSU sequences for *Coniothyriaceae* species. The isolates sequenced in this study are presented in **boldface**. Bootstrap support values ≥ 70 % for ML analyses and posterior probabilities values ≥ 0.9 obtained from Bayesian inference (BI) are indicated at the nodes as ML/BI. Bootstrap support values < 70 % or probability values < 0.9 are marked with "*", and nodes lacking support values are marked with "–". Isolates representing ex-type material are marked with "T". *Camarosporium celtidis* (MFLUCC 15-0444*)* represents the outgroup.

Systems Incorporated). The data for the diseased areas were analysed statistically using R software v. 4.2.1 (R Core Team 2020) by performing a One-way analysis of variance test and Games-Howell post hoc test for pairwise comparisons. To meet Koch's postulates, small pieces of tissue surrounding the toothpicks were then placed on 2 % MEA to allow for re-isolation of the inoculated fungi. A subset of these isolates was identified based on sequences of the ITS region.

RESULTS

Isolations from diseased tissues

Seventeen fungal strains were isolated from diseased stems and roots of dying quiver trees, with five originating from root samples and 12 from stems. The fungal strains were grown from the small pieces of tissue cut from the border of the lesions and healthy tissues. They were classified into five groups based on colony morphology. No visible fungal fruiting structures were found on the lesions of diseased plant parts.

Sequencing and phylogenetic analyses

Representative strains from each colony morph were sequenced and identified into five species based on BLAST results of ITS and/or *TUB2* sequence data (Supplementary data S1). The collection comprised two strains of *Paecilomyces formosus* (represented by CMW 56375; ITS PV405791, *TUB2* PV420379) from stems, five strains of *Phaeoacremonium parasiticum* (CMW 56380, 56382, 56378, 56384; ITS PV405792, *TUB2* PV420380) from roots and stems, two strains of *Pm. luteum* (CMW 56379; ITS PV405793, *TUB2* PV420381) from stems, and four strains of *Xylogone sphaerospora* (CMW 56383, 56385; ITS PV405794) from roots.

Two strains, CMW 56376 and CMW 56381, represented the fifth colony morph. They included two isolates clustered within the *Coniothyriaceae*, apart from other taxa, based on initial BLAST searches of the ITS sequences. The ML and BI analyses were performed based on the concatenated sequences of three loci (ITS, LSU and SSU). This concatenated dataset consisted of 29 ingroup taxa and 2362 characters, including alignment gaps. *Camarosporium celtidis* (MFLUCC 15-0444) was used as the outgroup taxon. Concatenated sequence alignments were deposited in Figshare (doi: 10.6084/m9.figshare.28532144). The TIM2+I+G models were selected for the ITS and LSU region, respectively, and TPM3uf+I for the SSU region. The ML and BI analyses generated phylogenetic trees with concordant topologies, revealing similar phylogenetic relationships among taxa. The ML tree with bootstrap support values for the ML and the posterior probabilities obtained from the BI is presented in Fig. 3. The two strains clustering in *Coniothyriaceae* represented a new lineage (ML/BI = 99/1.00), introduced here as a novel genus in *Coniothyriaceae*.

Taxonomy

Coniophoma N.Q. Pham, Marinc. & M.J. Wingf. ***gen. nov.*** MB 857753.

Etymology: The name 'Conio' is derived from its taxonomic affinity to the *Coniothyriaceae* and '*phoma*' from its morphological similarity to *Phoma*.

Conidiomata pycnidial, sub-globose to globose, brown. *Conidiophores* blastic, lining along peridial surface, simple, reduced to conidiogenous cells. *Conidiogenous cells* ampulliform. *Conidia* hyaline, oval to oblong, aseptate. *Sexual morph* not observed.

Type species: *Coniophoma aloidendri* N.Q. Pham, Marinc. & M.J. Wingf.

Coniophoma aloidendri N.Q. Pham, Marinc. & M.J. Wingf., ***sp. nov.*** MB 857754. Fig. 4.

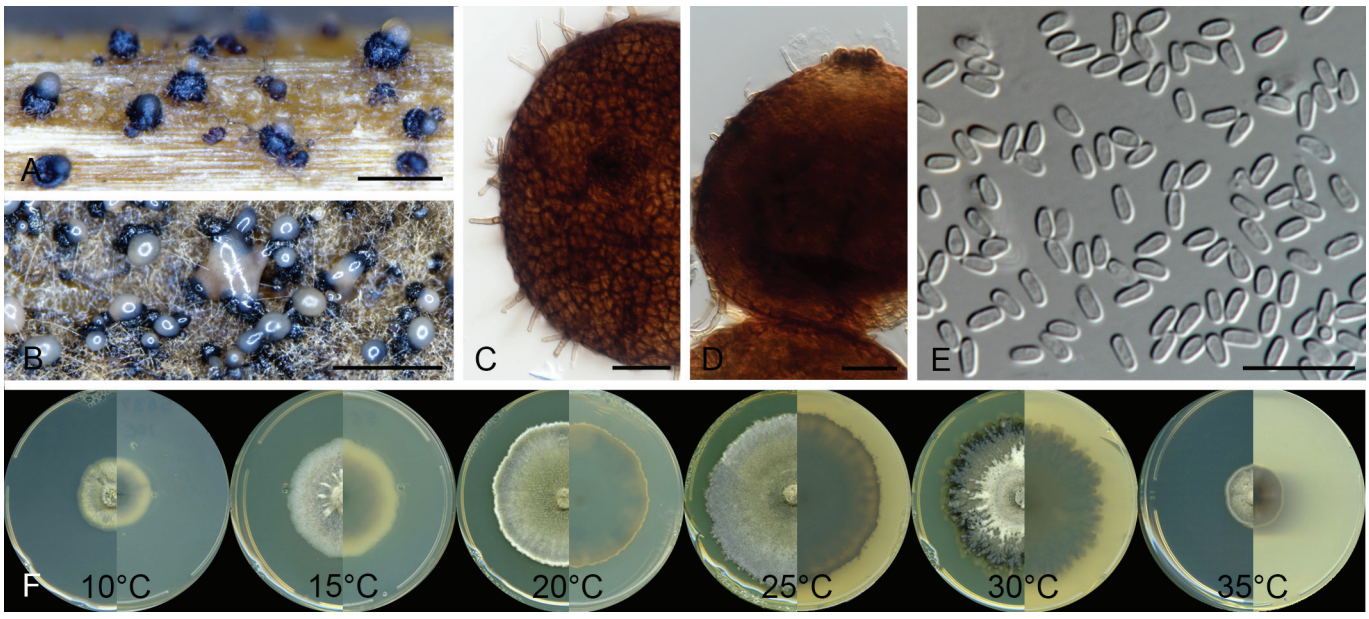

Fig. 4. Micrograph of *Coniophoma aloidendri gen. et sp. nov.* (CMW-IA 48, CMW 56381). **A, B.** Pycnidia formed on a toothpick (A) and WA (B). **C.** Pycnidium covered with hyphae. **D.** Pycnidium with prominent ostiole. **E.** Conidia. **F.** Cultures on 2 % MEA kept in the dark for 29 d (left: above, right: reverse). Scale bars: A = 250 µm; B = 500 µm; C, D = 25 µm; E = 10 µm.

Etymology: The name refers to *Aloidendron*, the host on which it occurs.

Diagnosis: Closely related to *Neoconiothyrium* but differs in having aseptate, hyaline conidia.

Typus: **South Africa**, Northern Cape Province, Nieuwoudtville, isolated from stem tissue of dying *Aloidendron dichotomum*, Aug. 2020. *M.J. Wingfield* [**holotype** PRU(M) 4619, dried culture; ex-holotype culture CMW-IA 48 = CMW 56381]. GenBank: ITS = PV405790; LSU = PV405796; SSU = PV405798.

Conidiomata formed on toothpicks or WA, pycnidial, sub-globose to globose, papillate, 69–183 × 66–186 (132.5 ± 30.44 × 121.6 ± 30.24) µm, pycnidial walls pseudoparenchymatous. *Conidiophores* lining along inside peridial walls, reduced to conidiogenous cells. *Conidiogenous cells* hyaline, ampulliform, 4–5 × 2.5–3.5 µm. *Conidia* hyaline, oval to oblong, mostly straight, aseptate, at times tapering to base and upper region swollen, exudating conidial mass creamy, 3–4 × 1–2 (3.3 ± 0.32 × 1.5 ± 0.15) µm.

Culture characteristics: Optimum growth temperature on 2 % MEA in the dark at 25 °C showing 3.1 mm/d, followed by 30 °C (2.4 mm/d), 20 °C (2.3 mm/d), 15 °C (1.41 mm/d) and 10 °C and 35 °C (0.6 mm/d). Cultures (29-d-old) sterile: *shape* circular (10–35 °C); *margins* entire (10–20, 35 °C), erose (20–30 °C); *elevation* flat (10–35 °C); *texture* velvety (10–35 °C); *colour* above yellowish white, yellowish grey with medium grey patches (10 °C), yellowish grey background with dark greyish reddish brown radial patches (15 °C), light yellow background with olive grey centre and purplish grey patches (20 °C), moderate yellow, brownish grey or olive grey in concentric areas (25 °C), dark reddish brown background with moderate yellow centre and pale yellow edges (30 °C), moderate yellow to olive grey or dark reddish brown (35 °C); *density* medium dense (10–30 °C), dense (35 °C); *pigmentation* on media absent.

Additional materials examined: **South Africa**, Northern Cape Province, Nieuwoudtville, isolated from stem tissue of dying *Aloidendron dichotomum,* Aug. 2020, *M.J. Wingfield*, fungarium PRU(M) 4617; culture CMW-IA 47 = CMW 56376. GenBank: ITS = PV405789; LSU = PV405795; SSU = PV405797.; ibid., fungarium PRU(M) 4618; culture CMW-IA 2602 = CMW 56377.

Notes: The strains of *Coniophoma aloidendri* formed a distinct lineage within a clade containing members of the *Coniothyriaceae*, i.e. coelomycete genera such as *Coniothyrium* s. str., *Coniothyrium* s. lat., *Coniothyrioides*, *Foliophoma*, *Neoconiothyrium*, *Querciphoma*, *Staurosphaeria*, and a hyphomycete genus *Ochrocladosporium.* Other than *Staurosphaeria*, only asexual morphs are known for these genera. High morphological variability in the family makes morphological delineation of genera difficult. However, *Coniophoma* can still be distinguished from other genera by its colourless aseptate conidia on simple blastic conidiogenous cells. In contrast, *Coniothyrioides* produces ellipsoidal to ovoid conidia that become pale to dark brown at maturity (Wijesinghe *et al.* 2023), *Coniothyrium* s. str. has brown conidia, *Neoconiothyrium* hyaline to medium brown conidia and ellipsoidal to sub-clavate conidia (Crous *et al.* 2017). *Querciphoma* has broadly ellipsoid conidia turning into brown with maturity (Crous & Kirk 2017), *Ochrocladosporium* has pale brown conidia and ramoconidia and is hyphomycetous (Crous *et al.* 2007), *Staurosphaeria* has red-brown macroconidia and hyaline globose to ellipsoidal microconidia (Wanasinghe *et al.* 2017), and *Foliophoma* has hyaline, aseptate, broadly ellipsoidal conidia produced in eustromatic conidiomata (Crous & Groenewald 2017). The members of *Coniothyrium* s. lat. such as *C. glycines*, *C. dolichi* and *C. telephii* are known to produce dark pycnidial setae (De Gruyter *et al.* 2013).

Pathogenicity

Eight weeks after inoculation, the *A. dichotomum* plants inoculated with six fungal strains of three fungi, *Ala. aloes*, *C. aloidendri* and *Pm. parasiticum*, showed distinct lesions on their stems. All the infected stems developed sepia to dark brown lesions around the inoculation points. Stems inoculated with clean toothpicks were used as controls and produced no lesions. Mean lesion areas caused by all six

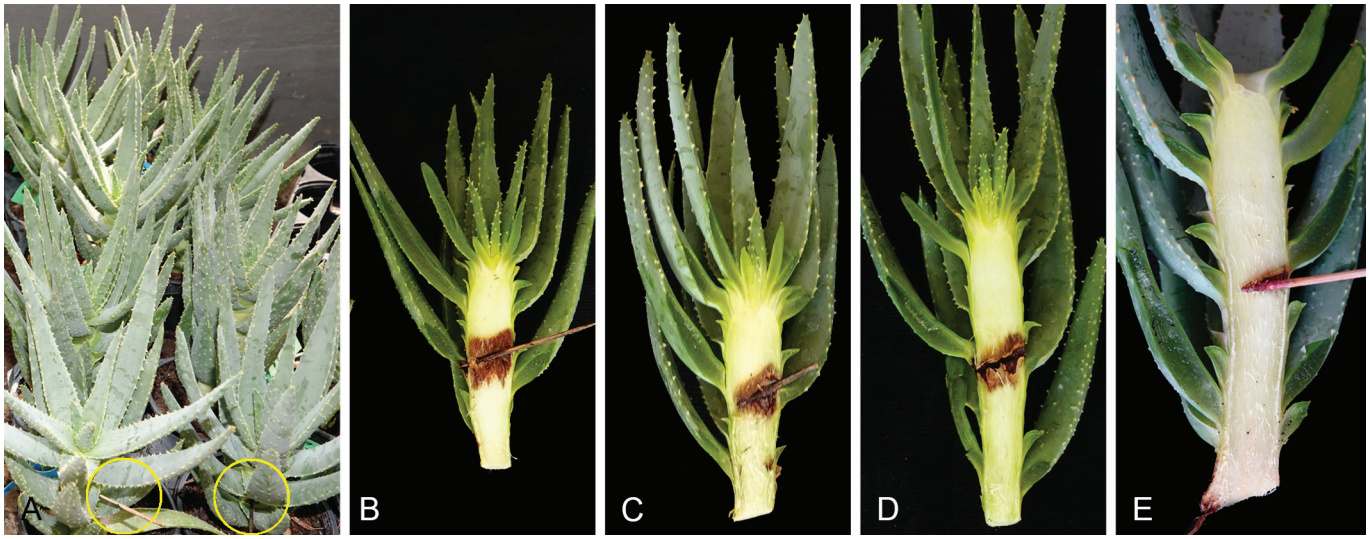

Fig. 5. Pathogenicity test on *Aloidendron dicotomum*. **A.** Seedlings inoculated with toothpick cultures (yellow circles). **B–D.** Lesions developed in 8-wk-old inoculated plants. **B.** *Phaeoacremonium parasiticum* (CMW 56378). **C.** *Coniophoma aloidendri* (CMW-IA 48, CMW 56381). **D.** *Alanphillipsia aloes* (CMW 56344). **E.** Control.

strains were significantly larger than the controls ($p < 0.05$) (Figs 5, 6). Two strains of *Pm. parasiticum* produced lesions significantly larger than those associated with *Ala. aloes* ($p < 0.05$) and *C. aloidendri* (Fig. 6). The inoculated fungi were easily re-isolated from the resulting lesions but never from the controls.

DISCUSSION

Five species of fungi were isolated from the edges of necrotic lesions of the stems and roots of dying *A. dichotomum* trees in this study. Among these, four species, *Paecilomyces formosus*, *Phaeoacremonium parasiticum*, *Pm. luteum* and *Xylogone sphaerospora* are reported from this tree for the first time. A fifth fungus represented a new species in a previously undescribed genus, described here as *Coniophoma aloidendri*. This study has increased the number of fungi known from *A. dichotomum* to fourteen. Nine previously reported species from these trees include *Alanphillipsia aloes* (*Botryosphaeriales*, Crous *et al.* 2013), *Dothiora aloidendri* (*Dothiorales*, Crous *et al.* 2020), *Hantamomyces aloidendri* (*Hypocreales*, Crous *et al.* 2020), *Lapidomyces aloidendricola* (*Capnodiales*, Crous *et al.* 2021), *Fusarium pharetrum* (*Hypocreales*, Lombard *et al.* 2019), *Neophaeococcomyces aloes* (*Chaetothyriales*, Crous *et al.* 2013, 2015), *Phoma aloes* (*Pleosporales*, Crous *et al.* 2013), *Staurosphaeria aloes* (*Pleosporales*, Crous *et al.* 2013), and *Neoscytalidium dimidiatum* (*Botryosphaeriales*, Crous *et al.* 2006).

Paecilomyces formosus was isolated less frequently than other species in this study and consequently excluded from the pathogenicity test. This is a common fungus known from various environmental samples, including soil, indoor air, and house dust (Samson 1974, Samson *et al.* 2004). It is also known to infect patients with underlying conditions (Sprute *et al.* 2021) and to cause diseases in woody plants, as well as post-harvest rot (Biango-Daniels & Hodge 2018). More recently, *P. formosus* has been reported in Iran, causing die-back of pistachio (*Pistacia vera*) in orchards (Heidarian *et al.* 2018, Sabernasab *et al.* 2019) and as a common endophyte on pistachio trees (Kavehnia *et al.* 2023). *Paecilomyces formosus* was initially considered a member of a species complex comprising three cryptic species (Samson *et al.* 2009, Heidarian *et al.* 2018), which has recently been resolved using whole-genome sequences (Urquhart & Idnurm 2023). The present study adds an unusual habitat for this fungus in South Africa. If it is isolated more consistently from *A. dichotomum* in the future, it would be useful to consider its pathogenicity on these trees.

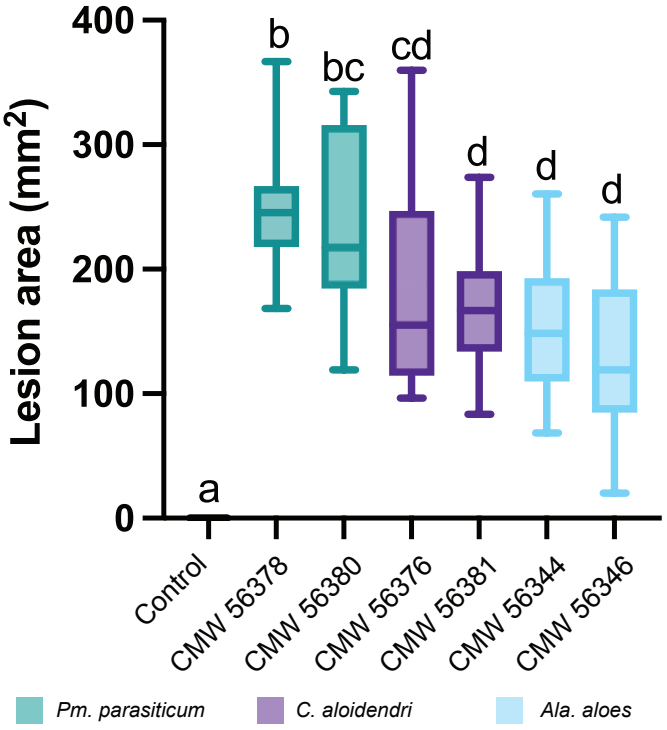

Fig. 6. Boxplots indicating lesion area resulting from the pathogenicity test of *Aloidendron dichotomum* inoculated with six different isolates and the control. Vertical bars represent the standard error of the means. Bars with different letters indicate statistical significance at $P \leq 0.05$.

Two *Phaeoacremonium* species, *Pm. parasiticum* and *Pm. luteum* were isolated in this study. *Phaeoacremonium parasiticum* was isolated from both stem and root samples more frequently than *Pm. luteum*, which was isolated only from stem tissues. *Phaeoacremonium* species occur on diverse substrates, including human tissue, woody plants, soil, and bark beetles (Crous *et al.* 2006, Gramaje *et al.* 2014). Several *Phaeoacremonium* species contribute to the notorious Petri and esca diseases of grapevines (Crous *et al.* 1996, Mostert *et al.* 2006) or cause subcutaneous phaeohyphomycosis in immunocompromised individuals (Mostert *et al.* 2005). Sixteen *Phaeoacremonium* species have been recorded from woody plants in South Africa, the best-known of which is *Pm. parasiticum* that has been isolated from necrotic wood tissue of apricot trees (*Prunus armeniaca*) and grapevines (*Vitis vinifera*) (Mostert *et al.* 2006, Damm *et al.* 2008, Cloete *et al.* 2011). *Phaeoacremonium parasiticum* strains isolated in this study gave rise to larger lesions, and it could be considered a possible candidate contributing to the decline of these trees.

Phaeoacremonium luteum was first reported from pruning wounds of tropical sandalwood (*Santalum album*) in Australia and was amongst five other species of the same genus isolated from wounds (Gramaje *et al.* 2014). Its name was derived from the yellow pigmentation on the media, which was absent in our strains. This is the first report of the fungus from South Africa.

Xylogone sphaerospora was frequently isolated in this study, occurring only on root tissues. The genus *Xylogone* belongs to the *Leotiomycetes* and is one of only two species in the genus. *Xylogone sphaerospora*, the type species, was initially reported from stored pulpwood chips in Sweden (von Arx & Nilsson 1969). The second species, *X. ganodermophthora,* was described from the basidiomycete fungus *Ganoderma lucidum* cultivated in Korea, which causes destructive yellow rot on the basidiocarps and associated wood logs (Kang *et al.* 2010). This is the first report of the fungus from the Southern Hemisphere.

Alanphillipsia aloes was first isolated from *A. dichotomum* in 2012 (Crous *et al.* 2013) and was recently re-isolated from *Euphorbia mauritanica* (*Euphorbiaceae*) in 2020 (Marincowitz *et al.* 2023). Both isolations were from necrotic lesions on the host plants. The genus was established to accommodate *Ala. aloes* (Crous *et al.* 2013), and five species have been added to the genus from *Aloidendron* and *Euphorbia* spp. in South Africa since its introduction (Crous

et al. 2013, 2014). Botryosphaeriaceous fungi are known to exist asymptomatically in host plants and cause diseases in the presence of environmental or biological stress (Slippers & Wingfield 2007, Mehl *et al.* 2013). The fungus was included in this study due to its previous occurrence on *A. dichotomum* and its association with disease in these and other plants. Interestingly, this was the least pathogenic of the three fungal species tested in this study. Its role in causing disease on *A. dichotomum* appears to be minimal, but under stress conditions, it could contribute to decline, consistent with its known opportunistic biology.

Quiver trees such as *A. dichotomum* have a long lifespan and occupy large habitats, extending over approximately 200,000 km^2 in the arid western regions of the Northern Cape in South Africa and south-central Namibia (Smith & Van Wyk 2008). Our samples were collected from a limited number of dying trees at one location. The results must be viewed as preliminary and are insufficient to attribute the death of *A. dichotomum* to the isolated fungi. However, they provide some insight into the role that fungi may play in the decline of these trees and suggest that a single plant pathogen is unlikely to be the sole cause. Based on this preliminary evidence and the earlier survey by Roux *et al.* (2009), we support the view raised in various previous studies (Foden *et al.* 2007, Guo *et al.* 2011, Jack *et al.* 2016, Van der Merwe & Geldenhuys 2017) that abiotic stress is likely an important factor to consider in the decline of these trees. Nonetheless, further studies on fungi and their potential role in contributing to this problem would be worthwhile.

ACKNOWLEDGEMENTS

We acknowledge funding from the DSI/NRF Chair in Fungal Genomics held by BDW and the Harry Oppenheimer Fellowship Award of the Oppenheimer Memorial Trust to MJW. We are also grateful to Dr Jason Samson for assistance in locating *A. dichotomum* plants used in the pathogenicity trials and Dr Konstanze Bensch at MycoBank for help naming the new taxon.

Conflict of interest: The authors declare that there is no conflict of interest.

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Supplementary Material: http://fuse-journal.org/

Data S1. Blast results of the different species treated in this study.

Table S1. Meta data and GenBank accession numbers of the strains used in the phylogenetic analyses.