



# Dieback and decline pathogens of olive trees in South Africa

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## Key words

*Celerioriella*  
five new taxa  
*Olea europaea* subsp. *cuspidata*  
*Olea europaea* subsp. *europaea*  
phylogenetics  
*Pseudophaeomoniella*  
taxonomy  
*Vredendaliella*  
*Xenocylindrosporium*

**Abstract** Trunk disease fungal pathogens reduce olive production globally by causing cankers, dieback, and other decline-related symptoms on olive trees. Very few fungi have been reported in association with olive dieback and decline in South Africa. Many of the fungal species reported from symptomatic olive trees in other countries have broad host ranges and are known to occur on other woody host plants in the Western Cape province, the main olive production region of South Africa. This survey investigated the diversity of fungi and symptoms associated with olive dieback and decline in South Africa. Isolations were made from internal wood symptoms of 145 European and 42 wild olive trees sampled in 10 and 9 districts, respectively. A total of 99 taxa were identified among 440 fungal isolates using combinations of morphological and molecular techniques. A new species of *Pseudophaeomoniella*, *P. globosa*, had the highest incidence, being recovered from 42.8 % of European and 54.8 % of wild olive samples. This species was recovered from 9 of the 10 districts where European olive trees were sampled and from all districts where wild olive trees were sampled. Members of the *Phaeomoniellales* (mainly *P. globosa*) were the most prevalent fungi in five of the seven symptom types considered, the only exceptions being twig dieback, where members of the *Botryosphaeriaceae* were more common, and soft/white rot where only *Basidiomycota* were recovered. Several of the species identified are known as pathogens of olives or other woody crops either in South Africa or elsewhere in the world, including species of *Neofusicoccum*, *Phaeoacremonium*, and *Pleurostoma richardsiae*. However, 81 of the 99 taxa identified have not previously been recorded on olive trees and have unknown interactions with this host. These taxa include one new genus and several putative new species, of which four are formally described as *Celerioriella umnquma* sp. nov., *Pseudophaeomoniella globosa* sp. nov., *Vredendaliella oleae* gen. & sp. nov., and *Xenocylindrosporium margaritarum* sp. nov.

**Article info** Received: 2 June 2020; Accepted: 3 October 2020; Published: 29 October 2020.

## INTRODUCTION

The first record of European olive (*Olea europaea* subsp. *europaea*) in South Africa dates back to Jan van Riebeeck in 1661. The first commercial olive farm was established in Paarl in 1925; however, initial expansion of the olive industry only occurred in the 1970s (Costa 1998). Although the olive industry in South Africa is still relatively small, a rapid expansion occurred during the last 11 yr with a 135 % increase in the area planted to the current 3 190 ha. Frantoio is the most frequently planted oil cultivar, accounting for 849 ha of the production area, while Mission is the most frequently planted dual cultivar (oil and table olives; 643 ha of the production area). Due to the recent growth in the olive industry, most of the olive trees in South Africa are relatively young, with 59 % of the trees aged 11–25 yr and only 6 % older than 25 yr. The main olive production region in South Africa is the Western Cape province (92 % of total plantings), where viticulture is the main agricultural enterprise (Viljoen 2020). This region has a Mediterranean climate with warm, dry summers and cool, wet winters. The indigenous wild olive (*O. europaea* subsp. *cuspidata* = *O. europaea* subsp. *africana*), a close relative of the European olive, commonly occurs in this region, often in close proximity to European olive orchards.

Research on decline diseases of olive trees has previously been dominated by investigations on Verticillium wilt caused by *Verticillium dahliae* (Jiménez-Díaz et al. 2012), and olive quick decline syndrome caused by the bacterium *Xylella fastidiosa* (Martelli et al. 2016). The latter pathogen has thus far only been associated with decline of olive trees in Argentina, Brazil, Italy, and the USA (Saponari et al. 2013, Kruger et al. 2014, Haelterman et al. 2015, Coletta-Filho et al. 2016). On the other hand, Verticillium wilt of olive has been reported in various countries in Europe, North Africa, and Central Asia, as well as in the USA (California) and Australia (Jiménez-Díaz et al. 2012). Neither of these olive tree diseases has been reported in South Africa.

In addition to the above-mentioned pathogens, species of *Basidiomycota*, *Botryosphaeriaceae*, *Cytospora*, *Diaporthe*, *Diatrypaeaceae*, *Phaeoacremonium*, *Phaeomoniellales*, and some other fungi such as *Comoclathris incompta* (= *Phoma incompta*), and *Pleurostoma richardsiae*, have also been associated with various decline-related symptoms of olive trees in Croatia, Greece, Italy, New Zealand, Spain, and the USA (Rumbos 1988, 1993, Taylor et al. 2001, Carlucci et al. 2008, 2013, 2015, Moral et al. 2010, 2017, Kaliterna et al. 2012, Nigro et al. 2013, Úrbez-Torres et al. 2013, 2020, Lawrence et al. 2018). Úrbez-Torres et al. (2013) identified 18 fungal species in a survey of fungi causing olive twig and branch dieback in California (USA), of which the *Botryosphaeriaceae* were found to be the most prevalent, followed by species of *Diaporthe* and the *Diatrypaeaceae*. When inoculated onto olive trees, all of these species caused lesions of various sizes, with the largest being produced by *Neofusicoccum mediterraneum*, followed by *Diplodia mutila* (Úrbez-Torres et al. 2013). Moral et al. (2010) also found *N. mediterraneum* to

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be an aggressive pathogen when inoculated on olive branches. More recent studies have also associated *Cytospora oleicola*, *C. olivarum*, *C. plurivora*, and *C. sorbicola* with branch cankers and dieback of olive trees in the USA (Lawrence et al. 2018, Úrbez-Torres et al. 2020). In some counties of California, Úrbez-Torres et al. (2020) recovered *Cytospora* spp. from almost 30 % of twig dieback and canker samples. Pathogenicity trials illustrated the ability of *C. oleicola* and *C. olivarum* to cause lesions when inoculated on olive branches (Úrbez-Torres et al. 2020); however, the reported lesion size was considerably smaller than that reported for some species of *Botryosphaeriaceae* by Úrbez-Torres et al. (2013). In Italy, *Pleurostoma richardsiae*, *Phaeoacremonium* spp., and members of the *Botryosphaeriaceae* have been identified as the most prevalent fungi associated with olive decline (Carlucci et al. 2013, 2015, Nigro et al. 2013). Carlucci et al. (2013) found *Pleurostoma richardsiae* to be more aggressive than *Neofusicoccum parvum* and *Phaeoacremonium minimum*. In a further study identifying *Phaeoacremonium* species as the most prevalent fungi on olive trees in Italy, Carlucci et al. (2015) found *Phaeoacremonium sicilianum*, *Pc. minimum*, and *Pc. italicum* to be more virulent than *Pc. alvesii*, *Pc. parasiticum*, and *Pc. scolyti*, although all six species caused significant lesions. Species of the *Phaeomoniellales* were identified from olive trees and reported to be pathogenic to this host in California and Italy (Carlucci et al. 2008, 2013, 2015, Saponari et al. 2013, Úrbez-Torres et al. 2013, Crous et al. 2015). Carlucci et al. (2008, 2013, 2015) initially reported isolates of *Pseudophaeomoniella* spp. from olive trees in Italy as *Lecythophora lignicola* (A. Carlucci pers. comm.). The genus *Pseudophaeomoniella* currently contains two species (*P. oleae* and *P. oleicola*) that were recovered from and shown to be pathogenic to olive trees in Italy (Crous et al. 2015). Úrbez-Torres et al. (2013) recovered *Phaeomoniella chlamydospora* at low incidences from olives in California, and found it to be weakly pathogenic.

Some other fungi have been recorded at lower incidences or only in incidental reports, but have been shown to cause dieback and decline related symptoms on olive trees. These include *Diaporthe foeniculina* (reported as *Phomopsis* sp. groups 1 and 2 by Úrbez-Torres et al. 2013 and as *Diaporthe* sp. by Moral et al. 2017), *Diaporthe rudis*, *Diatrype oregonensis*, *Diatrype stigma*, *Eutypa lata*, *Ilyonectria destructans*, *Comoclathris incompta* (reported as *Phoma incompta*), and members of the *Basidiomycota*, such as *Fomitiporia mediterranea*, *Schizophyllum commune*, and *Trametes versicolor* (Rumbos 1988, 1993, Ivic et al. 2010, Carlucci et al. 2013, Úrbez-Torres et al. 2013, Moral et al. 2017).

No formal survey of European olive dieback pathogens in South Africa has been published to date; however, there are some reports of fungi from decline-related symptoms on the closely related wild olive. Crous et al. (2000) lists three basidiomycete species (*Ganoderma lucidum*, *Phellinus linteus* = *Fomes yucatanensis*, and *Phellinus robiniae*) in association with wood rot, and *Hysteroglyphium fraxini* var. *oleastri* in association with dieback of wild olives in South Africa. Furthermore, Adams et al. (2006) reported the *Cytospora pruinosa* species complex (= *Valsa cypri* species complex) on dead twigs of the same host in South Africa. In a recent survey of *Phaeoacremonium* species in South Africa, Spies et al. (2018) reported *Phaeoacremonium africanum*, *Pc. minimum*, *Pc. parasiticum*, and *Pc. scolyti* on European olives, and *Pc. oleae*, *Pc. prunicola*, *Pc. scolyti* and *Pc. spadicum* on wild olives. With the exception of *Pc. minimum*, *Pc. parasiticum*, and *Pc. scolyti*, none of the fungi reported in association with olive decline diseases in other countries had been recorded on *Olea europaea* in South Africa before. Several of these fungi have, however, been associated with cankers,

dieback, and other decline related symptoms of grapevines and fruit trees in the Western Cape province of South Africa. These include *Diplodia seriata*, *Neofusicoccum australe*, *N. luteum*, *N. parvum*, *N. vitifusiforme*, *Diaporthe foeniculina*, *Eutypa lata*, *Ilyonectria destructans*, *Phaeoacremonium alvesii*, *Pc. rubrigenum*, *Pc. sicilianum*, *Phaeomoniella chlamydospora*, *Pleurostoma richardsiae*, and *Schizophyllum commune* (Crous et al. 2000, Van Niekerk et al. 2004, Damm et al. 2007, 2008a, Cloete et al. 2011, White et al. 2011a, Moyo et al. 2016, 2018a, b). The occurrence of these fungi on such crops, that are often grown in close proximity to European and wild olive trees, suggests that such pathogens could also contribute to olive dieback and decline in South Africa.

Therefore, the aim of this study was to determine the incidence and distribution of fungi associated with dieback and decline diseases of European and wild olive trees in the Western Cape province of South Africa. Furthermore, the association of some of the higher-level taxa with the internal wood symptoms was investigated, and novel taxa within the *Phaeomoniellales* were described.

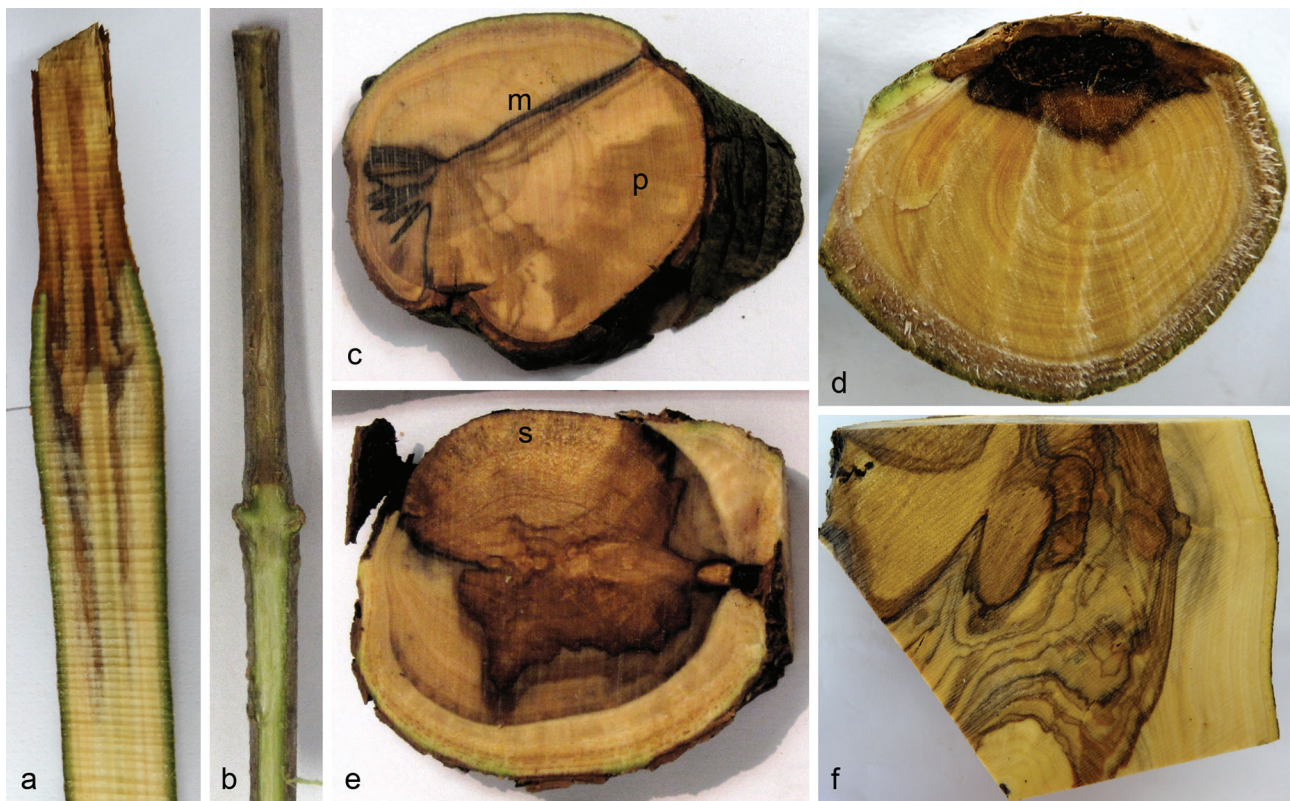
## MATERIALS AND METHODS

### Sampling and collection of fungal isolates

Symptomatic wood samples of 145 European olive trees (*Olea europaea* subsp. *europaea*) were collected from 10 districts (defined according to the Wine of Origin scheme, see [http://www.sawis.co.za/cert/download/Districts\\_-\\_Jan\\_2014.pdf](http://www.sawis.co.za/cert/download/Districts_-_Jan_2014.pdf)) in the Western Cape province of South Africa (Appendix 1). Sampled material consisted of cankerous branches or trunks, twigs showing dieback, and old wounds from pruning or other mechanical damage. Samples were collected in larger commercially producing orchards, as well as non-commercial, abandoned or neglected orchards, and trees in domestic gardens. Additional samples with similar symptoms were collected from 42 wild olive trees in nine districts (Appendix 1). Samples were processed as described by Moyo et al. (2016). In short, samples were cut to reveal internal symptoms that were photographed and marked prior to surface sterilisation (30 s in 70 % ethanol, 2 min in 3 % NaOCl, 30 s in 70 % ethanol) and plating of wood pieces from each marked symptom onto potato dextrose agar (PDA, Biolab, South Africa) containing 250 mg/L chloromycetin. Plates were incubated at 24 °C for 4 wk and inspected every 1–3 d. Emerging hyphae of possible fungal pathogens were transferred to fresh PDA plates to obtain pure isolates for identification. Isolates were stored as colonised agar plugs in sterile water at 4 °C and as colonised agar plugs in sterile 10 % glycerol at -84 °C.

Symptoms from which isolations were made, were classified in seven different types in order to investigate if certain fungi were associated with specific symptoms. Samples of European and wild olives were pooled for this aspect of the investigation. The seven symptom types are depicted in Fig. 1 and included twig dieback (n = 126), dark brown to black discolouration (n = 346), light brown to pink discolouration (n = 280), internal black lines (n = 149), the dark brown or black margin between healthy and discoloured tissue (n = 549), streaking (n = 100) and soft or white rot (n = 6). The recovery of isolates of specific fungi from the different symptom types was recorded and expressed as the percentage of symptoms of each type infected by the *Basidiomycota*, *Botryosphaeriaceae*, *Cytospora*, *Diaporthe*, *Diatrypaceae*, *Phaeoacremonium*, *Phaeomoniellales*, and *Pleurostoma*, respectively. Fungi not belonging to these genera, families, orders or classes were treated as a single group ('Other' fungi), and symptoms from which no fungi were obtained were also recorded.





**Fig. 1** Seven types of internal wood symptoms from which fungi were isolated during this study. a. Streaking; b. twig dieback; c. dark brown to black margin (m) and light brown to pink discoloration (p); d. dark brown to black discoloration; e. soft and white rot (s); f. internal black lines.

### Identification of isolates

Isolates were classified in morphological groups based on colony morphology and, in some cases, limited microscopic observations. Cultures morphologically identified as *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Penicillium*, and *Trichoderma*, that are generally not considered as dieback and decline pathogens, were discarded. Of the remaining isolates, representatives from all sampling sites and morphological groups were selected for sequencing of the translation elongation factor 1 alpha (*TEF1α*) region for the *Botryosphaeriaceae*, beta-tubulin (*TUB2*) for *Phaeoacremonium*, and the internal transcribed spacers ITS1 and ITS2 with the enclosed 5.8S ribosomal RNA gene for all remaining isolates (ITS). DNA was extracted using a CTAB-based protocol as described by Damm et al. (2008a). DNA samples were quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and diluted to a range of 5–15 ng/μL. PCR amplifications were performed in 10 μL reactions (15 μL for *TEF1α*) containing 1× KAPA Taq Ready Mix (KAPA Biosystems, Cape Town, South Africa), 0.08 μM of each primer (ITS: ITS5 and ITS4 (White et al. 1990); *TEF1α*: EF1-728F and EF1-986R (Carbone & Kohn 1999); *TUB2*: T1 (O'Donnell & Cigelnik 1997) and Bt2b (Glass & Donaldson 1995), and 1 μL DNA. Cycling conditions consisted of 5 min at 94 °C, 40 cycles of denaturation at 94 °C for 30 s, annealing for 30 s (ITS: 55 °C; *TEF1α*: 54.5 °C; *TUB2*: 58 °C), extension at 72 °C for 30 s, and final extension for 7 min at 72 °C. Successful amplifications were verified by gel electrophoresis and sequenced directly in one direction using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (PE Biosystems, Foster City, CA, USA). The sequencing product was analysed on an ABI PRISM 3130XL DNA sequencer (Perkin-Elmer, Norwalk, CT, USA) at the Central Analytical Facilities of Stellenbosch University. Trimming and editing of sequences were done with Geneious v. 9.1.7 (<http://www.geneious.com>, Kearse et al. 2012). Preliminary species identities were obtained by BLAST

analyses of sequences against the nucleotide database of GenBank for ITS and *TEF1α* sequences, and against a custom *Phaeoacremonium* database containing only reference *TUB2* sequences from Gramaje et al. (2015), Ariyawansa et al. (2015), Crous et al. (2016), Da Silva et al. (2017) and Spies et al. (2018). BLAST results were further confirmed through alignment of sequences with relevant reference sequences from GenBank using the MAFFT plugin in Geneious (Kato & Standley 2013) and phylogenetic analyses. The best fit substitution model for each alignment was estimated under the Akaike information criterion using jModeltest 2 (Darriba et al. 2012). Maximum likelihood analyses were performed using PhyML-MPI (Guindon et al. 2010) with support calculated from 100 bootstrap replicates. Phylogenies were viewed in FigTree v. 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). For a limited number of isolates, species were identified using species-specific PCR (*Diaporthe foeniculina* according to Lesuthu et al. 2019 and the new species of *Pseudophaeomoniella* according to Van Dyk 2020), DNA fingerprinting (*Botryosphaeriaceae* according to the protocols of Alves et al. 2007), or the morphology was compared to isolates that had been identified using molecular techniques. Representatives of all recovered species were included in phylogenetic analyses to confirm the inferred identities. All phylogenies are available on TreeBASE (study S26669 and S26950).

### Phylogenetic analyses of the *Phaeomoniellales*

A multi-gene phylogeny was generated for isolates in the *Phaeomoniellales* in an attempt to resolve the taxonomy of species in this order. Double strand consensus sequences of the actin (*ACT*), beta-tubulin (*TUB2*) and translation elongation factor 1-alpha (*TEF1α*) regions, as well as a fragment of the nuclear ribosomal RNA (rRNA) genes including ITS and the D1–D3 regions of the 28S ribosomal RNA gene (LSU), were generated for selected isolates in the *Phaeomoniellales*. The *ACT*, *TEF1α* and *TUB2* regions were amplified using the

**Table 1** GenBank accession numbers of isolates from the *Phaeoemoniellales* included in the multi-gene phylogeny. Sequences generated in this study are indicated in **bold**.

Species	Strain <sup>1</sup>	Country	Host	ITS	28S	GenBank accession numbers		
						TEF1 $\alpha$	ACT	TUB2
<i>Aequiabiella effusa</i>	CBS 120883 <sup>3</sup> = STE-U 6121	South Africa	<i>Prunus persica</i>	NR_132005	GQ154618	MN861676	n/a <sup>2</sup>	KR260451
<i>A. palatina</i>	CBS 145018 <sup>1</sup> = JKI-Ab36	Germany	spore trap attached to grapevine shoot	MH999506	MH999529	n/a	n/a	MK070469
<i>Celerionella dura</i>	CBS 120882 <sup>2</sup> = STE-U 6122	South Africa	<i>Prunus salicina</i>	NR_132004	GQ154617	MN861677	<b>MTT78367</b>	<b>MW017331</b>
<i>Ce. petrophilites</i>	CBS 142115 <sup>1</sup> = CPC 29256	Australia	<i>Petrophile teretifolia</i>	KY173394	KY173487	n/a	n/a	n/a
<i>Ce. prunicola</i>	CBS 120876 <sup>1</sup> = STE-U 6118	South Africa	<i>Prunus salicina</i>	NR_132003	GQ154614	n/a	<b>MTT78368</b>	KR260453
<i>Ce. umnquama</i>	STE-U 8442 = CSN801	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791052</b>	<b>MT797851</b>	<b>MT787395</b>	<b>MTT78370</b>	n/a
<i>Ceolothelium cinchonarum</i>	CBS 146756 <sup>1</sup> = STE-U 7966 = CSN1091	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791051</b>	<b>MT797850</b>	<b>MT787394</b>	<b>MTT78369</b>	n/a
<i>Dolabra nepheliae</i>	F 17105 f	Costa Rica	n/a	n/a	DQ329020	n/a	n/a	n/a
<i>Minutiella pruni-avium</i>	CBS 123297	Puerto Rico	<i>Litchi chinensis</i>	GU345749	GU332515	n/a	n/a	n/a
<i>M. simplex</i>	CBS 145513 <sup>1</sup>	Germany	<i>Prunus avium</i>	MN232957	MN232925	n/a	n/a	MN232985
<i>M. tardicola</i>	CBS 145008 <sup>1</sup> = JKI-Jn27	Germany	spore trap attached to grapevine shoot	MH999508	MH999531	n/a	n/a	MK070471
<i>Moristroma germanicum</i>	CBS 121757 <sup>1</sup> = STE-U 6123	South Africa	<i>Prunus armeniaca</i>	GQ154599	GQ154619	MN861680	<b>MTT78371</b>	KR260454
<i>Mo. japonicum</i>	CBS 145012 <sup>1</sup> = JKI-Feb06	Germany	spore trap attached to grapevine shoot	MH999512	MH999535	n/a	n/a	MK070475
<i>Mo. palatinum</i>	BN1674 <sup>1</sup>	Japan	<i>Quercus mongolica</i> var. <i>grossoserrata</i>	AY254052	AY254052	n/a	n/a	n/a
<i>Mo. quercinum</i>	CBS 145010 <sup>1</sup> = JKI-Feb17	Germany	spore trap attached to grapevine shoot	MH999510	MH999533	n/a	n/a	MK070473
<i>Neophaeoemoniella constricta</i>	BN1678 <sup>1</sup>	Sweden	<i>Quercus robur</i>	AY254051	AY254051	n/a	n/a	n/a
<i>Np. corymbiae</i>	CBS 145015 <sup>1</sup> = JKI-1Mz35	Germany	spore trap attached to grapevine shoot	MH999516	MH999539	n/a	n/a	MK070479
<i>Np. eucalypti</i>	CBS 145092 <sup>1</sup>	Germany	spore trap attached to grapevine shoot	MK047457	MK047507	n/a	n/a	n/a
<i>Np. eucalyptigena</i>	CBS 139919 <sup>1</sup>	Australia	<i>Corymbia citriodora</i>	NR_138001	KR476782	n/a	n/a	n/a
<i>Np. niveniae</i>	CBS 145093 <sup>1</sup>	USA	<i>Eucalyptus globulus</i>	NR_161148	MK047508	MK047569	n/a	MK047584
<i>Np. ossiformis</i>	CBS 131316 <sup>1</sup>	Australia	<i>Eucalyptus pilularis</i>	QJ044435	JQ044454	<b>MTT78396</b>	n/a	n/a
<i>Np. zymoides</i>	STE-U 7959 = CSN742	South Africa	<i>Nivenia stokoei</i>	<b>MT791053</b>	n/a	n/a	n/a	n/a
<i>Paraphaeoisaria alabamensis</i>	CBS 145013 <sup>1</sup> = JKI-May03	Germany	<i>Olea europaea</i> subsp. <i>cuspidata</i>	MH999514	MH999537	n/a	n/a	MK070477
<i>Paraphaeoemoniella capensis</i>	CBS 114904 <sup>1</sup> = AW304	Korea	spore trap attached to grapevine shoot	DQ270242	DQ270253	n/a	n/a	KR260455
<i>Phaeoemoniella chlamydospora</i>	CBS 121168	South Africa	<i>Pinus densiflora</i>	GQ154600	GQ154620	MN861679	n/a	<b>MW017332</b>
	STE-U 7960 = CSN743	South Africa	<i>Prunus salicina</i>	<b>MT791054</b>	n/a	<b>MTT78397</b>	n/a	n/a
	CBS 110.77A	USA	<i>Olea europaea</i> subsp. <i>cuspidata</i>	MH861028	MH872801	n/a	n/a	n/a
	CBS 110.77B <sup>1</sup>	USA	<i>Cronartium quercuum</i> f. sp. <i>fusiforme</i>	MH861029	n/a	n/a	n/a	n/a
	CBS 123535 <sup>1</sup>	USA	<i>Cronartium quercuum</i> f. sp. <i>fusiforme</i>	NR_137711	FJ372408	MN861681	<b>MTT78372</b>	KR260449
	CBS 229.95 <sup>1</sup>	Italy	<i>Encephalartos altensteinii</i>	NR_155612	NG_066265	n/a	n/a	AF253968
	CBS 117179	South Africa	<i>Vitis vinifera</i>	KF764544	n/a	KF764636	n/a	KF764683
	STE-U 7536	South Africa	<i>Vitis vinifera</i>	<b>MT791061</b>	<b>MT797852</b>	<b>MTT78398</b>	<b>MTT78373</b>	n/a
	CBS 114903 <sup>1</sup>	Korea	<i>Pinus densiflora</i>	DQ270240	MN861685	MN861678	n/a	KR260452
<i>'Phaeoemoniella' piniflorum</i>	STE-U 7946 = CSN18	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791062</b>	n/a	<b>MTT787403</b>	<b>MTT78378</b>	<b>MW017333</b>
<i>Pseudophaeoemoniella globosa</i>	CBS 146758 = STE-U 7947 = CSN41	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791066</b>	n/a	<b>MTT787400</b>	<b>MTT78375</b>	<b>MW017335</b>
	STE-U 7950 = CSN183	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791055</b>	n/a	<b>MTT787404</b>	<b>MTT78379</b>	n/a
	CBS 146755 <sup>1</sup> = STE-U 7951 = CSN185	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791056</b>	<b>MT797853</b>	<b>MTT787399</b>	<b>MTT87374</b>	<b>MW017337</b>
	STE-U 7952 = CSN186	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791067</b>	n/a	<b>MTT787405</b>	<b>MTT87380</b>	n/a
	CBS 146759 = STE-U 7953 = CSN329	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791069</b>	n/a	<b>MTT787401</b>	<b>MTT87376</b>	n/a
	STE-U 7954 = CSN334	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791068</b>	n/a	<b>MTT787406</b>	<b>MTT87381</b>	n/a
	STE-U 7955 = CSN349	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791063</b>	n/a	<b>MTT787407</b>	<b>MTT87382</b>	n/a
	STE-U 7956 = CSN386	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791057</b>	n/a	<b>MTT787408</b>	<b>MTT87383</b>	n/a
	STE-U 7957 = CSN435	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791064</b>	n/a	<b>MTT787409</b>	<b>MTT87384</b>	n/a
	STE-U 7958 = CSN451	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791058</b>	n/a	<b>MTT787410</b>	<b>MTT87385</b>	n/a
	STE-U 7962 = CSN806	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791059</b>	n/a	<b>MTT787411</b>	<b>MTT87386</b>	n/a
	STE-U 7963 = CSN808	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791070</b>	n/a	<b>MTT787412</b>	<b>MTT87387</b>	n/a
	STE-U 7964 = CSN824	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791065</b>	n/a	<b>MTT787413</b>	<b>MTT87388</b>	n/a
	STE-U 7965 = CSN960	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791071</b>	n/a	<b>MTT787414</b>	<b>MTT87389</b>	n/a
	STE-U 7968 = PMM1192	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	<b>MT791060</b>	n/a	<b>MTT787415</b>	<b>MTT87390</b>	<b>MW017338</b>
	PMM2484	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	<b>MT791072</b>	n/a	<b>MTT787402</b>	<b>MTT87377</b>	n/a



Table 1 (cont.)

Species	Strain <sup>1</sup>	Country	Host	GenBank accession numbers					
				ITS	28S	TEF1 $\alpha$	ACT	TUB2	
<i>P. oleae</i>	CBS 139191 <sup>T</sup> = FV84	Italy	<i>Olea europaea</i> subsp. <i>europaea</i>	NR_137966	KP635971	KP635968	KP635974	n/a	
<i>P. oleicola</i>	CBS 139192 <sup>T</sup> = M24	Italy	<i>Olea europaea</i> subsp. <i>europaea</i>	NR_137965	KP635970	KP411802	KP411805	n/a	
	STE-U 7933 = Ph58	Italy	<i>Olea europaea</i> subsp. <i>europaea</i>	MW008603	n/a	MW017340	MW017339	MW017336	
<i>Rhynchospora proteae</i>	CBS 112051 <sup>T</sup>	South Africa	<i>Protea laurifolia</i>	NR_132824	MN861683	n/a	MT787391	n/a	
<i>Streptiziana cliviae</i>	CBS 133577 <sup>T</sup> = CPC 19822	South Africa	<i>Clivia miniata</i>	NR_111823	NG_042750	n/a	n/a	n/a	
<i>S. malaysiana</i>	CBS 139902 <sup>T</sup> = CPC 24874	Malaysia	<i>Acacia mangium</i>	KR476731	KR476766	n/a	n/a	n/a	
<i>Vredendalilella oleae</i>	CBS 146757 <sup>T</sup> = STE-U 7969 = PMM1193	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791073	MT797854	MT787416	n/a	MW017334	
<i>Xenocylinthosporium kirstenboschense</i>	CBS 125545 <sup>T</sup>	South Africa	<i>Encephalartos friderici-guilielmi</i>	NR_132841	GU229891	n/a	n/a	n/a	
<i>X. margaritarum</i>	CBS 146848 <sup>T</sup> = STE-U 9059 = CSN1179	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791074	MT797855	MT787418	MT787393	n/a	
	CBS 146849 = STE-U 8437 = CSN1216	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791075	n/a	MT787417	n/a	n/a	
	CBS 146850 = STE-U 8440 = CSN1917	South Africa	<i>Olea europaea</i> subsp. <i>cuspidata</i>	MT791076	n/a	n/a	MT787392	n/a	
<i>X. sp. CF-JS-2015c</i>	CSN1180	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791077	MT797849	n/a	n/a	n/a	
	STE-U 8441 = CSN1184	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791078	MT797848	MT787420	n/a	n/a	
	STE-U 8436 = CSN1203	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791080	n/a	MT787419	n/a	n/a	
<i>X. sp. CF-JS-2015e</i>	STE-U 8438 = CSN1222	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791079	MT797847	MT787421	n/a	n/a	
<i>X. sp. CF-JS-2015f</i>	STE-U 8435 = CSN1191	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791082	MT797856	MT787422	n/a	n/a	
<i>X. sp. CF-JS-2015g</i>	STE-U 8446 = CSN1174	South Africa	<i>Olea europaea</i> subsp. <i>europaea</i>	MT791081	MT797846	n/a	n/a	n/a	

<sup>1</sup> CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CPC: Culture collection of Pedro Crous, housed at CBS; CSN: collection of Chris Spies at ARC-Nietvoorbij, Stellenbosch, South Africa; PMM: collection of Providence Moyo at the University of Stellenbosch, Department of Plant Pathology, Stellenbosch, South Africa; STE-U: fungal collection of the University of Stellenbosch, Department of Plant Pathology; <sup>2</sup> Ex-type strains. <sup>3</sup> Not available.

primers ACT-512F and ACT-783R (Carbone & Kohn 1999), EF1-728F and EF1-986R (Carbone & Kohn 1999), and Bt2a and Bt1b (Glass & Donaldson 1995), respectively. Cycling conditions were as described above, but annealing at 52 °C for *ACT* and *TEF1 $\alpha$*  and 58 °C for *TUB2*. For some isolates, *ACT* was amplified using a touch-down protocol with annealing temperatures decreasing from 66–58 °C in decrements of 2 °C every 5 cycles, followed by 20 cycles of annealing at 55 °C. The nuclear ribosomal RNA regions were amplified as a single fragment using the primers ITS5 (White et al. 1990) and LR7 (Vilgalys & Hester 1990) with cycling conditions as described above, but annealing at 50 °C and extending for 1 minute during every cycle. The ITS-LSU fragment was sequenced using the primers ITS3, ITS4, ITS5, LR0R, LR3, LR6 and LR7 (Vilgalys & Hester 1990, White et al. 1990). The *ACT*, *TEF1 $\alpha$* , and *TUB2* regions were sequenced using primers used for amplification. Sequences were assembled and edited using Geneious v. 9.1.7 (<http://www.geneious.com>, Kearse et al. 2012). Relevant reference sequences were obtained from GenBank and aligned with *de novo* generated data as described above (Table 1). The ITS, 28S, *ACT*, *TEF1 $\alpha$*  and *TUB2* regions were aligned separately and concatenated in Geneious v. 9.1.7 (<http://www.geneious.com>, Kearse et al. 2012). Maximum likelihood and Bayesian analyses of the concatenated and LSU only datasets were conducted in PhyML-MPI (Guindon et al. 2010) and PhyloBayes-MPI v. 1.8 (Lartillot et al. 2013), respectively. The GTR+I+G model was estimated as either the best fit model or one of the top three performing models for the different individual datasets using the Akaike Information Criterion in jModeltest 2 (Darriba et al. 2012). This model was consequently used for maximum likelihood analysis of the concatenated dataset, as well as the LSU dataset (best fit model), with support calculated from 1 000 bootstrap replicates. Bayesian analyses were performed under the CAT-GTR model. For each analysis, two chains were run for 10 000 (concatenated dataset) or 5 000 iterations (LSU dataset) of which the first 1 800 (concatenated dataset) or 800 (LSU dataset) were discarded as burn-in before assessing convergence using the bpcomp and tracecomp commands. The minimum effective sizes after running these commands were larger than 300 and maxdiff values were less than 0.1, indicating sufficient convergence as per the guidelines set out in the PhyloBayes-MPI manual. All phylogenies are available on TreeBASE (studies S26669 and S26950).

### Morphological characterisation of putative new species in the Phaeomoniellales

Representative isolates of putative new species in the *Phaeomoniellales* were selected for characterisation of micromorphological structures using a slide culture technique similar to that of Arzanlou et al. (2007). Colonised agar plugs (5–10 × 5–10 mm) were taken from 2-wk-old PDA cultures, placed on autoclaved microscope slides in Petri dishes containing two 90 mm filter paper disks moistened with 1.5 mL sterile deionised water, covered with autoclaved cover slips, and incubated at 25 °C for 10 d. Colonised microscope slides and cover slips were mounted separately in 70 % lactic acid, pressed for several hours to overnight under stacks of heavy books, and sealed with nail polish. Fungal growth on slides was inspected using a Nikon Eclipse Ni light microscope. Isolates were also grown on synthetic nutrient-poor agar (SNA) with autoclaved pine needles (Nirenberg 1976) for the production of conidiomata. Isolates of species that failed to produce conidia under these conditions were also cultured on SNA with autoclaved olive leaves and twigs in an attempt to induce sporulation. Images of vegetative hyphae, conidia, conidiogenous cells, collarettes, and conidiophores were captured at 1 000× and pycnidia at 11.25× magnification using a Nikon DS-Ri2 camera on a Nikon Eclipse Ni light microscope and a Nikon SMZ1500 stereo microscope,

respectively. Ten pycnidia and thirty individual structures of each type were viewed and measured using the NIS-Elements Viewer software (Nikon Instruments Inc.).

Colony morphology was evaluated on malt extract agar (MEA, Biolab), oatmeal agar (OA, Biolab) and PDA. Plates of the different media were inoculated with 4 mm diam plugs taken from actively growing PDA cultures and incubated at 25 °C in the dark for 21 d. In some cases, 4 mm diam plugs could not be used due to small colony sizes. For these species 1–2 mm diam colonies were picked from streaked cultures on PDA and transferred to the different media. Colony colours were evaluated using the colour charts of Rayner (1970).

Cardinal temperatures for growth were determined by incubating PDA plates at 25 °C in the dark for 2 d before marking colony margins on the bottom of each plate and incubating them at temperatures ranging from 5–40 °C at intervals of 5 °C in the dark. Each isolate was plated in triplicate for each temperature. Colony margins were marked on the bottom of each plate after 2, 3, and 4 wk. Plates that did not exhibit growth after 4 wk were incubated at 25 °C for an additional 7 d to establish viability of the cultures.

## RESULTS

### Sampling and collection of fungal isolates

Despite the presence of internal wood discolouration and other symptoms suggesting infection by pathogens in all samples, 43 European olive (30 %) and 12 wild olive (29 %) samples yielded no cultures of the fungi targeted in this survey. Some of these samples yielded putative saprophytes or endophytes that were not recorded; however, more often such samples yielded no fungi. Of the cultures recovered from the remaining samples, 440 representative isolates were identified to species level using sequencing and phylogenetic analyses (389 isolates), sequencing and BLAST (three isolates), DNA fingerprinting (six isolates), species-specific primers (seven isolates) or based on their morphological similarity to other sequenced isolates (25 isolates) (Appendix 2).

The incidence of fungi varied between the different symptom types, with twig dieback showing the highest incidence (63 % infection) while the lowest incidence was recorded for light brown or pink discolouration (21 % infection) (Table 2). All higher-level fungal taxa considered were recovered from all symptom types, except for streaking (no *Botryosphaeriaceae*, *Cytospora*, or *Diaporthe*), twig dieback (no *Basidiomycetes*, *Diatrypaceae*, or *Pleurostoma*) and soft and white rot (only *Basidiomycetes* recovered). For each symptom type the incidence of symptoms yielding no fungi was higher than the incidence of any of the fungal taxa taken into consideration.

The only exception to this was soft and white rot, where only six symptoms were considered of which half yielded no fungi, and the other half yielded *Basidiomycota*. The *Phaeomoniellales* had the highest incidence of all higher-level fungal taxa in all symptom types except for soft and white rot, where only *Basidiomycota* were recovered and twig dieback, where the *Botryosphaeriaceae* and 'Other' fungi had higher incidences (24 % and 27 %, respectively vs 17 % for the *Phaeomoniellales*). The highest incidence of the *Phaeomoniellales* was recorded for internal black lines (41 %), followed by streaking (33 %), dark brown or black discolouration (29 %), dark brown or black margins (25 %), twig dieback (17 %), and light brown or pink discolouration (14 %) (Table 2). Twig dieback yielded the highest incidences of *Botryosphaeriaceae* (23.8 %), *Diaporthe* (7.1 %), *Phaeoacremonium* (10.3 %), and 'Other' fungi (27.0 %).

### Identification of isolates

The list of isolates identified to species level is summarised in Appendix 2 and maximum likelihood phylogenies supporting these identifications are available on TreeBASE (study S26669). A total of 99 different fungal taxa were identified during this study, of which 85 were recovered from European olive trees, 33 from wild olive trees and 23 from both hosts (Table 3, Appendix 2). Forty-two of the recovered species belonged to higher level fungal taxa often associated with trunk disease or dieback of various hosts. These included the class *Basidiomycota* (six spp.), the families *Botryosphaeriaceae* (eight spp.) and *Diatrypaceae* (two spp.), the order *Phaeomoniellales* (10 spp.), and the genera *Biscogniauxia* (one sp.), *Cytospora* (two spp.), *Diaporthe* (two spp.), *Didymosphaeria* (two spp.), *Geosmithia* (one sp.), *Phaeoacremonium* (seven spp.), and *Pleurostoma* (one sp.) (Table 3). All species recovered at incidences of more than 5 % were among the classes, orders, families, or genera mentioned above, except for *Coniothyrium ferrarisianum* (phylogenetically a species of *Didymocyrtis*, TreeBASE study S26669 tree Tr125042) that was present on 7.6 % (n = 11) of the European olive trees sampled (Table 3). Based on the percentage of infected samples, the most prevalent fungal species infecting both European and wild olives in the Western Cape province of South Africa is a new species of *Pseudophaeomoniella* (Table 3). This fungus was isolated from 42.8 % (n = 62) and 54.8 % (n = 23) of the European olive and wild olive samples, respectively. Other fungi from these higher-level taxa that occurred in more than 5 % of the European olive samples were *Neofusicoccum cryptoaustrale/stellenboschiana* (11.7 %), *Diaporthe foeniculina* (10.3 %), *Neofusicoccum australe* (9 %), *Phaeoacremonium scolyti* (7.6 %), *Pleurostoma richardsiae* (6.9 %), and *Eutypa lata* (6.2 %) (Table 3). With the exception of *Neofusicoccum australe* and *Coniothyrium ferrarisianum*, all these fungi were also recovered from wild olives, although

**Table 2** Fungal incidence in each of seven different symptom types in European and wild olive wood. Numbers represent the number of symptoms from which the respective fungi were recovered, followed by the percentage in parentheses. Symptom types are depicted in Fig. 1.

Fungal group	Streaking (n=100)	Twig dieback (n=126)	Soft/white rot (n=6)	Dark brown or black margin (n=549)	Internal black lines (n=149)	Light brown or pink discolouration (n=280)	Dark brown or black discolouration (n=346)
<i>Basidiomycota</i>	2 (2.0 %)	–	3 (50.0 %)	10 (1.8 %)	6 (4.0 %)	3 (1.1 %)	17 (4.9 %)
<i>Botryosphaeriaceae</i>	–	30 (23.8 %)	–	15 (2.7 %)	8 (5.4 %)	8 (2.9 %)	9 (2.6 %)
<i>Cytospora</i>	–	1 (0.8 %)	–	12 (2.2 %)	3 (2.0 %)	2 (0.7 %)	7 (2.0 %)
<i>Diaporthe</i>	–	9 (7.1 %)	–	5 (0.9 %)	2 (1.3 %)	2 (0.7 %)	8 (2.3 %)
<i>Diatrypaceae</i>	1 (1.0 %)	–	–	10 (1.8 %)	3 (2.0 %)	2 (0.7 %)	2 (0.6 %)
<i>Phaeoacremonium</i>	2 (2.0 %)	13 (10.3 %)	–	14 (2.6 %)	3 (2.0 %)	6 (2.1 %)	6 (1.7 %)
<i>Phaeomoniellales</i>	33 (33.0 %)	22 (17.5 %)	–	137 (25.0 %)	61 (40.9 %)	38 (13.6 %)	102 (29.5 %)
<i>Pleurostoma</i>	3 (3.0 %)	–	–	10 (1.8 %)	6 (4.0 %)	2 (0.7 %)	9 (2.6 %)
Other	4 (4.0 %)	34 (27.0 %)	–	47 (8.6 %)	12 (8.1 %)	8 (2.9 %)	36 (10.4 %)
No fungi	60 (60.0 %)	47 (37.3 %)	3 (50.0 %)	341 (62.1 %)	73 (49.0 %)	220 (78.6 %)	190 (54.9 %)

not always at incidences of 5 % or more. In wild olive samples, the most prevalent fungi after the new *Pseudophaeomoniella* sp. were *Phaeoacremonium oleae* (19.1 %), *Diaporthe foeniculina* (9.5 %), *Eutypa lata* (9.5 %), *Biscogniauxia rosacearum* (7.1 %), *Neophaeomoniella niveniae* (7.1 %), and *Pleurostoma richardsiae* (7.1 %) (Table 3). With the exception of *Phaeoacremonium oleae* all these fungi were also recovered from European olives, although not necessarily at incidences of 5 % or more. Several other fungi from fungal groups often associated with trunk disease and dieback in various crops were also recovered from either European or wild olives. These included fungi from the *Basidiomycota* (*Fomitiporella viticola*, *Peniophora lycii*, *Phlebia acerina*, *T. versicolor*), the *Botryosphaeriaceae* (*Diplodia seriata*, *N. vitifusiforme*, and four undescribed species), *Cytospora* (*C. sp.* WvJ-2015a), *Diaporthe* (*D. ambigua*), the *Diatrypaceae* (*Cryptovalsa ampelina*), *Didymosphaeria* (*Dy. rubi-ulmifolii* and *Dy. variabile*), *Phaeoacremonium* (*Pc. afri-*

*canum*, *Pc. minimum*, *Pc. parasiticum*, *Pc. prunicola*, and *Pc. spadicum*) and the *Phaeomoniellales* (*Neophaeomoniella zymoides* and six undescribed species) (Table 3).

Most of the remaining fungal species not belonging to the higher-level taxa mentioned above occurred at incidences lower than 3 %. Exceptions include *Mycocalicium victoriae* (3.4 % on European olives, not recovered from wild olives) and *Teichospora* sp. CFJS-2015a (4.8 % on wild olives, not recovered from European olives) (Appendix 2).

### Phylogenetic analyses of the Phaeomoniellales

Phylogenetic analyses of the LSU region of the *Phaeomoniellales* provided good support ( $\geq 96$  % bootstrap support,  $\geq 0.97$  posterior probability) for most genera included, the only exceptions being *Celerioriella* that had low support ( $< 60$  % bootstrap support,  $< 0.6$  posterior probability) and *Xenocylindrosporium*

**Table 3** Incidence and distribution of 43 fungal taxa identified from 145 European and 42 wild olive trees in the Western Cape Province of South Africa. Only taxa from fungal groups often associated with dieback or decline diseases, and other taxa recovered at incidences of 5 % or more, are included here. Fifty-five additional fungal taxa that are not commonly considered as pathogens contributing to dieback and decline diseases, and that had incidences of less than 5 %, are included in Appendix 2.

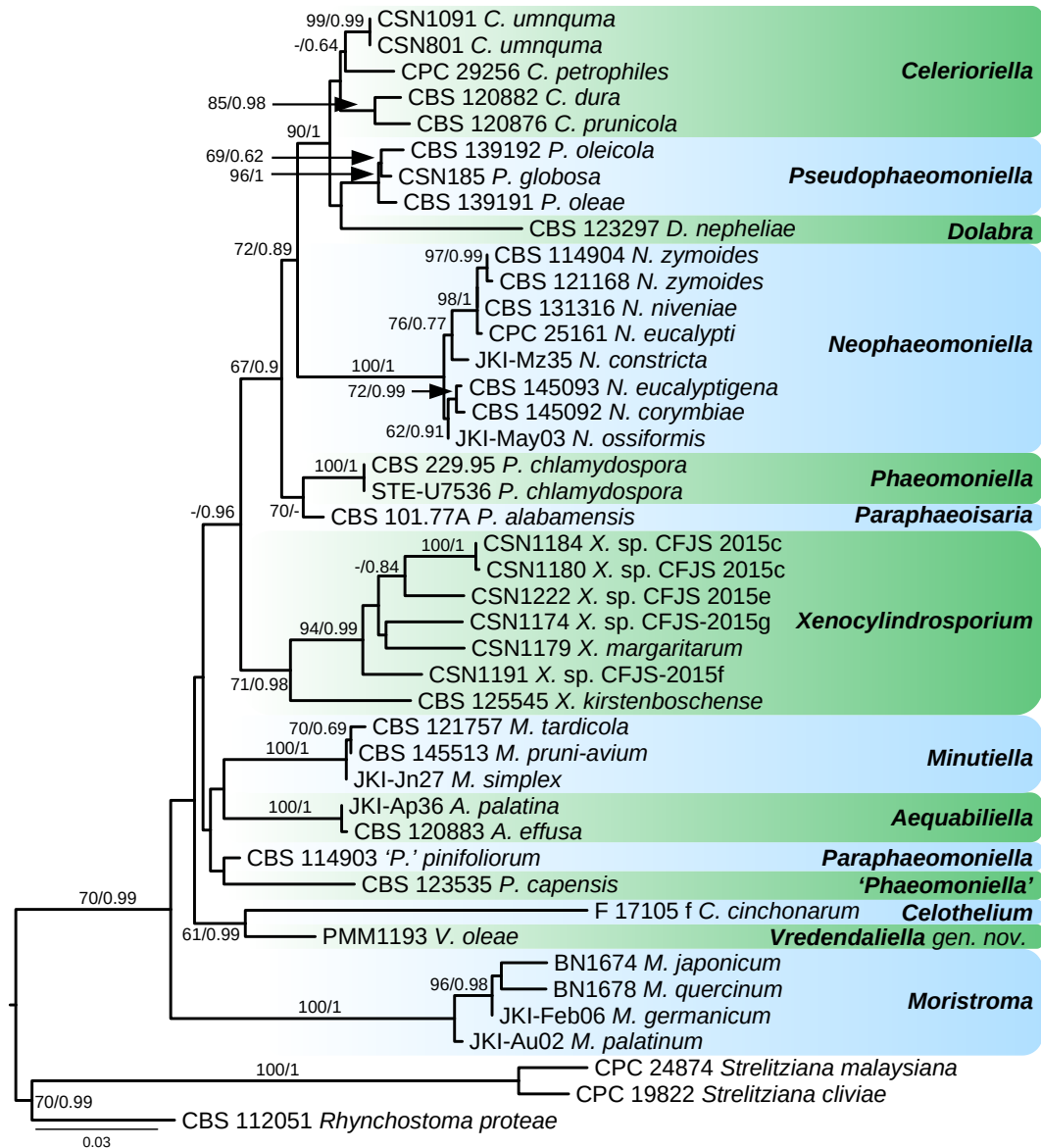
Fungal group	Species	Incidence <sup>1</sup>		Number of districts <sup>2</sup>	
		European olive (n=145)	Wild olive (n=42)	European olive (n=10)	Wild olive (n=9)
<i>Basidiomycota</i>	<i>Fomitiporella</i> sp. (Taxon 1)	6 (4.1 %)	–	3	–
	<i>Peniophora lycii</i>	2 (1.4 %)	–	1	–
	<i>Phlebia acerina</i>	1 (0.7 %)	–	1	–
	<i>Punctularia atropurpurascens</i>	–	2 (4.8 %)	–	2
	<i>Schizophyllum commune</i>	4 (2.8 %)	–	3	–
	<i>Trametes versicolor</i>	2 (1.4 %)	–	2	–
<i>Biscogniauxia</i>	<i>Biscogniauxia rosacearum</i>	2 (1.4 %)	3 (7.1 %)	1	2
<i>Botryosphaeriaceae</i>	<i>Diplodia seriata</i>	3 (2.1 %)	–	3	–
	<i>Neofusicoccum australe</i>	13 (9.0 %)	–	4	–
	<i>Neofusicoccum cryptoaustrale/stellenboschiana</i>	17 (11.7 %)	1 (2.4 %)	3	1
	<i>Neofusicoccum</i> sp. 4	1 (0.7 %)	–	1	–
	<i>Neofusicoccum</i> sp. 8	2 (1.4 %)	–	1	–
	<i>Neofusicoccum</i> sp. PMM-2014a	1 (0.7 %)	–	1	–
	<i>Neofusicoccum</i> sp. WvJ-2015a	4 (2.8 %)	1 (2.4 %)	4	1
<i>Neofusicoccum vitifusiforme</i>	1 (0.7 %)	1 (2.4 %)	1	1	
<i>Coniothyrium</i> s.lat.	<i>Coniothyrium ferrisianum</i> <sup>3</sup>	11 (7.6 %)	–	3	–
<i>Cytospora</i>	<i>Cytospora pruinosa</i>	6 (4.1 %)	1 (2.4 %)	3	1
	<i>Cytospora</i> sp. WvJ-2015a	6 (4.1 %)	–	3	–
<i>Diaporthe</i>	<i>Diaporthe ambigua</i>	1 (0.7 %)	–	1	–
	<i>Diaporthe foeniculina</i>	15 (10.3 %)	4 (9.5 %)	6	3
<i>Diatrypaceae</i>	<i>Cryptovalsa ampelina</i>	1 (0.7 %)	–	1	–
	<i>Eutypa lata</i>	9 (6.2 %)	4 (9.5 %)	4	2
<i>Didymosphaeria</i>	<i>Didymosphaeria rubi-ulmifolii</i>	1 (0.7 %)	1 (2.4 %)	1	1
	<i>Didymosphaeria variabile</i>	2 (1.4 %)	–	2	–
<i>Geosmithia</i>	<i>Geosmithia</i> sp. CFJS-2015a	2 (1.4 %)	1 (2.4 %)	2	1
<i>Phaeoacremonium</i>	<i>Phaeoacremonium africanum</i>	1 (0.7 %)	–	1	–
	<i>Phaeoacremonium minimum</i>	1 (0.7 %)	–	1	–
	<i>Phaeoacremonium oleae</i>	–	8 (19 %)	–	6
	<i>Phaeoacremonium parasiticum</i>	3 (2.1 %)	–	2	–
	<i>Phaeoacremonium prunicola</i>	–	1 (2.4 %)	–	1
	<i>Phaeoacremonium scolyti</i>	11 (7.6 %)	1 (2.4 %)	3	1
	<i>Phaeoacremonium spadicum</i>	–	1 (2.4 %)	–	1
<i>Phaeomoniellales</i>	<i>Celerioriella umnquma</i>	5 (3.4 %)	1 (2.4 %)	3	1
	<i>Neophaeomoniella niveniae</i>	1 (0.7 %)	3 (7.1 %)	1	2
	<i>Neophaeomoniella zymoides</i>	2 (1.4 %)	1 (2.4 %)	2	1
	<i>Pseudophaeomoniella globosa</i>	62 (42.8 %)	23 (54.8 %)	9	9
	<i>Vredendaliella oleae</i>	1 (0.7 %)	–	1	–
	<i>Xenocylindrosporium margaritarum</i>	2 (1.4 %)	1 (2.4 %)	2	1
	<i>Xenocylindrosporium</i> sp. CFJS-2015c	3 (2.1 %)	–	3	–
	<i>Xenocylindrosporium</i> sp. CFJS-2015e	1 (0.7 %)	–	1	–
	<i>Xenocylindrosporium</i> sp. CFJS-2015f	1 (0.7 %)	–	1	–
	<i>Xenocylindrosporium</i> sp. CFJS-2015g	1 (0.7 %)	–	1	–
<i>Pleurostoma</i>	<i>Pleurostoma richardsiae</i>	10 (6.9 %)	3 (7.1 %)	5	3

<sup>1</sup> Incidence values represent numbers of infected trees followed by percentages in parentheses.

<sup>2</sup> Districts sampled include Calitzdorp, Ceres Plateau, Franschoek (wild olives only), Lutzville Valley, Paarl, Robertson, Stellenbosch Swartland (European olives only), Tygerberg, Walker Bay (European olives only), Wellington (wild olives only), and Worcester (European olives only). The numbers of samples collected in each district are indicated in Appendix 1.

<sup>3</sup> Phylogenetically this species groups within the genus *Didymocyrtis* (see TreeBASE study S26669, tree Tr125042).





**Fig. 2** Generic classification of the *Phaeomoniellales* based on maximum likelihood analysis of the LSU gene. Bootstrap support and Bayesian posterior probability values higher than 60 % and 0.6, respectively, are indicated.

that only had moderate support in the maximum likelihood analysis (71 %), but good support in Bayesian analysis (0.98 posterior probability) (Fig. 2). Strain PMM1193 grouped with *Celothelium cinchonarium* with strong support in Bayesian analysis (0.99 posterior probability); however, this relationship only had low bootstrap support in maximum likelihood analysis (61 %), and *Ct. cinchonarium* was positioned on a long branch, indicating considerable phylogenetic distance between PMM1193 and that species (Fig. 2). The concatenated ITS-LSU-*ACT-TEF1 $\alpha$ -TUB2* phylogeny supported the initial identification of *Phaeomoniellales* strains collected during this survey based on ITS (TreeBASE study S26669, tree Tr125034). Most strains of the *Phaeomoniellales* collected in this study and included in the multi-gene phylogeny grouped in six well-supported clades, with four strains occupying unique positions (Fig. 3). Seventeen strains formed a strongly supported clade (98 % bootstrap support, 1.00 posterior probability) that did not include any reference sequences, but was related to *Pseudophaeomoniella oleae* and *Pseudophaeomoniella oleicola*. Strains CSN801 and CSN1091 formed a clade with good support (85 % bootstrap support, 0.98 posterior probability) that was related to, but distinct from *Celerioriella petrophiles*. Strains CSN743 and CSN742, respectively, grouped in clades containing the type strains of *Neophaeomoniella zymoides*

(100 % bootstrap support, 1.00 posterior probability) and *Neophaeomoniella niveniae* (98 % bootstrap support, 1.00 posterior probability). Nine strains collected in this study formed a diverse clade with weak support (67 % bootstrap support, 0.73 posterior probability) that did not include any reference sequences, but was related to *Xenocylindrosporium kirstenboschense*. Within this clade, six isolates formed two clades of three isolates each that had complete support (100 % bootstrap support, 1.00 posterior probability). The remaining three isolates in this clade (CSN1174, CSN1191, and CSN1222) occupied unique positions. As with the LSU phylogeny, strain PMM1193 grouped on its own in a position related to, but distinct from, *Celothelium cinchonarium*.

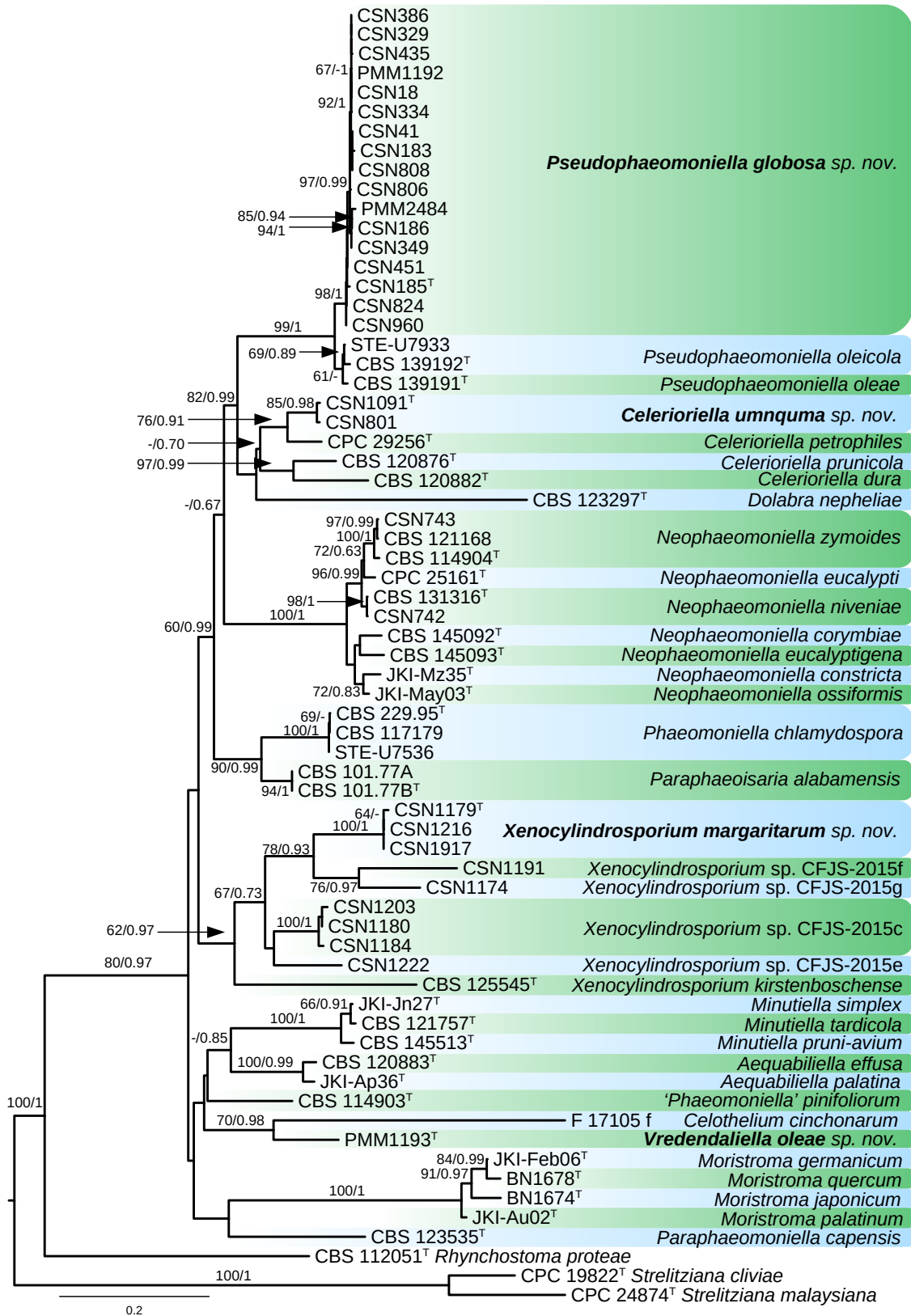
## TAXONOMY

***Celerioriella umnquma*** C.F.J. Spies, van Jaarsveld, L. Mostert & Halleen, sp. nov. — MycoBank MB836257; Fig. 4

*Etymology.* Referring to the Xhosa word for the host, olive, *umnquma*.

*Typus.* SOUTH AFRICA, Western Cape, Somerset-West, necrotic wood of European olive (*Olea europaea* subsp. *europaea*), 10 Mar. 2015, C.F.J. Spies (holotype CBS H-24370, culture ex-type CBS 146756 = STE-U 7966 = CSN1091).





**Fig. 3** Maximum likelihood phylogeny of the *Phaeoniellales* based on the concatenated LSU, ITS, *ACT*, *TEF1α* and *TUB2* regions. Bootstrap support and Bayesian posterior probability values higher than 60 % and 0.6, respectively, are indicated. Novel taxa described in this study are indicated in **bold**. Type strains are indicated with <sup>T</sup>.

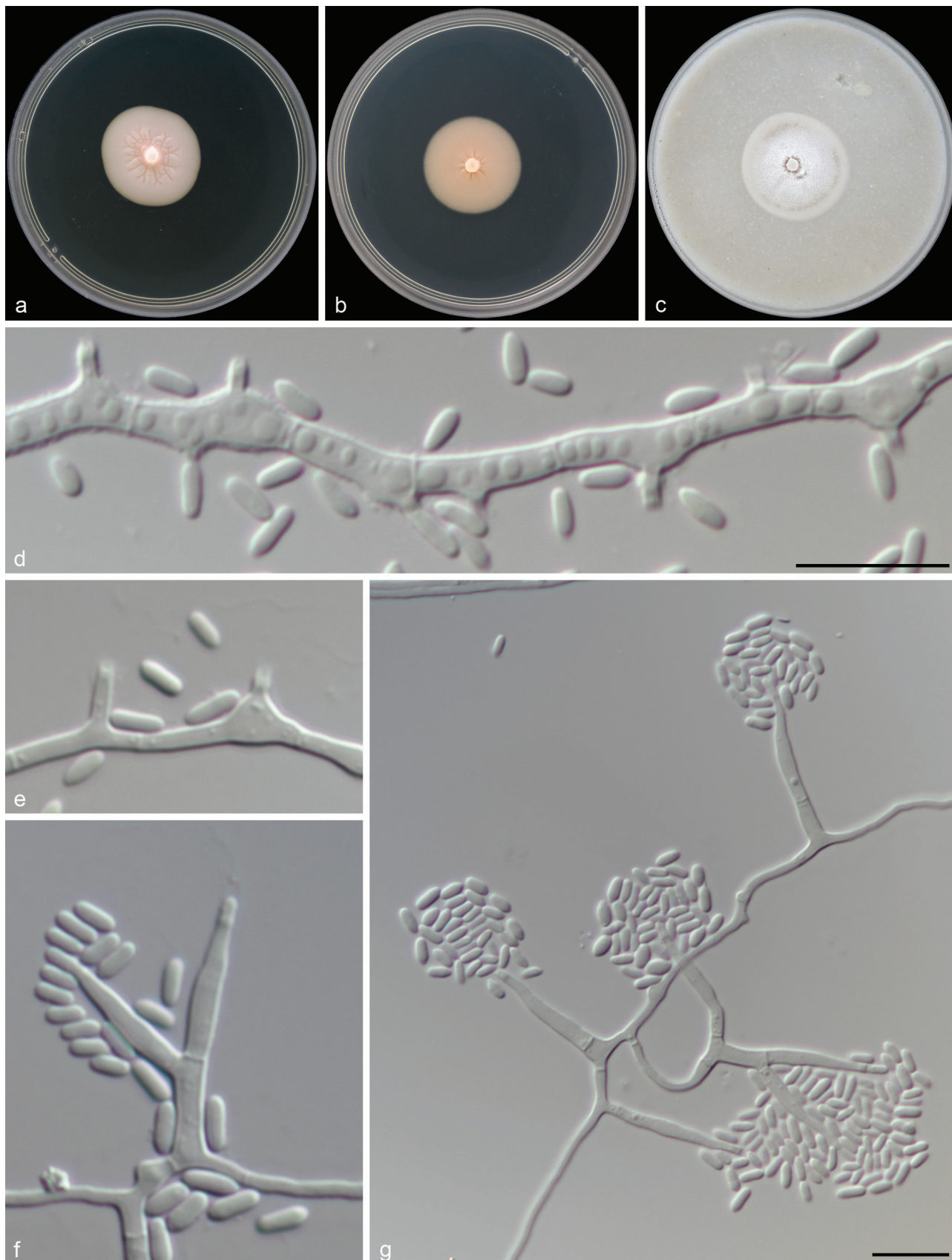
*Mycelium* smooth-walled to verruculose, hyaline, 1–1.5(–2) (av. 2) μm diam. *Pycnidia* not observed. Conidia on hyphae borne in slimy heads on intercalary adelophialides and on terminal or lateral phialides. *Terminal and lateral phialides* smooth-walled, hyaline to pale brown, mainly slender elongate ampulliform to navicular, (8–)8.5–17.5(–19.5) × 1.5–2(–2.5) (av. 13.5 × 2) μm. *Adelophialides* abundant, mainly cylindrical, sometimes conical or cylindrical with an inflated base,

1–8(–9.5) × 1–2(–3.5) (av. 2.5 × 1.5) μm. *Collarettes* cylindrical, 0.5–1 × 0.5–1(–1.5) (av. 1 × 1) μm (only 23 measured). *Conidia* smooth-walled, hyaline, subcylindrical to oblong ellipsoidal, ovoid, obovoid, 2.5–4(–4.5) × 1–2 (av. 3.5 × 1.5) μm. *Conidiphores* branched or unbranched, up to 4 septa, 15.5–37.5 × 2–2.5 (av. 22.5 × 2) μm (only 12 measured).

Culture characteristics — Colonies on PDA spreading, reaching 20, 31 and 42 mm diam in 2, 3 and 4 wk, respectively;

surface smooth, flat, with some central folds, without aerial mycelium, with entire edge, after 3 wk pale rosy buff above and in reverse. On MEA flat, surface smooth with central folds, without aerial mycelium, with entire margin, after 3 wk pale rosy vinaceous above, rosy buff in reverse. On OA flat, with felty aerial mycelia, white with pale hazel sections near the centre and margins of the colony.

Notes — Despite the fact that none of the phylogenies presented provides good support for the *Celerioriella* clade including *Ce. umnquma*, this species is included in *Celerioriella* based on morphological similarities to this genus (e.g., the abundance of adelophialides) and differences to the phylogenetically closely related genera *Pseudophaeomoniella* that develops a yeast-like synasexual morph in culture (Crous et al. 2015) and *Dolabra*



**Fig. 4** *Celerioriella umnquma*. a–c. Colony morphology on a. MEA; b. PDA; c. OA; d–e. adelophialides; f–g. conidiophores with slimy heads of conidia. — Scale bars: d, g = 10  $\mu$ m, d applies to e–f.



that has long, fusiform conidia (Rossman et al. 2010). *Celerioriella umnquma* is phylogenetically related to, but distinct from, *Ce. petrophiles*. Morphologically, these species can be distinguished based on the thinner hyphae of *Ce. umnquma* and colony pigmentation on PDA and MEA. The pycnidial conidiomata reported for *Ce. petrophiles* and other *Celerioriella* spp. have not been observed in *Ce. umnquma*. A BLAST search of the ITS region of *Ce. umnquma* against the Nucleotide database of GenBank revealed probable conspecificity (98–99 % similarity over 419 and 454 bases) with two strains of an unidentified ‘*Phaeomoniella*’ species recovered from olive twigs in Portugal (KT804064 and KU325017; Gomes et al. 2019). In the current investigation this species was recovered from both European and wild olives, but at low incidences (< 4 %).

***Pseudophaeomoniella globosa*** C.F.J. Spies, Carlucci, Moyo, van Jaarsveld, Halleen & L. Mostert, *sp. nov.* — MycoBank MB836258; Fig. 5

*Etymology.* Referring to the globose conidiogenous cells observed in pycnidia produced on olive wood.

*Typus.* SOUTH AFRICA, Western Cape, Robertson, necrotic wood of European olive (*Olea europaea* subsp. *europaea*), 2 Nov. 2014, P. Moyo (holotype CBS H-24369, culture ex-type CBS 146755 = STE-U 7951 = CSN185).

*Mycelium* smooth-walled to finely verruculose, hyaline, (1–)1.5–2.5 (av. 2)  $\mu\text{m}$  diam. Yeast-like growth observed occasionally. Conidia forming on hyphal cells and in pycnidia. *Pycnidia* produced on pine needles on SNA after incubation for 3–4 wk, (66.5–)67.5–149.5(–151)  $\mu\text{m}$  diam, dark brown to black, seemingly opening by irregular rupture, exuding clear conidial suspension, wall of 1–4 layers of brown *textura angularis*; conidiogenous cells mainly ampulliform, sometimes subcylindrical, navicular, globose to sub-globose, lageniform, pyriform, or irregular shaped, 3.5–9(–10)  $\times$  2–4.5 (av. 6  $\times$  3)  $\mu\text{m}$ ; collarettes inconspicuous, short, cylindrical, 0.5–1.5  $\times$  1–1.5 (av. 1  $\times$  1)  $\mu\text{m}$  (only five characterised); conidia smooth-walled, hyaline, subcylindrical to oblong ellipsoidal, 2.5–3  $\times$  1–1.5 (av. 3  $\times$  1)  $\mu\text{m}$ . *Conidia* on hyphae borne in slimy heads on intercalary adelophialides and terminal or lateral phialides, or in rows within empty hyphae (endoconidia). *Terminal and lateral phialides* mainly elongate ampulliform to subcylindrical with tapering apex, occasionally navicular to ovoid, obovoid or with irregular shape, 4–16.5  $\times$  (1–)1.5–3 (av. 8.5  $\times$  2)  $\mu\text{m}$ . *Adelophialides* mainly conical, sometimes subcylindrical or elongate ampulliform, 1–3.5(–4)  $\times$  1–3 (av. 2  $\times$  2)  $\mu\text{m}$ . Phialides and adelophialides often constricted at the collarette. *Collarettes* cylindrical, 0.5–1.5  $\times$  0.5–2 (av. 1  $\times$  1)  $\mu\text{m}$  (only 18 measured). Conidia smooth-walled, hyaline, subcylindrical to oblong ellipsoidal to obovoid, (2–)2.5–3.5(–4)  $\times$  1–2 (av. 3  $\times$  1.5)  $\mu\text{m}$ . Endoconidia subcylindrical to oblong ellipsoidal, 2–3.5  $\times$  1–1.5 (av. 3  $\times$  1.5)  $\mu\text{m}$ . *Conidiophores* uncommon, branched or unbranched, up to 3 septa, 7.5–21  $\times$  2–2.5 (av. 13.5  $\times$  2.5)  $\mu\text{m}$  (only 4 measured).

*Culture characteristics* — Colonies on PDA spreading, reaching 25, 37 and 47 mm diam in 2, 3 and 4 wk, respectively; surface smooth, flat, without aerial mycelium, with entire edge, after 3 wk pale buff above and buff to pale honey in reverse. On MEA smooth, flat with some folds in the centre, without aerial mycelium, with entire edge, after 3 wk white above, pale buff with pale honey centre in reverse. On OA smooth with woolly aerial mycelium in the centre, with entire edge, after 3 wk white with greenish olivaceous centre.

*Additional materials examined.* SOUTH AFRICA, Western Cape, Strand, internal wood necrosis of wild olive (*Olea europaea* subsp. *cuspidata*), 25 Sept. 2014, P. Moyo, cultures CBS 146758 = STE-U 7947 = CSN41; Western Cape, Stellenbosch, Jonkershoek, internal wood necrosis of wild olive (*Olea europaea* subsp. *cuspidata*), 12 Feb. 2015, C.F.J. Spies, cultures CBS 146759 = STE-U 7953 = CSN329.

*Notes* — *Pseudophaeomoniella globosa* is widespread and occurs frequently on European and wild olives in the Western Cape province of South Africa. Phylogenetically, this species is very closely related to *P. oleae* and *P. oleicola*. This was also confirmed by a BLAST search using the ITS region. Of the four gene regions used here for phylogenetic analyses, *TEF1a* provides the highest support for the distinction between the species. Morphologically, *P. globosa* can be distinguished by the production of endoconidia, which has not been reported for the other species of *Pseudophaeomoniella*. Strains CSN41 and CSN329 produced phialides and adelophialides with more diverse and irregular shapes than the type strain, e.g., some phialides were sub-globose, ovoid or obovoid. This is reflected in the slightly shorter and wider dimensions recorded for these two strains: 3.5–10.5(–11.5)  $\times$  (1.5–)2–3(–3.5) (av. 6.5  $\times$  2.5)  $\mu\text{m}$  and (3–)3.5–9.5(–10.5)  $\times$  2–3(–3.5) (av. 6  $\times$  2.5)  $\mu\text{m}$  for strains CSN41 and CSN329, respectively. Hyphae of strain CSN329 sometimes had pale to golden brown pigmentation and individual hyphal segments were sometimes inflated and irregular shaped. Pale brown pigmentation of some phialides was also observed in this strain. Three additional strains were included in studies of culture morphology, but not micromorphology. Strain CSN808 on PDA after 3 wk was pale buff with a pale rosy buff centre and pale vinaceous buff to fawn concentric rings. Pale primrose pigmentation was observed on the PDA colony of CSN960. Some strains had radial folds on PDA and/or MEA. CSN824 on MEA after 3 wk with pale olivaceous buff centre. Strain CSN960 on MEA after 3 wk with concentric folds. Central pigmentation on OA varying from none to sulphur yellow, citrine green, grey olivaceous, pale olivaceous grey, or greenish black.

***Vredendaliella*** C.F.J. Spies, Moyo, Halleen & L. Mostert, *gen. nov.* — MycoBank MB836261

*Etymology.* In reference to the location where this genus was first recovered.

*Type species.* *Vredendaliella oleae* C.F.J. Spies, Moyo, Halleen & L. Mostert.

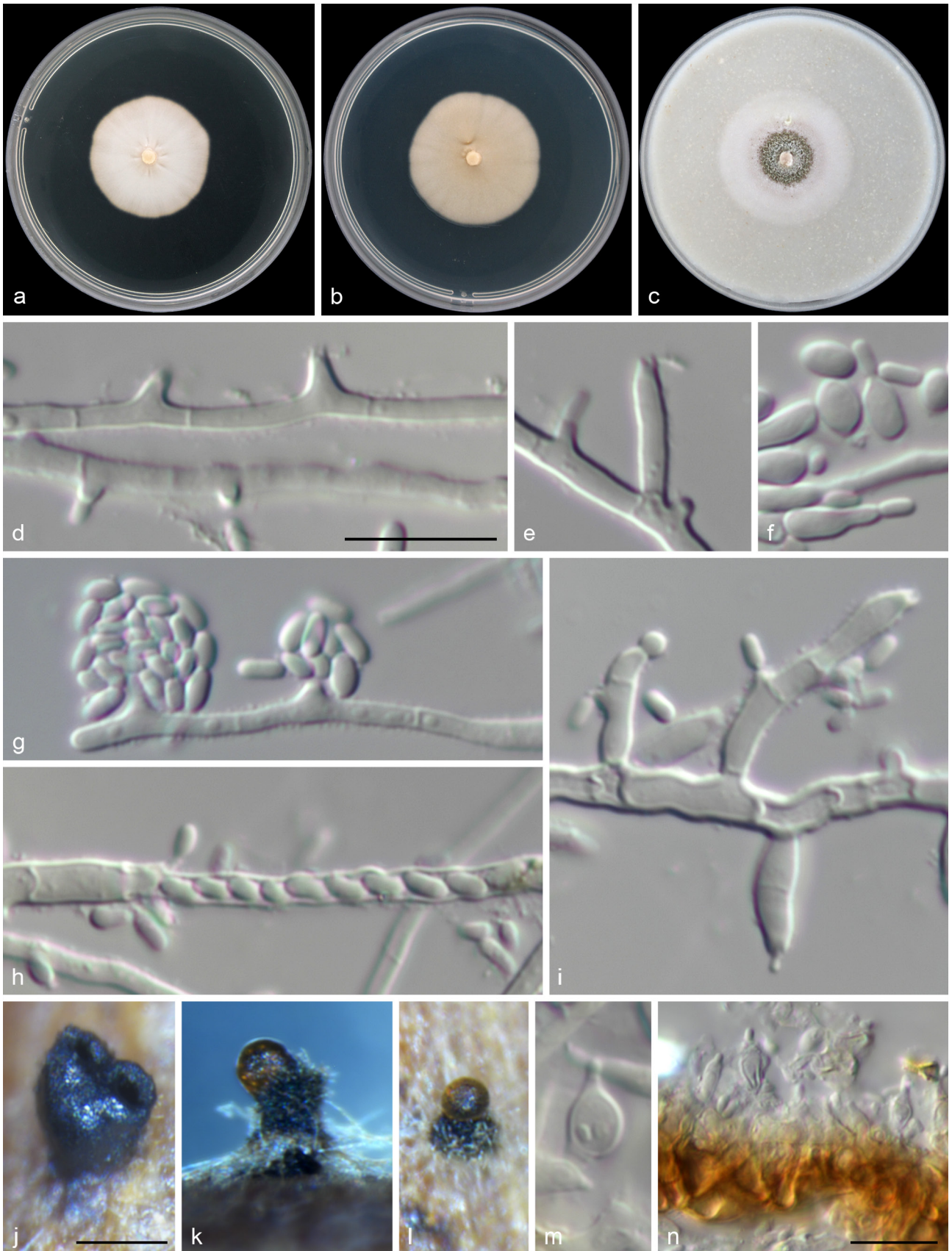
*Mycelium* consisting of hyaline to dark brown septate hyphae. Conidia formed on hyphae and in pycnidia. *Conidiogenous cells* on hyphae mostly reduced to adelophialides. *Conidia* borne on slimy heads on conidiogenous cells, aseptate, hyaline, smooth-walled, subcylindrical, oblong-ellipsoidal to obovoid. *Conidiomata* pycnidial, dark brown to black, semi-immersed or superficial, sub-globose or irregularly shaped. *Conidiogenous cells* brown, smooth-walled, ellipsoidal to broadly ellipsoidal. *Conidia* smooth-walled, hyaline, subcylindrical to oblong-ellipsoidal to obovoid.

***Vredendaliella oleae*** C.F.J. Spies, Moyo, Halleen & L. Mostert, *sp. nov.* — MycoBank MB836263; Fig. 6

*Etymology.* Referring to the host from which this species was recovered.

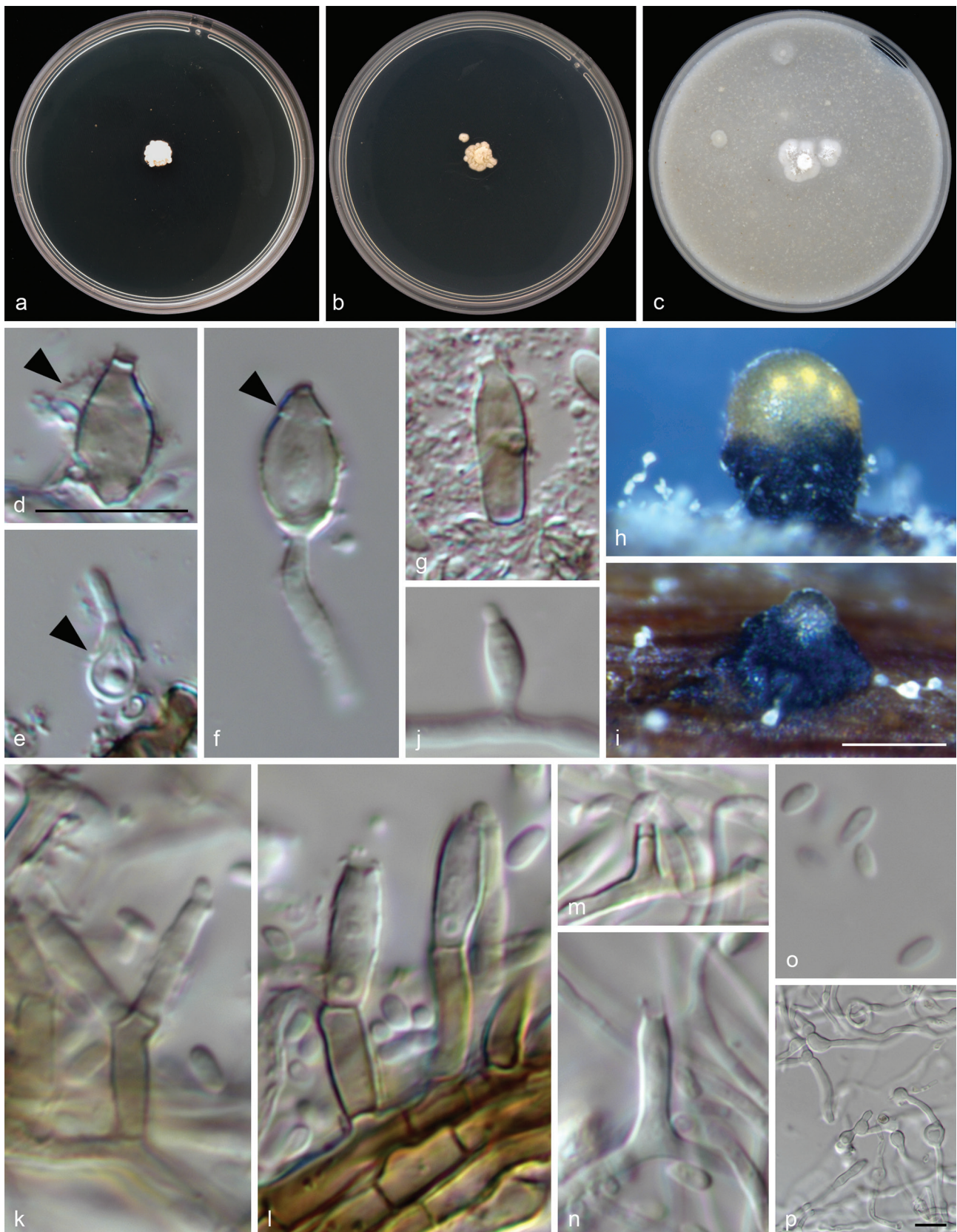
*Mycelium* smooth-walled, forming irregularly swollen hyphal cells on PDA, hyaline, sometimes dark brown, 1–2.5 (av. 1.5)  $\mu\text{m}$ . *Conidia* forming on hyphal cells and in pycnidia. *Pycnidia* forming on pine needles on SNA after 4 wk, globose to irregularly globose (50–)60.5–160.5(–170.5) (av. 106.5)  $\mu\text{m}$ . Seemingly opening by irregular rupture to exude clear conidial suspension. *Conidiogenous cells* in pycnidia usually dark brown, ellipsoidal to broadly ellipsoidal, oval or lens-shaped, sometimes ampulliform, fusiform or cylindrical, often with bib-like collar, (4.5–)5–11(–12.5)  $\times$  2–5 (av. 7.5  $\times$  4)  $\mu\text{m}$ ; collarettes inconspicuous, cylindrical, 0.5–1.5  $\times$  0.5–1.5 (av. 0.5  $\times$  1)  $\mu\text{m}$  (only 11 characterised); conidia smooth-walled, hyaline, ellipsoidal to oblong-ellipsoidal or subcylindrical, 2.5–4.5(–5)  $\times$





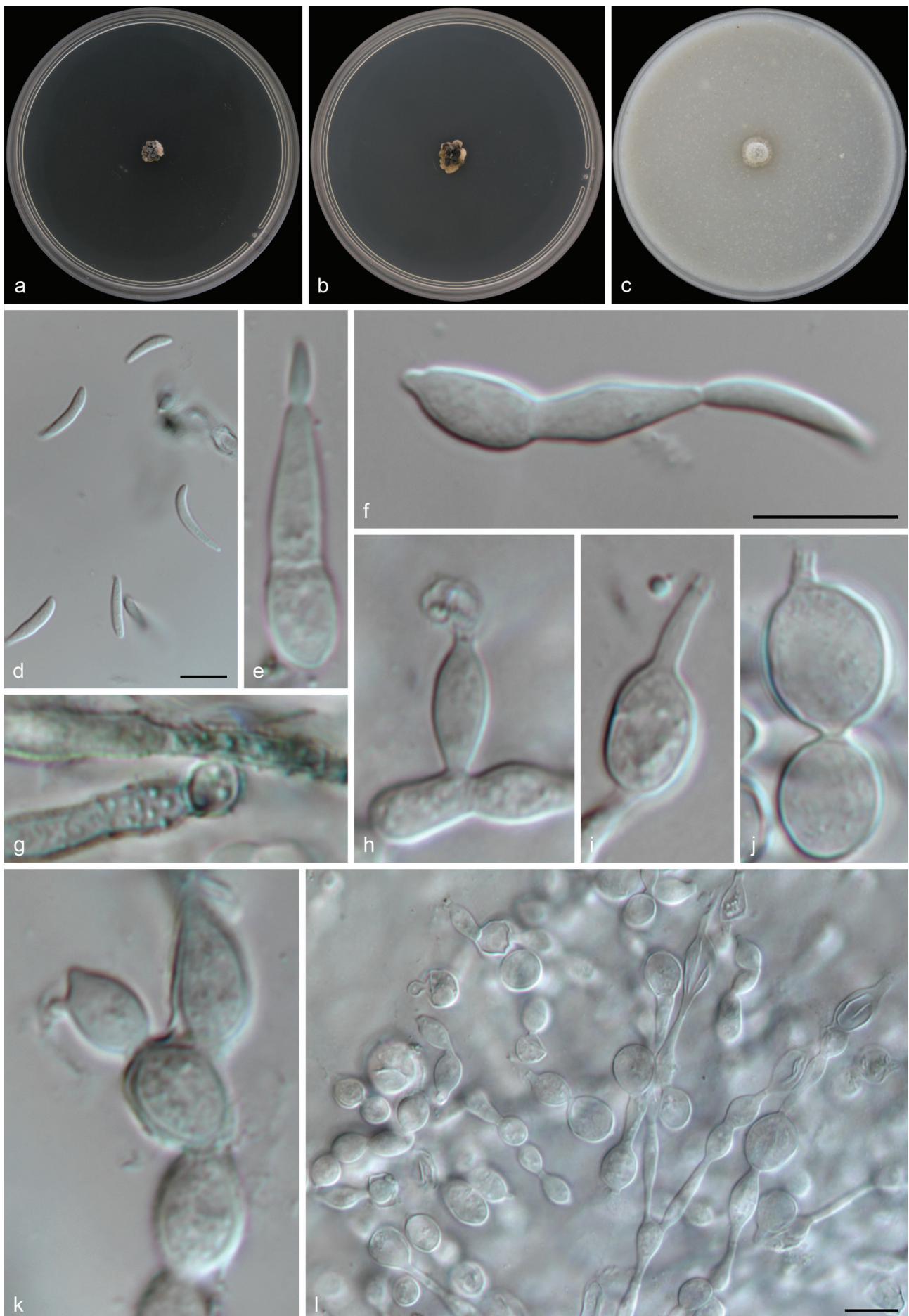
**Fig. 5** *Pseudophaeomoniella globosa*. a–c. Colony morphology on a. MEA; b. PDA; c. OA; d–i. conidia and conidiogenous cells on hyphal growth; d. adelophialides; e. subcylindrical phialide; f. microcyclic conidiation; g. slimy heads of conidia on adelophialides; h. endoconidia produced within empty hyphae; i. conidiophores; j–l. pycnidia produced on pine needles on SNA; j. empty pycnidium; k–l. pycnidia oozing clear conidial suspension; m–n. conidiogenous cells produced within pycnidia; m. globose conidiogenous cell; n. sub-cylindrical to sub-globose conidiogenous cells on pycnidial wall. — Scale bars: d, n = 10  $\mu$ m, d applies to e–i and m; j = 100  $\mu$ m, applies to k–l.





**Fig. 6** *Vredendaliella oleae*. a–c. Colony morphology on a. MEA; b. PDA; c. OA; d–g. pigmented conidiogenous cells in pycnidia; d–f. lens-shaped to ovoid conidiogenous cells with bib-like collars (indicated by arrowheads); g. sub-cylindrical conidiogenous cell; h–i. pycnidia on pine needles on SNA oozing clear conidial suspension; j–p. hyphal growth on pine needles on SNA; j, m, n. phialides; k, l. conidiophores; o. conidia; p. hyphae with irregular swollen segments. — Scale bars: d, p = 10  $\mu$ m, d applies to e–g and j–o; i = 100  $\mu$ m, applies to h.





**Fig. 7** *Xenocylindrosporium margaritarum*. a–c. Colony morphology on a. MEA; b. PDA; c. OA; d–l. hyphae, conidia and conidiogenous cells on hyphal growth; d. conidia; e, f, h–j. phialides; g. verruculose hyphal segments; k–l. chains of swollen hyphal segments. — Scale bars: d, f, l = 10  $\mu$ m, f applies to e and g–k.



1.5–2 (av.  $3 \times 1.5$ )  $\mu\text{m}$ . *Conidia* on hyphae borne in slimy heads on intercalary adelophialides and terminal or lateral phialides. *Terminal and lateral phialides* mainly subcylindrical to navicular, (4–)6–12(–14)  $\times$  1–3(–3.5) (av.  $9 \times 2$ )  $\mu\text{m}$  (only 29 measured). *Adelophialides* mainly subcylindrical, sometimes conical, 1–8  $\times$  1–2.5 (av.  $3.5 \times 1.5$ )  $\mu\text{m}$ . *Collarettes* cylindrical, 0.5–1.5  $\times$  1–2 (av.  $1 \times 1.5$ )  $\mu\text{m}$  (only 9 measured). *Conidia* smooth-walled, hyaline, subcylindrical to oblong-ellipsoidal to obovoid, 2.5–3.5  $\times$  1–2 (av.  $3 \times 1.5$ )  $\mu\text{m}$ . *Conidiophores* uncommon, branched or unbranched, usually brown, up to 1 septum, 8–17  $\times$  1–3.5 (av.  $15 \times 2.5$ )  $\mu\text{m}$  (only 5 measured).

**Culture characteristics** — Colonies on PDA slow growing, without aerial mycelium, creased, with undulate margin, after 3 wk white, pale buff in reverse. On MEA restricted, without aerial mycelium, creased, with undulate margin, after 3 wk white above, pale buff in reverse. On OA smooth with sparse woolly mycelium in the centre, with entire margin, after 3 wk white.

**Specimens examined.** SOUTH AFRICA, Western Cape, Vredendal, necrotic wood of European olive (*Olea europaea* subsp. *europaea*), 13 Aug. 2013, P. Moyo (holotype CBS H-24371, culture ex-type CBS 146757 = STE-U 7969 = PMM1193).

**Notes** — *Vredendaliella oleae* is currently known only from the ex-type strain reported here. An ITS BLAST search on the Nucleotide database of GenBank revealed that the closest match to this species only had 95 % sequence identity over 483 bases (KP992094), suggesting that there are currently no other records of the ITS region of *Vredendaliella oleae* on GenBank. The closest BLAST match (KP992094) is of an unclassified *Eurotiomycetes* species from *Juniperus deppeana* in the USA (Huang et al. 2016). The LSU phylogeny presented here suggests that *Vredendaliella* is related to *Celothelium* as represented by *Ct. cinchonarum*, although bootstrap support for this relationship is not very strong (61 % bootstrap support, 0.99 posterior probability) and long branch lengths suggests considerable evolutionary distance between the two taxa. Unfortunately, the only sequenced *Celothelium* species (*Ct. aciculiferum* and *Ct. cinchonarum*) are only known from their ascomata (no data are available on conidiomata) and *Vredendaliella oleae* is currently only known from its conidiomata, since no ascomata were observed in this study. This complicates morphological comparisons between these species. Conidiomata in other *Celothelium* species are described as pycnidial or stromatic with thin-walled, lageniform conidiogenous cells, and multi-septate, filiform macroconidia, but no microconidia (Aguirre-Hudson 1991). *Vredendaliella oleae* differs from them in the shape of the conidiogenous cells, absence of macroconidia and presence of microconidia.

***Xenocylindrosporium margaritarum*** C.F.J. Spies, van Jaarsveld, Halleen & L. Mostert, *sp. nov.* — MycoBank MB836260; Fig. 7

**Etymology.** Latin, meaning 'of pearls', a dual reference to the chains of globose vegetative hyphal cells that resemble strings of pearls, and the location from which the type strain was recovered (Paarl, meaning 'pearl').

**Typus.** SOUTH AFRICA, Western Cape, Paarl, necrotic wood of European olive (*Olea europaea* subsp. *europaea*), 4 Feb. 2015, C.F.J. Spies (holotype CBS H-24372, culture ex-type CBS 146848 = STE-U 9059 = CSN1179).

**Mycelium** on PDA after 4 wk consisting mainly of branched chains of hyaline, smooth-walled, globose to irregular cylindrical hyphal cells, individual hyphal cells sometimes include inflated and non-inflated sections (4.5–)5.5–15.5(–18.5)  $\times$  (2.5–)3–10(–11) (av.  $10 \times 5$ )  $\mu\text{m}$ . Hyaline to dark green hyphae consisting of smooth-walled to verruculose cylindrical cells were occasionally observed. Conidia produced on vegetative hyphae. *Conidiogenous cells* monophialidic, smooth-walled, hyaline, similar in shape and size to vegetative hyphal cells, globose,

ampulliform to cylindrical, sometimes with a narrow elongated cylindrical neck, (6–)6.5–14.5(–16.5)  $\times$  3–8.5 (av.  $10 \times 5$ )  $\mu\text{m}$ . *Collarettes* rarely observed, cylindrical, 0.5–1  $\times$  1–2 (av.  $1 \times 1.5$ )  $\mu\text{m}$  (only 7 characterised). *Conidia* solitary, smooth-walled, hyaline, single-celled, curved, tapering to rounded apex and truncate base, (10.5–)12–20(–20.5)  $\times$  2–3(–3.5) (av.  $16 \times 2.5$ )  $\mu\text{m}$ . Swollen conidia becoming septate and differentiating to become vegetative hyphal or conidiogenous cells.

**Culture characteristics** — Colonies on PDA very slow growing. On MEA without prominent aerial mycelium, irregularly raised, with undulate margin, after 3 wk white and leaden grey above, white and olivaceous grey in reverse. On PDA uneven, irregularly raised, smooth surface, with some felty aerial mycelium, with undulate margin, after 3 wk white and grey olivaceous to iron grey above, white in reverse. On OA raised, felty to woolly aerial mycelium, with entire edge, after 3 wk buff, a clear exudate is produced around the colony.

**Notes** — This species was recovered from both European and wild olives in this study, but at low incidences (< 3 %). An ITS BLAST on the Nucleotide database of GenBank suggests that there are no other ITS representatives of *X. margaritarum* on GenBank (closest match < 91.5 % sequence identity over ~600 bases). Although generic concepts within the *Phaeomoniellales* have not been resolved, phylogenetic analyses of the LSU gene region groups *X. margaritarum* with *X. kirstenboschense* (type species of *Xenocylindrosporium*; 71 % bootstrap support, 0.98 posterior probability). Conidiogenous cells and conidia of *X. margaritarum* also conform to the generic description provided by Crous et al. (2009). Unfortunately, the ex-type strain of *X. margaritarum* did not produce acervuloid conidiomata typical of the genus when cultured on MEA, PDA, SNA with autoclaved pine needles, SNA with autoclaved olive twigs and leaves, or OA. Alternative culturing methods might be required to induce these structures. Sporulation of the ex-type strain was only observed on PDA after 4 wk. Two additional strains of *X. margaritarum* (CSN1216 and CSN1917) produced pale buff to pale rosy buff colonies on OA, but exhibited colony morphologies similar to that of the type strain on MEA and PDA. These strains did not sporulate on MEA, PDA, or OA and were not characterised with regards to other micromorphological characteristics.

## DISCUSSION

This survey has revealed a unique community of fungi associated with dieback and decline related symptoms on European and wild olive trees in South Africa. On European olive trees, species of the *Phaeomoniellales* were the most prevalent, being isolated from 48 % of the samples, followed by species of *Botryosphaeriaceae* (19 %), while all other higher-level fungal taxa including *Phaeoacremonium* and the *Diatrypaceae* occurred at incidences of 11 % or less. Similar surveys of fungi associated with wilt, dieback and cankers on European olive trees in Europe and the USA found the dominant fungi to be the *Botryosphaeriaceae* in Spain and the USA (Úrbez-Torres et al. 2013, Moral et al. 2017) and *Phaeoacremonium* or the *Botryosphaeriaceae* in Italy (Carlucci et al. 2013, 2015). Recently published data further suggest that species of *Cytospora* are also important contributors to cankers and dieback of olive trees in the USA (Úrbez-Torres et al. 2020). There are several possible reasons why the fungi associated with olive dieback and decline in South Africa are so different to those reported in the countries mentioned above. The age of the olive industry and the olive trees planted is likely to be an important contributing factor, since Carlucci et al. (2013, 2015) found higher incidences of some pathogens such as the *Botryosphaeriaceae* and *Phaeoacremonium* in older trees (25–35 yr in Carlucci et al.

(2013) and > 50 yr in Carlucci et al. (2015)), whereas most South African olive trees are younger than 25 yr. Úrbez-Torres et al. (2020) suggested that climatological conditions and the availability of susceptible hosts may have influenced the distribution of *Cytospora* species across olive producing counties in California. Similarly, environmental differences between South Africa, the USA and Europe may have contributed to differences in the profiles of fungal species associated with olive decline and dieback. The fact that *P. globosa* was recovered frequently from both European and indigenous wild olive trees in South Africa, has a wide distribution within the Western Cape province, and has not been reported from any other host or country, suggests that this species might be indigenous to South Africa, with the native wild olive trees as its primary host. However, it is also possible that this species was introduced from abroad along with the European olive host, and has since adopted the indigenous wild olive as a new host. Further studies are required to confirm this.

A surprisingly high number of novel taxa in the *Phaeomoniellales* were discovered during the current survey. The order *Phaeomoniellales* was recently introduced by Chen et al. (2015) to accommodate the genera *Celothelium*, *Dolabra*, *Moristroma*, *Phaeomoniella*, and *Xenocylindrosporium*. Crous et al. (2015) further split the genus *Phaeomoniella* into six different genera (*Aequabiliella*, *Celerioriella*, *Minutiella*, *Neophaeomoniella*, *Paraphaeomoniella*, and *Phaeomoniella*), and also introduced *Pseudophaeomoniella* as a new genus. Phylogenetic relationships among the genera within the *Phaeomoniellales* have not been resolved (Chen et al. 2015), which complicates the generic classification within this order. None of the gene regions used in our analyses resolved all genus-level relationships with good support. However, phylogenetic analyses of the D1–D3 regions of the LSU region provided moderate to good support for almost all genera in the *Phaeomoniellales* for which data were included from more than one species, i.e., *Moristroma*, *Neophaeomoniella*, *Pseudophaeomoniella*, and *Xenocylindrosporium*. The only exception was *Celerioriella* (*Ce. dura*, *Ce. petrophiles*, *Ce. prunicola*, and *Ce. umnquma*) that had low support in maximum likelihood and Bayesian analyses. The fact that most genera with more than one species in the *Phaeomoniellales* were well-supported in phylogenetic analyses of the LSU region suggests that this region is currently adequate for the delineation of genera in the *Phaeomoniellales*. Kraus et al. (2020) recently described six new species of known genera in the *Phaeomoniellales* collected in German vineyards. Such collections and descriptions broaden the available knowledge on genera in the *Phaeomoniellales* and help to consolidate generic concepts within this order. The future discovery and description of taxa in the *Phaeomoniellales* will no doubt further improve the resolution of evolutionary relationships and generic boundaries among taxa within this order.

Members of the *Phaeomoniellales* are generally associated with plants as endophytes, saprophytes or plant pathogens (Chen et al. 2015). Species that have been associated with vascular discoloration and other trunk disease or decline symptoms in various hosts include *Phaeomoniella chlamydospora* (one of the causal agents of Petri disease and esca in grapevines), *Aequabiliella effusa*, *Celerioriella dura*, *Celerioriella prunicola*, *Minutiella tardicola*, *Neophaeomoniella zymoides*, *Pseudophaeomoniella oleae*, and *Pseudophaeomoniella oleicola* (Larignon & Dubos 1997, Damm et al. 2010, Úrbez-Torres et al. 2013, Crous et al. 2015). Symptom associations in the current survey indicated a high incidence of *Phaeomoniellales* (mainly *Pseudophaeomoniella globosa*) in streaking symptoms of European and wild olives (33 % incidence) while all other fungi occurred at low incidences ( $\leq 4$  %) in this symptom type. Similarly, vascular streaking of grapevines and olives have been associated

with infections by *Phaeomoniella chlamydospora* (Mugnai et al. 1999, White et al. 2011b, Úrbez-Torres et al. 2013). The *Phaeomoniellales* also had high incidences in other symptoms of branches and trunks of European and wild olives where black or dark brown discoloration of the wood was observed (25–41 %). The recently described *Pseudophaeomoniella oleae* and *Pseudophaeomoniella oleicola* were both recovered from European olive trees in Italy and reported to cause extensive wood discoloration (Crous et al. 2015). Carlucci et al. (2008) reported the development of brown streaking, chlorosis, loss of leaves and shoot dieback in European olive trees six years after inoculation with a species of *Pseudophaeomoniella* that had incorrectly been identified as *Lecytophora lignicola* at the time (Carlucci pers. comm.). *Pseudophaeomoniella globosa*, the dominant species recovered in the current survey, is closely related to the other two species in this genus. This close evolutionary relationship, together with the wide distribution, high incidence and strong association of *P. globosa* with internal wood symptoms of olives as observed in the current survey, implicates this species as an important role player in olive dieback and decline in South Africa. Other members of the *Phaeomoniellales* were recovered at much lower incidences, and only two of the eight species are known (*Neophaeomoniella niveniae* and *Np. zymoides*). The only previous record of *Np. niveniae* is that of the type, which was collected from leaves of *Nivenia stokoei*, also in the Western Cape Province of South Africa (Crous et al. 2011). The pathogenic ability of this species is unknown. *Neophaeomoniella zymoides*, although initially reported as an endophyte of pine needles in Korea (Lee et al. 2006), was later associated with necrotic wood of plum trees in South Africa (Limpopo Province), and shown to cause significant lesions when inoculated on peach shoots, but not on plum (Damm et al. 2010). More recently this species was also recovered from spore traps in German vineyards, but found to be non-pathogenic to grapevine (Kraus et al. 2020). Of the remaining *Phaeomoniellales* species collected from olive trees in this study, one is a new genus here described as *Vredendaliella*, and five are previously undescribed species of *Xenocylindrosporium*. Formal descriptions of four of the undescribed species of *Xenocylindrosporium* were not possible in this study due to a failure of isolates to sporulate on a variety of media. Prior to this study, *Xenocylindrosporium* was only known from the collection and description of the type species, *X. kirstenboschense*, from leaf spots of *Encephalartos friderici-guilielmi* (Crous et al. 2009). Although the culturing techniques and media are not clearly outlined in that study, the production of acervuloid conidiomata was reported on the host material and on MEA. In the current investigation, sporulation of *Xenocylindrosporium* was successfully induced only on PDA and only in one strain of *X. margaritarum*. Furthermore, all *Xenocylindrosporium* isolates exhibited slow to very slow growth on agar media. This suggests alternative culturing techniques or media would probably more ideal for investigating these fungi.

Species of the *Botryosphaeriaceae* were reported as the most common pathogens associated with olive dieback in the USA (Úrbez-Torres et al. 2013) and Spain (Moral et al. 2017). Species that have been reported from dieback and decline symptoms of European olives in these countries, Croatia, Italy, and New Zealand include *Botryosphaeria dothidea*, *Diplodia mutila*, *Di. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum luteum*, *N. mediterraneum*, *N. parvum*, *N. ribis*, and *N. vitifusiforme* (Taylor et al. 2001, Romero et al. 2005, Lazzizzera et al. 2008, Moral et al. 2010, 2017, Kaliterna et al. 2012, Carlucci et al. 2013, 2015, Úrbez-Torres et al. 2013). Of these species only *Di. seriata* and *N. vitifusiforme* were recovered from olive trees in the current survey, and at very low incidences (1–2 %). Nevertheless, the pathogenicity of



both these species to olive trees has been shown (Carlucci et al. 2013, Úrbez-Torres et al. 2013). *Neofusicoccum cryptoaustrale/stellenboschiana* and *N. australe* were the most common species of the *Botryosphaeriaceae* on European olives in this survey. *Neofusicoccum australe* has been associated with trunk diseases of grapevines, Japanese persimmons and stone fruit in South Africa (Van Niekerk et al. 2004, Damm et al. 2007, Moyo et al. 2016). This species was also reported as one of the causal agents of drupe rot of olives in Italy by Lazzizzera et al. (2008). However, a re-examination of some of the isolates revealed them to be *N. cryptoaustrale* and *N. stellenboschiana* (Yang et al. 2017). In the current investigation, these two species could not be distinguished using ITS, *TEF1α*, and *TUB2* sequence data alone or in combination. *Neofusicoccum cryptoaustrale* was originally isolated from *Eucalyptus* leaves (Crous et al. 2013) and shown to be pathogenic to this host by Pavlic-Zupanc et al. (2017). *Neofusicoccum stellenboschiana* was described by Yang et al. (2017) using a strain originally isolated from, and shown to be pathogenic to grapevines in South Africa by Van Niekerk et al. (2004). Four undescribed *Neofusicoccum* species were also recovered during the current survey, but at low incidences. Two of these, *Neofusicoccum* sp. 4 and *Neofusicoccum* sp. 8 have, respectively, previously been reported from grapevines and *Proteaceae* in South Africa (Van Niekerk et al. 2004, Marincowitz et al. 2008, Yang et al. 2017). The *Botryosphaeriaceae* were the most common fungi isolated from twig dieback symptoms in this survey. This is also in agreement with the results of Úrbez-Torres et al. (2013) who found a considerably higher incidence of *Botryosphaeriaceae* compared to other fungi in olive twig dieback samples in the USA. However, these authors also found a higher incidence of *Botryosphaeriaceae* in perennial cankers than in twig dieback samples. In our survey perennial cankers were not assessed as a single symptom type, but isolates of the *Botryosphaeriaceae* were also recovered from various internal wood symptoms that could have been associated with perennial cankers, although at very low incidences ( $\leq 5\%$ ).

*Diaporthe* species have been associated with dieback and decline symptoms of European olives in Italy, Spain, and the USA (Carlucci et al. 2013, Úrbez-Torres et al. 2013, Moral et al. 2017). Aside from *D. rudis* (reported as *D. viticola* by Úrbez-Torres et al. 2013), isolates of *Diaporthe* reported in those surveys were not conclusively identified to the species-level. Both Moral et al. (2017) and Úrbez-Torres et al. (2013) identified some isolates as *Diaporthe* sp. or *Phomopsis* sp. groups 1 and 2. However, inclusion of the ITS sequences of those isolates in our *Diaporthe* phylogeny suggests that these are in fact *D. foeniculina*. In the current survey, this species was the most prevalent *Diaporthe* species and the third most prevalent fungus overall on European olives. It was also recovered from three wild olive trees. Úrbez-Torres et al. (2013) found that both *D. foeniculina* (reported as *Phomopsis* sp. groups 1 and 2) and *D. rudis* caused significant lesions on olive branches, but these were considerably smaller than those caused by *N. mediterraneum* and *D. mutila*. Moral et al. (2017) on the other hand, reported asymptomatic infections by inoculated *D. foeniculina* isolates. *Diaporthe ambigua* has not been reported on olives globally, but has been associated with trunk disease and decline-related symptoms in apple, Japanese persimmon, grapevine, pear and plum trees and grapevines in South Africa (Smit et al. 1996, Van Niekerk et al. 2005, White et al. 2011a, Moyo et al. 2016). This species was only recovered from a single European olive tree during the current survey and its pathogenicity to this host is currently unknown.

All *Phaeoacremonium* species recorded on European and wild olive trees during this survey were previously reported on these hosts by Spies et al. (2018). Elsewhere in the world, *Phaeoacremonium* species have been implicated in olive dieback

and decline in Italy and the USA (Carlucci et al. 2008, 2013, 2015, Nigro et al. 2013, Úrbez-Torres et al. 2013). Species reported from these countries include *Pc. alvesii*, *Pc. italicum*, *Pc. minimum*, *Pc. parasiticum*, *Pc. rubrigenum*, *Pc. scolyti*, and *Pc. sicilianum*. In South Africa, *Pc. minimum*, *Pc. parasiticum* and *Pc. scolyti* have also been recovered from European olives (Spies et al. 2018; this study). With the exception of *Pc. rubrigenum*, all *Phaeoacremonium* species reported on European olives globally also occur on various woody hosts in South Africa (Mostert et al. 2006, Damm et al. 2008a, Cloete et al. 2011, White et al. 2011a, Moyo et al. 2016, Spies et al. 2018) and the aggressiveness of all species except *Pc. rubrigenum* has been confirmed on European olive trees (Carlucci et al. 2013, 2015, Úrbez-Torres et al. 2013). The only additional species on European olive in South Africa that have not been reported elsewhere in the world is *Pc. africanum* (Spies et al. 2018). Prior to that study, *Pc. africanum* had only been reported from apricot and was shown to be pathogenic to this host as well as to plum (Damm et al. 2008a). In Italy, Carlucci et al. (2013) reported the recovery of *Phaeoacremonium* (only *Pc. minimum*) mainly from olive trees older than 25 yr during a survey that included trees aged 18–35 yr. The incidence was not reported as the number of infected trees, but the overall percentage of tissue segments infected by *Pc. minimum* was low (2.1%). Two years later, Carlucci et al. (2015) reported high incidences of *Phaeoacremonium* spp. in olive trees both younger and older than 50 yr (respectively 73% and 100% of plants infected) in Italy. Compared to the latter study, the incidences of *Phaeoacremonium* in European olive trees in South Africa and the USA are quite low (11% and < 1.8%, respectively; this study, Úrbez-Torres et al. 2013). One possible explanation for this difference could be the age of the trees, since the majority of European olive trees sampled in the current survey were younger than 25 yr. This could also be a contributing factor to the higher incidence of *Phaeoacremonium* observed in the wild olive trees during this study, since, although the exact ages are not known, many of these trees appeared to be very old. However, the species of *Phaeoacremonium* most frequently recovered from wild olives was *P. oleae*, a species that is not known to occur on European olives, even though these two hosts are often found in close proximity in South Africa.

A wide range of additional fungi were recovered at lower incidences from European and wild olives during the current survey. These include some species reported as olive trunk pathogens elsewhere in the world, such as *Cytospora pruinosa* complex, *Eutypa lata*, *Pleurostoma richardsiae*, *Schizophyllum commune*, and *Trametes versicolor* (Rumbos 1993, Carlucci et al. 2008, 2013, 2015, Moral et al. 2010, 2017, Kaliterna et al. 2012, Úrbez-Torres et al. 2013). Several of the remaining fungi, however, have not previously been reported in association with dieback or decline of European olive trees, but are known as dieback or canker pathogens of other woody hosts. Examples of these include *Biscogniauxia rosacearum* (Raimondo et al. 2016), *Cryptovalsa ampelina* (Moyo et al. 2018a, b), *Didymosphaeria rubi-ulmifolii* and *Didymosphaeria variabile* (Damm et al. 2008b, Cloete et al. 2011). The pathogenicity of these and other fungi recovered in the current survey need to be confirmed on European olive trees in a South African context.

A total of 81 of the 99 fungal taxa identified during this survey had not previously been isolated from olive or wild olive trees globally (Yang et al. 2017, Farr & Rossman continuously updated). Some of these species are known or suspected trunk disease, dieback or decline pathogens of other crops; however, their pathogenicity to olive trees need to be established in order to determine the potential threat these species pose to the olive industry in South Africa. Based on the incidence and distribution of fungi recorded in this survey, *P. globosa* is likely to be of major concern, if it is shown to be pathogenic.



**Acknowledgements** The authors would like to express their gratitude to Palesa Lebenya, Danie Marais, Julia Marais, Bongwiwe Sokwaliwa, and Carine Vermeulen for assistance with sampling and isolations. We are also extremely grateful to Maria Luisa Raimondo (University of Foggia, Italy) for providing reference material and sequence data of *Pseudophaeoconiella oleicola* Ph58. CFJS was supported financially by the Department of Science and Technology (DST) and National Research Foundation (NRF).

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**Appendix 1** Numbers of European and wild olive samples collected from different districts in the Western Cape Province of South Africa. Districts are defined according to the Wine of Origin scheme, see [http://www.sawis.co.za/cert/download/Districts\\_-\\_Jan\\_2014.pdf](http://www.sawis.co.za/cert/download/Districts_-_Jan_2014.pdf).

	European olive	Wild olive
Calitzdorp	2	2
Ceres Plateau	3	4
Franschhoek	0	2
Lutzville Valley	24	10
Paarl	21	1
Robertson	2	1
Stellenbosch	27	16
Swartland	12	0
Tygerberg	14	4
Walker Bay	39	0
Wellington	0	2
Worcester	1	0
<b>TOTAL</b>	<b>145</b>	<b>42</b>



**Appendix 2** Species identities, host and location information for 440 fungal strains identified during this survey.

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>
<i>Anteaglonium</i> sp. CFJS-2015a	CSN641	Stellenbosch	European olive	MT813895	TreeBASE S26669, tree Tr125025
	CSN649	Stellenbosch	European olive	MT813897	TreeBASE S26669, tree Tr125025
<i>Anteaglonium</i> sp. CFJS-2015b	CSN642	Stellenbosch	European olive	MT813896	TreeBASE S26669, tree Tr125025
<i>Biscogniauxia rosacearum</i>	CSN1052	Stellenbosch	European olive	MT813910	TreeBASE S26669, tree Tr125028
	CSN1054	Wellington	Wild olive	MT813911	TreeBASE S26669, tree Tr125028
	CSN1055	Wellington	Wild olive	MT813912	TreeBASE S26669, tree Tr125028
	CSN1056	Stellenbosch	Wild olive	MT813913	TreeBASE S26669, tree Tr125028
	PMM2071	Stellenbosch	European olive	MT813997	TreeBASE S26669, tree Tr125028
<i>Calosphaeria africana</i>	CSN33	Robertson	European olive	MT813858	TreeBASE S26669, tree Tr125029
<i>Capronia</i> sp. CFJS-2015b	CSN1167	Paarl	European olive	MT813953	TreeBASE S26669, tree Tr125030
	CSN1168	Paarl	European olive	MT813954	TreeBASE S26669, tree Tr125030
	CSN1171	Stellenbosch	European olive	Not available	Morphological similarity to CSN1172
	CSN1172	Stellenbosch	European olive	MT814032	TreeBASE S26669, tree Tr125030
<i>Celerioriella umnquma</i>	CSN801	Durbanville	Wild olive	See Table 1	Fig. 3
	CSN1091	Somerset West	European olive	See Table 1	Fig. 3
	CSN1092	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN1901	Piketberg	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN1918	Vredendal	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN1922	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
<i>Clonostachys byssicola</i>	CSN1133	Durbanville	Wild olive	MT813941	TreeBASE S26669, tree Tr125032
<i>Colletotrichum acutatum</i>	CSN1066	Durbanville	European olive	MT813920	TreeBASE S26669, tree Tr125033
<i>Coniochaeta decumbens</i>	CSN654	Durbanville	Wild olive	MT813899	TreeBASE S26669, tree Tr125035
<i>Coniochaeta mutabilis</i>	PMM2016	Paarl	European olive	MT813987	TreeBASE S26669, tree Tr125035
<i>Coniochaeta velutina</i>	PMM2036	Stellenbosch	European olive	MT813993	TreeBASE S26669, tree Tr125035
<i>Coniothyrium ferrarisianum</i>	CSN587	Paarl	European olive	MT813876	TreeBASE S26669, tree Tr125042
	CSN588	Paarl	European olive	MT813877	TreeBASE S26669, tree Tr125042
	CSN590	Paarl	European olive	MT813878	TreeBASE S26669, tree Tr125042
	CSN632	Stellenbosch	European olive	MT813893	TreeBASE S26669, tree Tr125042
	CSN1063	Somerset West	European olive	MT813917	TreeBASE S26669, tree Tr125042
	CSN1064	Somerset West	European olive	MT813918	TreeBASE S26669, tree Tr125042
	CSN1067	Somerset West	European olive	MT813921	TreeBASE S26669, tree Tr125042
	CSN1069	Somerset West	European olive	MT813922	TreeBASE S26669, tree Tr125042
	CSN1070	Somerset West	European olive	MT813923	TreeBASE S26669, tree Tr125042
	CSN1071	Durbanville	European olive	MT813924	TreeBASE S26669, tree Tr125042
	CSN1072	Somerset West	European olive	MT813925	TreeBASE S26669, tree Tr125042
	CSN1073	Somerset West	European olive	MT813926	TreeBASE S26669, tree Tr125042
	PMM2039	Stellenbosch	European olive	MT813995	TreeBASE S26669, tree Tr125042
<i>Cosmospora</i> sp. CFJS-2015a	CSN1162	Stellenbosch	Wild olive	MT813948	TreeBASE S26669, tree Tr125036
<i>Cryptovalsa ampelina</i>	CSN1924	Vredendal	European olive	MT813973	TreeBASE S26669, tree Tr125046
<i>Cytospora pruinosa</i> complex	CSN577	Stellenbosch	European olive	MT813875	TreeBASE S26669, tree Tr125037
	CSN623	Riebeeck-Kasteel	European olive	MT814030	TreeBASE S26669, tree Tr125037
	ID0203	Ceres	Wild olive	MT813983	TreeBASE S26669, tree Tr125037
	PMM2025	Stellenbosch	European olive	MT814036	TreeBASE S26669, tree Tr125037
	PMM2026	Stellenbosch	European olive	MT813988	TreeBASE S26669, tree Tr125037
	PMM2029	Paarl	European olive	MT813989	TreeBASE S26669, tree Tr125037
	PMM2030	Paarl	European olive	MT813990	TreeBASE S26669, tree Tr125037
	PMM2033	Stellenbosch	European olive	MT813992	TreeBASE S26669, tree Tr125037
	PMM2077	Stellenbosch	European olive	MT813999	TreeBASE S26669, tree Tr125037
<i>Cytospora</i> sp. WvJ-2015a	CSN619	Stellenbosch	European olive	MT814028	TreeBASE S26669, tree Tr125037
	CSN620	Stellenbosch	European olive	MT813885	TreeBASE S26669, tree Tr125037
	CSN621	Durbanville	European olive	MT814029	TreeBASE S26669, tree Tr125037
	CSN622	Stellenbosch	European olive	MT813886	TreeBASE S26669, tree Tr125037
	CSN625	Stellenbosch	European olive	MT813887	TreeBASE S26669, tree Tr125037
	CSN627	Stellenbosch	European olive	MT813889	TreeBASE S26669, tree Tr125037
	CSN1153	Hermanus	European olive	MT813944	TreeBASE S26669, tree Tr125037
	<i>Diaporthe ambigua</i>	PMM2078	Stellenbosch	European olive	MT814000
<i>Diaporthe foeniculina</i>	CSN223	Calitzdorp	European olive	MT814020	TreeBASE S26669, tree Tr125044
	CSN224	Franschhoek	Wild olive	MT814021	TreeBASE S26669, tree Tr125044
	CSN225	Franschhoek	Wild olive	MT814022	TreeBASE S26669, tree Tr125044
	CSN296	Durbanville	European olive	MT813863	TreeBASE S26669, tree Tr125044
	CSN297	Durbanville	European olive	MT813864	TreeBASE S26669, tree Tr125044
	CSN301	Durbanville	European olive	MT814023	TreeBASE S26669, tree Tr125044
	CSN306	Durbanville	European olive	MT814024	TreeBASE S26669, tree Tr125044
	CSN307	Stellenbosch	European olive	MT813865	TreeBASE S26669, tree Tr125044
	CSN321	Riebeeck-Kasteel	European olive	MT814025	TreeBASE S26669, tree Tr125044
	CSN338	Stellenbosch	Wild olive	Not available	Species specific PCR, assay of Lesuthu et al. (2019)
	CSN343	Stellenbosch	Wild olive	MT813866	TreeBASE S26669, tree Tr125044
	CSN348	Paarl	European olive	MT813867	TreeBASE S26669, tree Tr125044
	CSN549	Somerset West	European olive	MT814026	TreeBASE S26669, tree Tr125044
	CSN550	Somerset West	European olive	MT814027	TreeBASE S26669, tree Tr125044
	CSN867	Hermanus	European olive	MT813903	TreeBASE S26669, tree Tr125044
	CSN866	Hermanus	European olive	MT813902	TreeBASE S26669, tree Tr125044
	PMM2076	Stellenbosch	European olive	MT813998	TreeBASE S26669, tree Tr125044
	PMM2079	Stellenbosch	European olive	MT814001	TreeBASE S26669, tree Tr125044
	PMM2080	Stellenbosch	European olive	MT814002	TreeBASE S26669, tree Tr125044

## Appendix 2 (cont.)

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>
<i>Diaporthe foeniculina</i> (cont.)	PMM2081	Paarl	European olive	MT814003	TreeBASE S26669, tree Tr125044
	PMM2083	Stellenbosch	European olive	MT814004	TreeBASE S26669, tree Tr125044
	PMM2161	Bonnievale	Wild olive	MT814011	TreeBASE S26669, tree Tr125044
<i>Didymocyrtis banksiae</i>	CSN1049	Hermanus	European olive	MT813909	TreeBASE S26669, tree Tr125042
	CSN1050	Hermanus	European olive	Not available	Morphological similarity to CSN1049
	CSN1065	Wellington	Wild olive	MT813919	TreeBASE S26669, tree Tr125042
<i>Didymosphaeria rubi-ulmifolii</i>	CSN634	Somerset West	European olive	MT813894	TreeBASE S26669, tree Tr125047
	CSN1150	Paarl	Wild olive	MT813942	TreeBASE S26669, tree Tr125047
<i>Didymosphaeria variabile</i>	CSN618	Riebeeck-Kasteel	European olive	MT813884	TreeBASE S26669, tree Tr125047
	CSN1932	Vredendal	European olive	MT813980	TreeBASE S26669, tree Tr125047
<i>Diplodia seriata</i>	ID0683	Hermanus	European olive	MT813193 (EF), MT813986 (ITS)	TreeBASE S26669, tree Tr125045
	PMM2093	Paarl	European olive	MT814037	TreeBASE S26669, tree Tr125045
<i>Eutypa lata</i>	ID0305	Ceres	European olive	Not available	Morphologically similar to ID0318
	ID0318	Ceres	Wild olive	MT813985	TreeBASE S26669, tree Tr125046
	ID0319	Ceres	Wild olive	Not available	Morphologically similar to ID0318
	PMM2905	Riebeeck-Kasteel	European olive	MT814012	TreeBASE S26669, tree Tr125046
	PMM2907	Durbanville	Wild olive	Not available	Morphologically similar to PMM2905
	PMM3064	Stellenbosch	European olive	Not available	Morphologically similar to PMM3071
	PMM3066	Stellenbosch	European olive	Not available	Morphologically similar to PMM3071
	PMM3067	Stellenbosch	European olive	Not available	Morphologically similar to PMM3071
	PMM3068	Hermanus	European olive	Not available	Morphologically similar to PMM3071
	PMM3069	Hermanus	European olive	Not available	Morphologically similar to PMM3071
	PMM3070	Hermanus	European olive	Not available	Morphologically similar to PMM3071
PMM3071	Hermanus	European olive	MT814013	TreeBASE S26669, tree Tr125046	
<i>Exophiala sideris</i>	CSN1190	Hermanus	European olive	MT813960	TreeBASE S26669, tree Tr125030
<i>Exophiala</i> sp. CFJS-2015a	CSN1170	Paarl	European olive	MT814031	TreeBASE S26669, tree Tr125030
<i>Exophiala</i> sp. CFJS-2015b	CSN995	Hermanus	European olive	MT813908	TreeBASE S26669, tree Tr125030
<i>Exophiala xenobiotica</i>	CSN1930	Vredendal	European olive	MT813978	TreeBASE S26669, tree Tr125030
<i>Fomitiporella</i> sp. (Taxon 1)	CSN503	Paarl	European olive	Not available	Morphologically similar to PMM2086
	CSN505	Paarl	European olive	Not available	Morphologically similar to PMM2086
	CSN518	Paarl	European olive	Not available	Morphologically similar to PMM2086
	CSN944	Hermanus	European olive	MT813904	TreeBASE S26669, tree Tr125048
	CSN1936	Vredendal	European olive	MT813982	TreeBASE S26669, tree Tr125048
	PMM2086	Paarl	European olive	MT814042	TreeBASE S26669, tree Tr125048
<i>Geosmithia</i> sp. CFJS-2015a	CSN158	Calitzdorp	Wild olive	MT813861	TreeBASE S26669, tree Tr125049
	CSN159	Calitzdorp	European olive	MT813862	TreeBASE S26669, tree Tr125049
	PMM2037	Paarl	European olive	MT813994	TreeBASE S26669, tree Tr125049
<i>Helminthosporium asterinum</i>	CSN1166	Stellenbosch	European olive	MT813952	BLAST – 97.13 %) ITS identity to <i>Ellisembia asterinum</i> CBS 203.35 AF073918 (98 %) coverage. No suitable reference sequences available for phylogenetic analysis.
<i>Herpotrichiellaceae</i> sp. CFJS-2015a	CSN1211	Durbanville	Wild olive	MT813965	TreeBASE S26669, tree Tr125030
<i>Heterophoma</i> sp.	CSN1929	Vredendal	European olive	MT813977	TreeBASE S26669, tree Tr125050
<i>Hysterium</i> sp. CFJS-2015a	CSN1227	Hermanus	European olive	MT813971	TreeBASE S26669, tree Tr125038
<i>Hysterium</i> sp. CFJS-2015b	CSN1108	Paarl	Wild olive	MT813937	TreeBASE S26669, tree Tr125038
<i>Jattaea</i> sp. CFJS-2015a	CSN1152	Stellenbosch	European olive	MT813943	TreeBASE S26669, tree Tr125029
<i>Kirschsteiniothelia</i> sp. CFJS-2015a	CSN602	Paarl	European olive	MT813880	TreeBASE S26669, tree Tr125039
	CSN604	Wellington	Wild olive	MT813881	TreeBASE S26669, tree Tr125039
	CSN605	Paarl	European olive	MT813882	TreeBASE S26669, tree Tr125039
<i>Lembosiniella</i> sp. CFJS-2015a	CSN1210	Hermanus	European olive	MT813964	TreeBASE S26669, tree Tr125040
	CSN1225	Hermanus	European olive	MT813970	TreeBASE S26669, tree Tr125040
<i>Leptosillia</i> sp. CFJS-2015a	PMM2101	Paarl	European olive	MT814010	TreeBASE S26669, tree Tr125041
<i>Lophiostoma cynaroidis</i>	CSN1107	Wellington	Wild olive	MT813936	TreeBASE S26669, tree Tr125051
	CSN1178	Paarl	European olive	MT813958	TreeBASE S26669, tree Tr125051
<i>Meyerozyma guilliermondii</i>	CSN1219	Hermanus	European olive	MT813966	TreeBASE S26669, tree Tr125052
	CSN1223	Hermanus	European olive	MT813968	TreeBASE S26669, tree Tr125052
<i>Mycocalicium victoriae</i>	CSN1128	Hermanus	European olive	MT813939	TreeBASE S26669, tree Tr125053
	CSN1129	Somerset West	European olive	Not available	Morphological similarity to CSN1128
	CSN1130	Hermanus	European olive	MT813940	TreeBASE S26669, tree Tr125053
	CSN1131	Hermanus	European olive	Not available	Morphological similarity to CSN1128
	CSN1194	Hermanus	European olive	MT813961	TreeBASE S26669, tree Tr125053
<i>Neocucurbitaria cava/juglandicola</i>	CSN631	Stellenbosch	European olive	MT813892	TreeBASE S26669, tree Tr125054
<i>Neocucurbitaria unguis-hominis</i>	CSN629	Paarl	European olive	MT813890	TreeBASE S26669, tree Tr125054
<i>Neodevriesia fraseriae</i>	CSN1169	Somerset West	European olive	MT813955	TreeBASE S26669, tree Tr125055
<i>Neofusicoccum australe</i>	ID0395	Riebeeck-Kasteel	European olive	MT274485, MT295262	TreeBASE S26669, tree Tr125056
	ID0403	Stellenbosch	European olive	MT274487, MT295264	TreeBASE S26669, tree Tr125056
	ID0493	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
	ID0498	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
	ID0499	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
	ID0500	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
	ID0507	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
	ID0508	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056
					DNA fingerprinting, protocol of Alves et al. (2007)



## Appendix 2 (cont.)

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>	
<i>Neofusicoccum australe</i> (cont.)	ID0656	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0663	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0671	Hermanus	European olive	Not available	DNA fingerprinting, protocol of Alves et al. (2007)	
	ID0672	Hermanus	European olive	Not available	DNA fingerprinting, protocol of Alves et al. (2007)	
	ID0677	Hermanus	European olive	Not available	DNA fingerprinting, protocol of Alves et al. (2007)	
	ID0678	Hermanus	European olive	Not available	DNA fingerprinting, protocol of Alves et al. (2007)	
	ID0681	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2094	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2095	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125056	
<i>Neofusicoccum cryptoaustrale/stellenboschiana</i> <sup>3</sup>	CSN179	Strand	Wild olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0416	Stellenbosch	European olive	MT274489, MT295266	TreeBASE S26669, tree Tr125056	
	ID0489	Durbanville	European olive	MT274491, MT295268	TreeBASE S26669, tree Tr125056	
	ID0490	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0491	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0492	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0494	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0496	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0658	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0661	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0664	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0665	Hermanus	European olive	Not available	DNA fingerprinting, protocol of Alves et al. (2007)	
	ID0666	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0668	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0669	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0673	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0674	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0680	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0744	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0837	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2089	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2096	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125056	
	<i>Neofusicoccum</i> sp. 4	ID0660	Hermanus	European olive	MT274493, MT295270	TreeBASE S26669, tree Tr125056
<i>Neofusicoccum</i> sp. 8	ID0828	Hermanus	European olive	MT274494, MT295271	TreeBASE S26669, tree Tr125056	
	ID0847	Hermanus	European olive	MT274495, MT295272	TreeBASE S26669, tree Tr125056	
<i>Neofusicoccum</i> sp. PMM-2014a	PMM2097	Paarl	European olive	MT814007	TreeBASE S26669, tree Tr125056	
	PMM2098	Paarl	European olive	MT814008	TreeBASE S26669, tree Tr125056	
	PMM2100	Paarl	European olive	MT814009	TreeBASE S26669, tree Tr125056	
<i>Neofusicoccum</i> sp. WvJ-2015a	CSN180	Franschhoek	Wild olive	MT274479, MT295256	TreeBASE S26669, tree Tr125056	
	ID0396	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125056	
	ID0402	Stellenbosch	European olive	MT274486, MT295263	TreeBASE S26669, tree Tr125056	
	ID0417	Stellenbosch	European olive	MT274490, MT295267	TreeBASE S26669, tree Tr125056	
	ID0495	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2090	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2091	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2092	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125056	
	PMM2099	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125056	
	<i>Neofusicoccum vitifusiforme</i>	CSN182	Franschhoek	Wild olive	MT274497, MT295274	TreeBASE S26669, tree Tr125056
ID0827		Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125056	
<i>Neophaeomoniella niveniae</i>	CSN742	Stellenbosch	Wild olive	See Table 1	Fig. 3	
	CSN985	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034	
	CSN1916	Klawer	Wild olive	Not available	TreeBASE S26669, tree Tr125034	
	CSN1919	Klawer	Wild olive	Not available	TreeBASE S26669, tree Tr125034	
<i>Neophaeomoniella zymoides</i>	CSN743	Stellenbosch	Wild olive	See Table 1	Fig. 3	
	CSN986	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034	
	CSN1913	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034	
<i>Nigrograna</i> sp. CFJS-2015a	CSN591	Stellenbosch	European olive	MT813879	TreeBASE S26669, tree Tr125057	
<i>Nigrospora zimmermanii</i>	CSN1157	Riebeeck-Kasteel	European olive	MT813945	TreeBASE S26669, tree Tr125058	
<i>Parapyrenochaeta protearum</i>	CSN1911	Stellenbosch	European olive	MT813972	TreeBASE S26669, tree Tr125059	
<i>Peniophora lycii</i>	CSN371	Stellenbosch	European olive	MT813868	TreeBASE S26669, tree Tr125061	
	CSN509	Stellenbosch	European olive	Not available	Morphologically similar to CSN371	
<i>Phaeoacremonium africanum</i>	CSN946	Durbanville	European olive	KY906773	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
<i>Phaeoacremonium minimum</i>	PMM2073	Stellenbosch	European olive	KY906895	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
<i>Phaeoacremonium oleae</i>	CSN403	Paarl	Wild olive	KY906719	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
	CSN413	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	CSN703	Stellenbosch	Wild olive	KY906751	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
	CSN720	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	CSN721	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	CSN945	Durbanville	Wild olive	KY906771	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
	CSN1154	Durbanville	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	ID0231	Ceres	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	PMM1980	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125062	
	PMM1981	Stellenbosch	Wild olive	KY906891	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
	PMM2440	Bonnievale	Wild olive	KY906937	TreeBASE S26669, tree Tr125062; Spies et al. 2018	
	<i>Phaeoacremonium parasiticum</i>	CSN418	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125062
		CSN476	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125062
CSN624		Durbanville	European olive	KY906731	TreeBASE S26669, tree Tr125062; Spies et al. 2018	

## Appendix 2 (cont.)

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>
<i>Phaeoacremonium prunicola</i>	ID0230	Ceres	Wild olive	KY906817	TreeBASE S26669, tree Tr125062; Spies et al. 2018
<i>Phaeoacremonium scolyti</i>	CSN676	Paarl	European olive	KY906743	TreeBASE S26669, tree Tr125062; Spies et al. 2018
	CSN1193	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1196	Hermanus	European olive	KY906779	TreeBASE S26669, tree Tr125062; Spies et al. 2018
	CSN1199	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1200	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1201	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1205	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1206	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1208	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1212	Stellenbosch	Wild olive	KY906781	TreeBASE S26669, tree Tr125062; Spies et al. 2018
	CSN1213	Paarl	European olive	KY906783	TreeBASE S26669, tree Tr125062; Spies et al. 2018
	CSN1214	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1215	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1217	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125062
	CSN1218	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125062
<i>Phaeoacremonium spadicum</i>	ID0208	Ceres	Wild olive	KY906815	TreeBASE S26669, tree Tr125062; Spies et al. 2018
<i>Phaeoannellomyces elegans</i>	CSN1921	Klaver	Wild olive	MT814034	TreeBASE S26669, tree Tr125030
<i>Phialemoniopsis comearis</i>	CSN1175	Somerset West	European olive	MT813956	TreeBASE S26669, tree Tr125063
<i>Phialemoniopsis ocularis</i>	CSN1177	Riebeeck-Kasteel	European olive	MT813957	TreeBASE S26669, tree Tr125063
	CSN1183	Durbanville	Wild olive	MT814033	TreeBASE S26669, tree Tr125063
	CSN1224	Hermanus	European olive	MT813969	TreeBASE S26669, tree Tr125063
<i>Phialocephala oblonga</i>	CSN630	Stellenbosch	European olive	MT813891	TreeBASE S26669, tree Tr125064
<i>Phialocephala</i> sp. CFJS-2015b	CSN1185	Stellenbosch	European olive	MT813959	TreeBASE S26669, tree Tr125064
<i>Phlebia acerina</i>	PMM2070	Stellenbosch	European olive	MT813996	TreeBASE S26669, tree Tr125065
<i>Pleosporineae</i> sp. CFJS-2015a	CSN650	Riebeeck-Kasteel	European olive	MT813898	TreeBASE S26669, tree Tr125059
	CSN1923	Stellenbosch	European olive	MT814035	TreeBASE S26669, tree Tr125059
<i>Pleurostoma richardsiae</i>	CSN144	Robertson	European olive	MT813859	TreeBASE S26669, tree Tr125029
	CSN145	Robertson	European olive	MT813860	TreeBASE S26669, tree Tr125029
	CSN493	Paarl	European olive	MT813870	TreeBASE S26669, tree Tr125029
	CSN495	Paarl	Wild olive	Not available	Morphological characteristics
	CSN496	Paarl	European olive	MT813871	TreeBASE S26669, tree Tr125029
	CSN500	Durbanville	European olive	MT813872	TreeBASE S26669, tree Tr125029
	CSN501	Botrivier	European olive	MT813873	TreeBASE S26669, tree Tr125029
	CSN514	Paarl	European olive	MT813874	TreeBASE S26669, tree Tr125029
	CSN515	Stellenbosch	Wild olive	Not available	Morphological characteristics
	CSN947	Hermanus	European olive	MT813905	TreeBASE S26669, tree Tr125029
	CSN1101	Hermanus	European olive	MT813934	TreeBASE S26669, tree Tr125029
	CSN1161	Hermanus	European olive	MT813947	TreeBASE S26669, tree Tr125029
	CSN1925	Klaver	Wild olive	MT813974	TreeBASE S26669, tree Tr125029
	PMM2011	Stellenbosch	European olive	Not available	Morphological characteristics
	PMM2012	Paarl	European olive	Not available	Morphological characteristics
	PMM2013	Paarl	European olive	Not available	Morphological characteristics
<i>Preussia africana</i>	CSN626	Riebeeck-Kasteel	European olive	MT813888	TreeBASE S26669, tree Tr125066
<i>Preussia minima</i>	CSN1111	Riebeeck-Kasteel	European olive	MT813938	TreeBASE S26669, tree Tr125066
<i>Pseudocamarosporium africanum</i>	CSN1104	Paarl	Wild olive	MT813935	TreeBASE S26669, tree Tr125067
<i>Pseudolophiostoma</i> sp. CFJS-2015a	CSN1198	Hermanus	European olive	MT813962	TreeBASE S26669, tree Tr125051
<i>Pseudophaeoniella globosa</i>	CSN18	Franschhoek	Wild olive	See Table 1	Fig. 3
	CSN19	Franschhoek	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN41	Strand	Wild olive	See Table 1	Fig. 3
	CSN183	Calitzdorp	Wild olive	See Table 1	Fig. 3
	CSN185	Robertson	European olive	See Table 1	Fig. 3
	CSN186	Calitzdorp	European olive	See Table 1	Fig. 3
	CSN294	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN299	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN304	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN305	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN310	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN314	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN315	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN319	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN325	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN329	Stellenbosch	Wild olive	See Table 1	Fig. 3
	CSN334	Paarl	Wild olive	See Table 1	Fig. 3
	CSN339	Paarl	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN344	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN349	Paarl	European olive	See Table 1	Fig. 3
	CSN375	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN377	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN381	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN382	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN385	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN386	Wellington	Wild olive	See Table 1	Fig. 3
	CSN390	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN391	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN395	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN396	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034



## Appendix 2 (cont.)

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>
<i>Pseudophaeomoniella globosa</i> (cont.)	CSN397	Riebeeck-Kasteel	European olive	Not available	Species specific PCR (Van Dyk 2020)
	CSN400	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN401	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN405	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN409	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN410	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN412	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN424	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN427	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN435	Riebeeck-Kasteel	European olive	See Table 1	Fig. 3
	CSN441	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN446	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN448	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN451	Stellenbosch	European olive	See Table 1	Fig. 3
	CSN463	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN726	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN727	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN728	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN729	Paarl	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN730	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN731	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN733	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN735	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN736	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN737	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN738	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN739	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN746	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN750	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN751	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN752	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN753	Stellenbosch	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN754	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN755	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN756	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN757	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN759	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN765	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN766	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN769	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN771	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN788	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN791	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN792	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN799	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN800	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN802	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN803	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN804	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN806	Durbanville	Wild olive	See Table 1	Fig. 3
	CSN808	Durbanville	European olive	See Table 1	Fig. 3
	CSN816	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN818	Durbanville	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN824	Somerset West	European olive	See Table 1	Fig. 3
	CSN825	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN831	Riebeeck-Kasteel	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN834	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN835	Durbanville	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN838	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN950	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN952	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN954	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN955	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN956	Wellington	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN960	Hermanus	European olive	See Table 1	Fig. 3
	CSN961	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN962	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN965	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN966	Hermanus	European olive	Not available	Species specific PCR (Van Dyk 2020)
	CSN968	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN971	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN972	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN973	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN976	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN979	Somerset West	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN982	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN991	Hermanus	European olive	Not available	TreeBASE S26669, tree Tr125034
	CSN1900	Piketberg	European olive	Not available	Species specific PCR (Van Dyk 2020)
	CSN1914	Klaver	Wild olive	Not available	TreeBASE S26669, tree Tr125034
	CSN1915	Klaver	Wild olive	Not available	Species specific PCR (Van Dyk 2020)
	CSN1920	Lutzville	Wild olive	Not available	Species specific PCR (Van Dyk 2020)
	ID0250	Ceres	Wild olive	Not available	TreeBASE S26669, tree Tr125034

## Appendix 2 (cont.)

Species	Strain <sup>1</sup>	Location	Host	GenBank	Basis for identification <sup>2</sup>	
<i>Pseudophaeomoniella globosa</i> (cont.)	ID0251	Ceres	European olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0253	Ceres	European olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0255	Ceres	Wild olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0256	Ceres	European olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0258	Ceres	European olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0263	Ceres	Wild olive	Not available	TreeBASE S26669, tree Tr125034	
	ID0264	Ceres	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM1192	Vredendal	European olive	See Table 1	Fig. 3	
	PMM2017	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM2018	Paarl	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM2044	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM2047	Paarl	European olive	Not available	Morphological similarity to PMM2044	
	PMM2052	Paarl	European olive	Not available	Morphological similarity to PMM2044	
	PMM2057	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM2060	Stellenbosch	European olive	Not available	TreeBASE S26669, tree Tr125034	
	PMM2061	Stellenbosch	European olive	Not available	Species specific PCR (Van Dyk 2020)	
	PMM2484	Bonnievale	Wild olive	See Table 1	Fig. 3	
	PMM2485	Bonnievale	Wild olive	Not available	TreeBASE S26669, tree Tr125034	
	<i>Punctularia atropurpurascens</i>	CSN1060	Durbanville	Wild olive	MT813915	TreeBASE S26669, tree Tr125068
		CSN1061	Wellington	Wild olive	MT813916	TreeBASE S26669, tree Tr125068
<i>Sarocladium strictum</i>	CSN1202	Hermanus	European olive	MT813963	TreeBASE S26669, tree Tr125069	
	CSN1220	Hermanus	European olive	MT813967	TreeBASE S26669, tree Tr125069	
<i>Schizophyllum commune</i>	CSN336	Paarl	European olive	Not available	Morphological similarity to PMM2088.	
	CSN528	Paarl	European olive	Not available	Morphological similarity to PMM2088.	
	CSN1160	Hermanus	European olive	MT813946	TreeBASE S26669, tree Tr125070	
	PMM2087	Stellenbosch	European olive	MT814005	TreeBASE S26669, tree Tr125070	
<i>Symbiotaphrina microtheca</i>	CSN615	Stellenbosch	European olive	MT813883	TreeBASE S26669, tree Tr125071	
	CSN1163	Hermanus	European olive	MT813949	TreeBASE S26669, tree Tr125071	
	CSN1164	Hermanus	European olive	MT813950	TreeBASE S26669, tree Tr125071	
	CSN1165	Hermanus	European olive	MT813951	TreeBASE S26669, tree Tr125071	
<i>Teichospora</i> sp. CFJS-2015a	CSN953	Durbanville	Wild olive	MT813906	TreeBASE S26669, tree Tr125051	
	CSN1083	Stellenbosch	European olive	MT813927	TreeBASE S26669, tree Tr125051	
	CSN1084	Durbanville	Wild olive	MT813928	TreeBASE S26669, tree Tr125051	
	CSN1085	Stellenbosch	European olive	MT813929	TreeBASE S26669, tree Tr125051	
	CSN1086	Paarl	European olive	MT813930	TreeBASE S26669, tree Tr125051	
	CSN1087	Paarl	Wild olive	MT813931	TreeBASE S26669, tree Tr125051	
	CSN1088	Paarl	European olive	MT813932	TreeBASE S26669, tree Tr125051	
<i>Torula ficus</i>	PMM2032	Stellenbosch	European olive	MT813991	TreeBASE S26669, tree Tr125072	
<i>Trametes versicolor</i>	CSN1058	Stellenbosch	European olive	MT813914	TreeBASE S26669, tree Tr125073	
	ID0244	Ceres	European olive	MT813984	TreeBASE S26669, tree Tr125073	
<i>Tympanis</i> sp. CFJS-2015a	CSN1093	Hermanus	European olive	MT813933	TreeBASE S26669, tree Tr125074	
Unknown – aff. <i>Anthopsis catenata</i>	CSN406	Paarl	European olive	MT813869	BLAST – 81.89 % ITS identity to <i>Anthopsis catenata</i> CBS 492.81 NR_159623 (87 % coverage).	
Unknown – aff. <i>Phaeomoniellales</i>	CSN783	Riebeeck-Kasteel	European olive	MT813672 (18S), MT814041 (ITS)	Partial 18S BLAST (404bp) – 94.43 % identity to <i>Pseudophaeomoniella oleicola</i> CBS 139192 KP411807 (88 % coverage). ITS BLAST – No significant similarity found.	
Unknown – <i>Pleosporales</i> sp.	CSN1927	Vredendal	European olive	MT813976	TreeBASE S26669, tree Tr125075	
Unknown – <i>Pleosporales</i> sp.	CSN1933	Vredendal	European olive	MT813981	TreeBASE S26669, tree Tr125075	
Unknown – <i>Pleosporales</i> sp.	CSN1926	Vredendal	European olive	MT813975	TreeBASE S26669, tree Tr125075	
Unknown – putative <i>Bezerromycetales</i> sp.	CSN1931	Klawer	Wild olive	MT813979	TreeBASE S26669, tree Tr125076	
Unknown – putative <i>Debaryomycetaceae</i> sp.	CSN781	Stellenbosch	European olive	MT813901	TreeBASE S26669, tree Tr125052	
Unknown – putative <i>Verrucariaceae</i> sp.	CSN741	Paarl	European olive	MT813900	TreeBASE S26669, tree Tr125060	
<i>Vredendaliella oleae</i>	PMM1193	Vredendal	European olive	See Table 1	Fig. 3	
<i>Xenocyliandrosporium margaritarum</i>	CSN1179	Paarl	European olive	See Table 1	Fig. 3	
	CSN1216	Somerset West	European olive	See Table 1	Fig. 3	
	CSN1917	Klawer	Wild olive	See Table 1	Fig. 3	
<i>Xenocyliandrosporium</i> sp. CFJS-2015c	CSN1180	Paarl	European olive	See Table 1	Fig. 3	
	CSN1184	Stellenbosch	European olive	See Table 1	Fig. 3	
	CSN1203	Hermanus	European olive	See Table 1	Fig. 3	
<i>Xenocyliandrosporium</i> sp. CFJS-2015e	CSN1222	Hermanus	European olive	See Table 1	Fig. 3	
<i>Xenocyliandrosporium</i> sp. CFJS-2015f	CSN1191	Hermanus	European olive	See Table 1	Fig. 3	
<i>Xenocyliandrosporium</i> sp. CFJS-2015g	CSN1174	Somerset-West	European olive	See Table 1	Fig. 3	
<i>Xylonomycetes</i> sp. CFJS-2015a	CSN958	Hermanus	European olive	MT813907	TreeBASE S26669, tree Tr125071	

<sup>1</sup> CSN: collection of Chris Spies at ARC-Nietvoorbij, Stellenbosch, South Africa; ID: collection of Ihan du Plessis at ARC-Nietvoorbij, Stellenbosch, South Africa; PMM: collection of Providence Moyo at the University of Stellenbosch, Department of Plant Pathology, Stellenbosch, South Africa.

<sup>2</sup> Phylogenies are referred to by figure reference or TreeBASE accession numbers. Details of other methods of identification are provided.

<sup>3</sup> In the current investigation, these two species could not be distinguished using ITS, *TEF1α*, and *TUB2* sequence data alone or in combination.