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RESEARCH ARTICLE

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Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees

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Key words

Diaporthales
molecular systematics
pathogenicity
Togninia
Togniniaceae

Abstract The genus *Phaeoacremonium* is associated with opportunistic human infections, as well as stunted growth and die-back of various woody hosts, especially grapevines. In this study, *Phaeoacremonium* species were isolated from necrotic woody tissue of *Prunus* spp. (plum, peach, nectarine and apricot) from different stone fruit growing areas in South Africa. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS1, ITS2, β-tubulin, actin and 18S rDNA) were used to identify known, and describe novel species. From the total number of wood samples collected (257), 42 *Phaeoacremonium* isolates were obtained, from which 14 species were identified. *Phaeoacremonium scolyti* was most frequently isolated, and present on all *Prunus* species sampled, followed by *Togninia minima* (anamorph: *Pm. aleophilum*) and *Pm. australiense.* Almost all taxa isolated represent new records on *Prunus*. Furthermore, *Pm. australiense*, *Pm. iranianum*, *T. fraxinopennsylvanica* and *Pm. griseorubrum* represent new records for South Africa, while *Pm. griseorubrum*, hitherto only known from humans, is newly reported from a plant host. Five species are newly described, two of which produce a *Togninia* sexual state. *Togninia africana, T. griseo-olivacea* and *Pm. pallidum* are newly described from *Prunus armeniaca*, while *Pm. prunicolum* and *Pm. fuscum* are described from *Prunus salicina*.

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INTRODUCTION

The genus *Phaeoacremonium* was established 12 years ago (Crous et al. 1996) to accommodate cephalosporium-like fungi known from grapevine (Petri 1912) and human infections (Ajello et al. 1974). In spite of the exclusion of *Pm. chlamydospora* (now *Phaeomoniella chlamydospora*, Crous & Gams 2000), the number of known species increased quickly. The genus *Togninia* was confirmed as teleomorph (Mostert et al. 2003), and their phylogenetic position clarified within the Togniniaceae in the Diaporthales (Réblová et al. 2004). Presently 23 *Phaeoacremonium* species have been described, of which 10 have been linked to *Togninia* teleomorphs (Mostert et al. 2006a, Réblová & Mostert 2007). *Togninia* species known to date have been shown to have either a homo- or heterothallic mating strategy (Mostert et al. 2003, 2006a, Rooney-Latham et al. 2005). In species where the mating strategy has been studied in more detail, like *T. minima*, both mating types have been found to occur in the same field, and even the same grapevine (Mostert et al. 2003, Pascoe et al. 2004, Rooney-Latham et al. 2005). Recent research has focused on the development of genus- and species-specific primers to facilitate early detection (Mostert et al. 2006a, Aroca & Raposo 2007), and the development of an online polyphasic identification system (www.cbs.knaw.nl/phaeoacremonium/biolomics.aspx).

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Phaeoacremonium species have been associated with human infections, often skin- or nail-infections, so-called phaeohyphomycoses (Ajello et al. 1974, Guarro et al. 2003, Hemashettar et al. 2006), as well as disease symptoms of a number of woody hosts worldwide (Rumbos 1986, Di Marco et al. 2004, Kubátová et al. 2004), especially with grapevine diseases such as Petri disease and esca (Pascoe et al. 2004, Rooney-Latham et al. 2005, Whiting et al. 2005, Mostert et al. 2006b, Aroca & Raposo 2007). Only two *Phaeoacremonium* species are known from *Prunus*. *Phaeoacremonium aleophilum* (*T. minima*) was reported on *Prunus pennsylvanica* in Canada (Hausner et al. 1992) and *P. armeniaca* in South Africa (Mostert et al. 2006a). *Phaeoacremonium parasiticum* (*T. parasitica*) was isolated from wilting trees of *P. armeniaca* in Tunisia (Hawksworth et al. 1976) and from *P. avium* in Greece (Rumbos 1986). Rumbos (1986) described *Pm. parasiticum* as causal agent of a serious dieback disease of cherry trees in different locations in Greece in the 1980s. Several cherry cultivars were found to be susceptible to the disease, which caused leaf drop, wilting and wood discolouration. In one orchard, were the fungus was closely associated with bark beetles (Scolytidae) and metallic wood-boring beetles (Buprestidae), the majority of the trees were affected and died. *Phaeoacremonium parasiticum* caused xylem lesions in cherry, apricot, olive and peach trees (Rumbos 1986).

In South Africa, stone fruit orchards are often established in close proximity of vineyards. Thirteen *Phaeoacremonium* species have been reported from *Vitis vinifera*, eight of which are known from South Africa, where *T. minima* was found on *Vitis* and *Prunus* (Mostert et al. 2006a). It is possible that this pathogen disseminates from one host to another. This phenomenon may be more common among phytopathogenic ascomycetes than previously accepted, as several species of Botryosphaeriaceae,

Table 1 Names, accession numbers and collection details of isolates studied.

Species	Accession No.[1]	Host	Location	Pathotest[2]	GenBank accessions			
					ITS	TUB	ACT	SSU
Phaeoacremonium australiense	STE-U 5960	*Prunus salicina*	Paarl, Western Cape, South Africa		EU128021	EU128069	EU128111	
	STE-U 5961	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128022	EU128070	EU128112	
	STE-U 5839	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128023	EU128071	EU128113	
	STE-U 5838	*P. salicina*	Paarl, Western Cape, South Africa		EU128024	EU128072	EU128114	EU128055
	STE-U 5959, CBS 120861	*P. salicina*	Stellenbosch, Western Cape, South Africa		EU128025	EU128073	EU128115	EU128054
Pm. fuscum	STE-U 5969, CBS 120856*	*P. salicina*	Mookgopong, Limpopo, South Africa	x	EU128050	EU128098	EU128141	EU128059
	STE-U 6366	*P. salicina*	Mookgopong, Limpopo, South Africa		EU128051	EU128199	EU128140	
Pm. griseorubrum	STE-U 5957, CBS 120860	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128026	EU128074	EU128116	
	STE-U 5958	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128027	EU128075	EU128117	
Pm. iranianum	STE-U 6092	*P. armeniaca*	Robertson, Western Cape, South Africa	x	EU128028	EU128076	EU128118	
	STE-U 6179	*P. armeniaca*	Montagu, Western Cape, South Africa	x	EU128029	EU128077	EU128119	
	STE-U 6091, CBS 120864	*P. armeniaca*	Robertson, Western Cape, South Africa		EU128030	EU128078	EU128120	
Pm. pallidum	STE-U 6104, CBS 120862*	*P. armeniaca*	Bonnievale, Western Cape, South Africa	x	EU128053	EU128103	EU128144	EU128061
Pm. prunicolum	STE-U 5967, CBS 120858*	*P. salicina*	Mookgopong, Limpopo, South Africa	x	EU128047	EU128095	EU128137	EU128056
	STE-U 5968	*P. salicina*	Mookgopong, Limpopo, South Africa	x	EU128048	EU128096	EU128138	EU128057
Pm. scolyti	STE-U 5955, CBS121755	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	x	EU128034	EU128082	EU128124	
	STE-U 6095, CBS 121438	*P. armeniaca*	Robertson, Western Cape, South Africa		EU128035	EU128083	EU128125	
	STE-U 6096	*P. armeniaca*	Bonnievale, Western Cape, South Africa		EU128036	EU128084	EU128126	
	STE-U 6097	*P. persica*	Modimolle, Limpopo, South Africa		EU128037	EU128085	EU128127	
	STE-U 6098, CBS121756	*P. persica*	Modimolle, Limpopo, South Africa		EU128038	EU128086	EU128128	
	STE-U 6099	*P. persica*	Modimolle, Limpopo, South Africa		EU128039	EU128087	EU128129	
	STE-U 6100	*P. persica*	Modimolle, Limpopo, South Africa		EU128040	EU128088	EU128130	
	STE-U 5834	*P. salicina*	Stellenbosch, Western Cape, South Africa	x	EU128041	EU128089	EU128131	
	STE-U 5954, CBS 121439	*P. salicina*	Paarl, Western Cape, South Africa		EU128042	EU128090	EU128132	
	STE-U 5956	*P. salicina*	Mookgopong, Limpopo, South Africa		EU128043	EU128091	EU128133	
Pm. subulatum	STE-U 6094, CBS 120866	*P. armeniaca*	Robertson, Western Cape, South Africa	x	EU128044	EU128092	EU128134	
Togninia africana	STE-U 6177, CBS 120863*	*P. armeniaca*	Montagu, Western Cape, South Africa	x	EU128052	EU128100	EU128142	EU128060
	STE-U 6364	*P. armeniaca*	Montagu, Western Cape, South Africa			EU128101	EU128143	
	STE-U 6365	*P. armeniaca*	Montagu, Western Cape, South Africa			EU128102		
T. fraxinopennsylvanica (Pm. mortoniae)	STE-U 6101, CBS 120865	*P. salicina*	Franschhoek, Western Cape, South Africa	x	EU128031	EU128079	EU128121	
	STE-U 6102	*P. salicina*	Franschhoek, Western Cape, South Africa	x	EU128032	EU128080	EU128122	
T. griseo-olivacea	STE-U 5966, CBS 120857*	*P. armeniaca*	Mookgopong, Limpopo, South Africa	x	EU128049	EU128097	EU128139	EU128058
T. minima (*Pm. aleophilum*)	STE-U 6088	*P. armeniaca*	Robertson, Western Cape, South Africa		EU128014	EU128062	EU128104	
	STE-U 6089, CBS 121434	*P. armeniaca*	Bonnievale, Western Cape, South Africa		EU128015	EU128063	EU128105	
	STE-U 6090	*P. armeniaca*	Bonnievale, Western Cape, South Africa		EU128016	EU128064	EU128106	
	STE-U 5836, CBS 121435	*P. salicina*	Paarl, Western Cape, South Africa		EU128017	EU128065	EU128107	
	STE-U 5962	*P. salicina*	Paarl, Western Cape, South Africa		EU128018	EU128066	EU128108	
	STE-U 5963	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128019	EU128067	EU128109	
	STE-U 5964, CBS 121436	*P. persica*	Paarl, Western Cape, South Africa	x	EU128020	EU128068	EU128110	
T. parasitica (Pm. parasiticum)	STE-U 6093, CBS 121437	*P. armeniaca*	Montagu, Western Cape, South Africa	x	EU128033	EU128081	EU128123	
T. viticola (*Pm. viticola*)	STE-U 5965, CBS 121440	*P. salicina*	Paarl, Western Cape, South Africa	x	EU128045	EU128093	EU128135	
	STE-U 6180	*P. salicina*	Franschoek, Western Cape, South Africa	x	EU128046	EU128094	EU128136	

[1] STE-U: Culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands.

[2] Isolates studied in the pathogenicity test, * ex-type cultures.

Cryphonectriaceae and Valsaceae have been dispersed from branches and stems of fruit trees to other woody hosts in the vicinity (Adams et al. 2005, Crous et al. 2006b, Gryzenhout et al. 2006, Damm et al. 2007).

The comparatively slow-growing and, until recently, relatively unknown species of *Phaeoacremonium* were probably often excluded from surveys of fungi on woody plants in South Africa, and subsequently only *Phaeomoniella chlamydospora* is listed in the most recent compilation of phytopathogenic fungi from South Africa (Crous et al. 2000). Because of the highly diverse, endemic vegetation and different climatic regions, more than 200 000 species of fungi have been estimated to occur in South Africa (Crous et al. 2006a), which was acknowledged by the authors as rather conservative. As a recent study on Botryosphaeriaceae has shown (Damm et al. 2007), *Prunus* represents a rich catch-crop for many of these fungi, and thus would also be a good host to sample for novel species of *Phaeoacremonium*. Therefore, the aim of the present study is to determine the diversity of *Phaeoacremonium* species on *Prunus* wood in South Africa and to describe five new species isolated from *P. armeniaca* and *P. salicina*. A further aim of this study was to determine which species of *Phaeoacremonium*, formerly known from grapevines, would have *Prunus* spp. as alternate hosts.

MATERIAL AND METHODS

Isolates

Branches with wood symptoms (e.g. die-back, canker, necrosis) were sampled from plum (*Prunus salicina*), peach (*P. persica*), nectarine (*P. persica* var. *nucipersica*) and apricot (*P. armeniaca*) orchards in the Western Cape and the Limpopo province of South Africa. Wood pieces with necrosis symptoms were prepared according to Damm et al. (2007) and incubated on potato-dextrose agar (2 % PDA; Biolab, Midrand, South Africa, supplemented with 100 mg/L streptomycin sulphate and 100 mg ampicillin) and synthetic nutrient-poor agar medium (SNA; Nirenberg 1976) supplemented with 100 mg penicillin G, 50 mg streptomycin sulphate, 10 mg chlortetracycline hydrochloride (pH 6), under cool fluorescent white light at 25 °C. Single-conidial isolates were obtained from all strains for further study. Reference strains are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U), Stellenbosch, South Africa, and the Centraalbureau voor Schimmelcultures (CBS) Utrecht, The Netherlands. Isolates used for morphological and sequence analysis are presented in Table 1.

Morphology

The *Phaeoacremonium* anamorphs were morphologically characterised on malt extract plates (MEA; 2 % malt extract, Oxoid Ltd., England; 1.5 % agar, Difco, USA) incubated at 25 °C in the dark for 2–3 wks as described in Mostert et al. (2006a). Teleomorph structures were described from PDA and SNA plates incubated for 3 or 2 mo at 25 °C in the laboratory under diffuse daylight. Vertical sections through perithecia and photographs of characteristic structures were made as described in Damm et al. (2007). Colony characters and pigment production on MEA, PDA and oatmeal agar (OA; Gams et al. 2007) incubated at 25 ºC were noted after 8 and 16 d. Colony colours were determined using the colour charts of Rayner (1970). Cardinal temperatures for growth were determined by incubating MEA plates in the dark at temperatures ranging from 5 to 40 °C in 5 °C intervals, also including 37 °C, emulating human body temperature. Radial growth was measured after 8 d at 25 °C.

DNA isolation, amplification and analysis

Genomic DNA of all isolates was isolated from fungal mycelium grown on PDA plates, placed in a 1.5 mL tube with glass beads and 600 µL hexadecyltrimethyl ammonium bromide (CTAB) extraction buffer (0.2 M Tris, 1.4 M NaCl, 20 mM EDTA, 0.2 g/L CTAB) and crushed 3 min at 30 vibrations per second in a Retsch Mixer Mill MM301 (Retsch, Haan, Germany). Before adding 400 µL chloroform : isoamylalcohol (24 : 1), the tube was placed in a 65 °C water bath for 15 min. The fungal matrix was spun down for 5 min at 15 800 *x g*. The watery supernatant was transferred into a new centrifuge tube and cold ammonium acetate solution (final concentration 2.5 M) and 600 µL cold isopropanol were added. After 15 min incubation at room temperature, the precipitate was spun down for 5 min at 15 800 *x g* and the supernatant discarded. One millilitre cold 70 % ethanol was added to the pellet, spun down for 5 min at 15 800 *x g* and the supernatant discarded. The DNA pellet was dried and resuspended in 100 µL ddH_2O.

The 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS1 and ITS2), the β-tubulin gene (TUB), the actin gene (ACT) and a partial sequence of the 18S rDNA gene (SSU) were amplified and sequenced using the primer pairs ITS-1F (Gardes & Bruns 1993) + ITS-4 (White et al. 1990), primers T1 (O'Donnell & Cigelnik 1997) + Bt2b (Glass & Donaldson 1995), ACT-512F + ACT-783R (Carbone & Kohn 1999) and NS1 + NS8 (White et al. 1990), according to the conditions and protocols explained in Mostert et al. (2006a). Additional primers used for sequencing the SSU were: NS2, NS3, NS4, NS5 (White et al. 1990). The ITS region was sequenced for preliminary identification of the fungi isolated from *Prunus* wood. Even though the ITS region has shown not to be robust for all species determination in the genus *Phaeoacremonium* (Groenewald et al. 2001, Mostert et al. 2005), we did found it valuable information for future ITS comparisons and lodged it in GenBank (Table 1).

The sequences generated in this study and additional sequences obtained from GenBank (www.ncbi.nlm.gov) were manually aligned using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). *Pleurostomophora richardsiae* (CBS 270.33) and *Wuestneia molokaiensis* (CBS 114877) were used as outgroup in the TUB and ACT phylogenies, while *Cochliobolus sativus* (U42479) and *Pleospora betae* (U3466) were used as outgroup in the SSU phylogeny. Two introns, only present in the outgroups (sequence positions 205–267, 388–421) were excluded from the SSU analysis. Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). The TUB and ACT data were analysed for each region separately, as well as with a combined data set. Alignment gaps in all analyses were treated as missing data and all characters were unordered and

of equal weight. Maximum parsimony analysis was performed using the heuristic search option with 100 random sequence additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. The robustness of the trees obtained was evaluated by 1 000 bootstrap replications with 100 random sequence additions (Hillis & Bull 1993). Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for the resulting tree. A partition homogeneity test with the same search criteria was conducted in PAUP to examine the possibility of a joint analysis of the TUB and ACT data sets. Sequences derived in this study were lodged at GenBank (Table 1) and the alignments in TreeBASE.

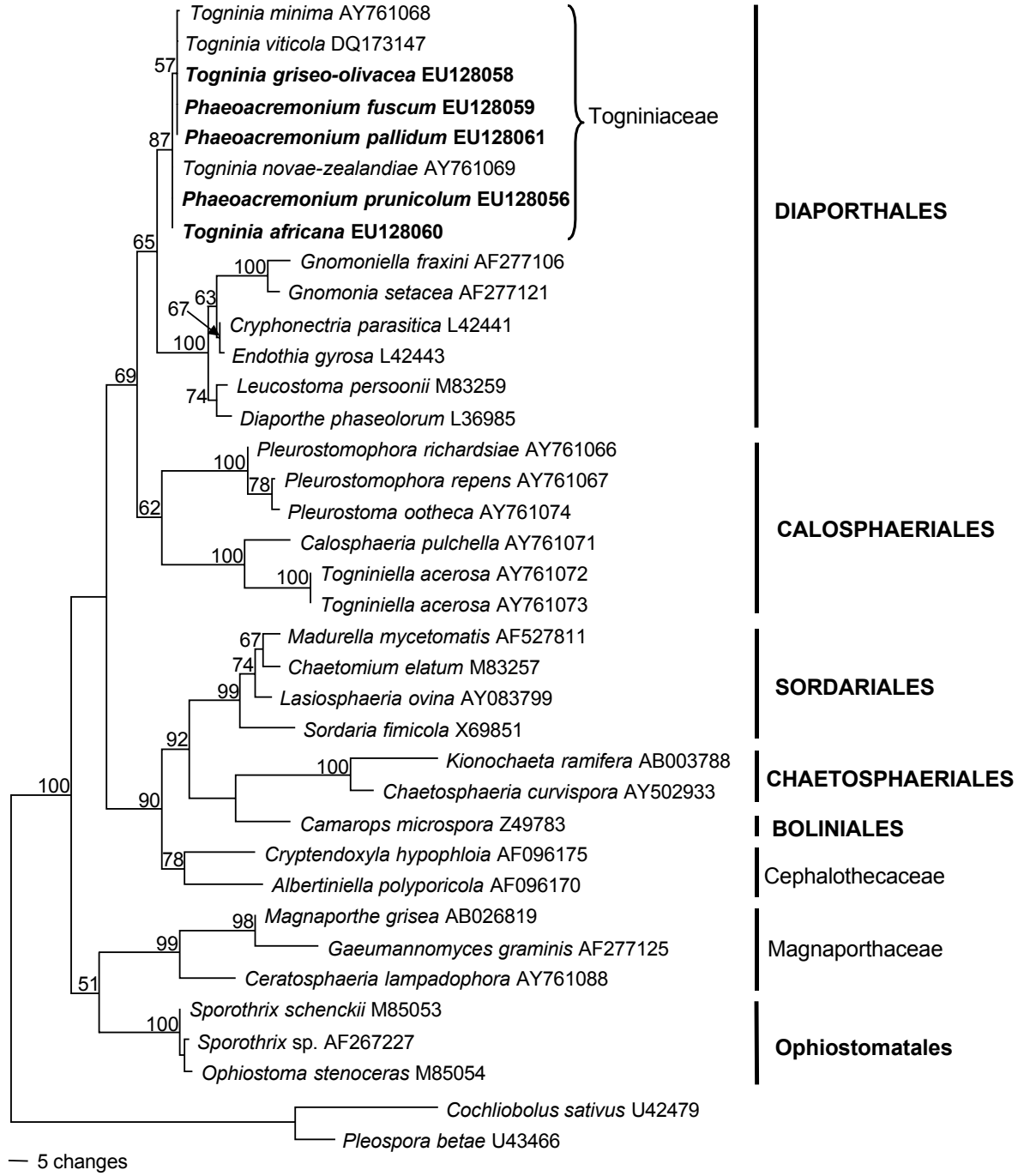

Fig. 1 One of 12 most parsimonious trees obtained from heuristic searches of the SSU gene sequences (Length = 591 steps, CI = 0.633, RI = 0.772, RC = 0.489, HI = 0.367). Bootstrap support values (1 000 replicates) above 50 % are shown at the nodes. *Cochliobolus sativus* U42479 and *Pleospora betae* U3466 were used as outgroups. Isolates analysed in this study are emphasised in **bold**.

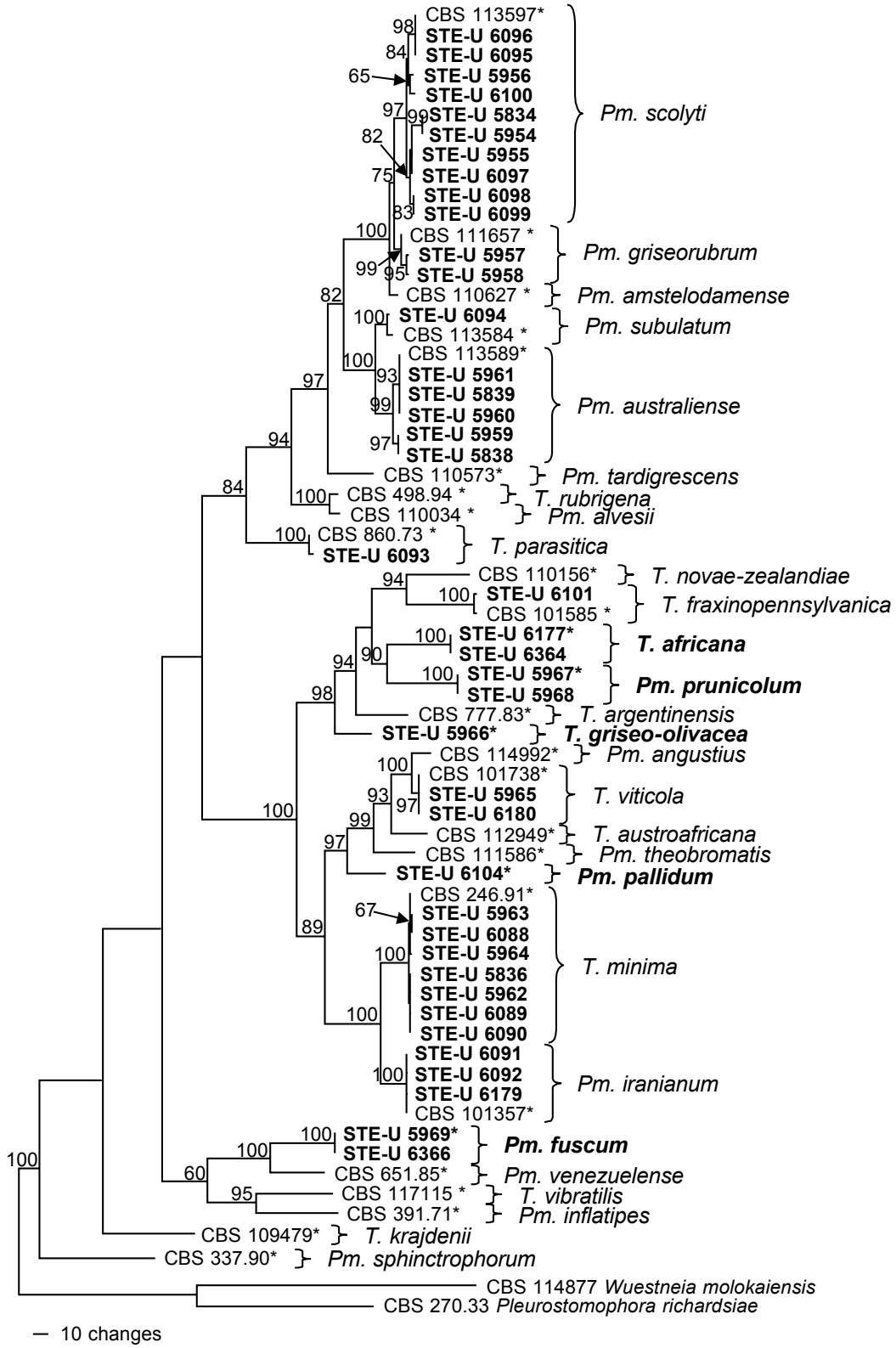

Fig. 2 One of 312 most parsimonious trees obtained from heuristic searches of a combined alignment of the TUB and ACT gene sequences (Length = 1807 steps, CI = 0.522, RI = 0.833, RC = 0.435, HI = 0.478). Bootstrap support values (1 000 replicates) above 60 % are shown at the nodes. *Pleurostomophora richardsiae* and *Wuestneia molokaiensis* were used as outgroups. Isolates analysed in this study are emphasised in **bold**. Ex-type strains are indicated with asterisks.

Pathogenicity tests

Preliminary pathogenicity tests were conducted with 14 taxa on detached apricot (cv. 'Belida', 4-year-old trees) and plum (cv. 'Southern Bell', 5-year-old trees) shoots. Depending on strain availability, one or two isolates per taxon were used and treated as sub-samples in the statistical analysis. Fresh vegetative shoots were collected from the trees shortly after harvest, cut into 12 cm pieces (5–8 mm diam), treated and inoculated with colonised agar plugs from 2-wk-old PDA cultures according to Damm et al. (2007), except for the surface sterilisation (40 s in 0.1 % solution of a patented didecyldimethylammonium chloride formulation, Sporekill, ICA International Chemicals Pty. Ltd., Stellenbosch, South Africa). *Acremonium strictum* (STE-U 6296) and uncolonised PDA plugs were used as negative controls. Shoots were incubated at 25 °C in moist chambers (> 93 % RH) for 2 wks, after which the bark was peeled off and lesions visible on the xylem tissue were measured. Each treatment combination consisted of one shoot, which was replicated four times in each of three blocks (= moist chambers). Re-isolations were made from the leading edges of lesions and the resulting cultures identified. The layout of the trial was a randomised block design. Lesion length data were subjected to analyses of variance using SAS v. 8.1 (SAS Institute, Cary, North Carolina USA) and Student's t-test for Least Significant Difference was calculated at the 5 % significance level to compare the treatment means for the different taxa.

RESULTS

Phylogenetic analysis

Five SSU sequences produced in this study were added to the 32 sequences obtained from GenBank comprising an alignment of 2266 characters including the gaps, of which 244 characters were parsimony-informative, 80 variable (parsimony-uninformative) and 1942 constant. The heuristic search of the SSU data resulted in 12 most parsimonious trees (Length = 591 steps, CI = 0.633, RI = 0.772, RC = 0.489, HI = 0.367), of which one is shown in Fig. 1. The clades represent eight classes or families, respectively, within the Sordariomycetes. The unknown *Phaeoacremonium* species found on *Prunus* trees grouped with species of Togniniaceae, *Togninia minima*, *T. novae-zealandiae* and *T. viticola*, forming the Togniniaceae-subclade (87 % bootstrap support). This subclade grouped with other Diaporthales (65 %) forming a sister clade to the Calosphaeriales.

The partition homogeneity test (p-value = 0.192) led us to combine the TUB and ACT data sets (633 characters in data

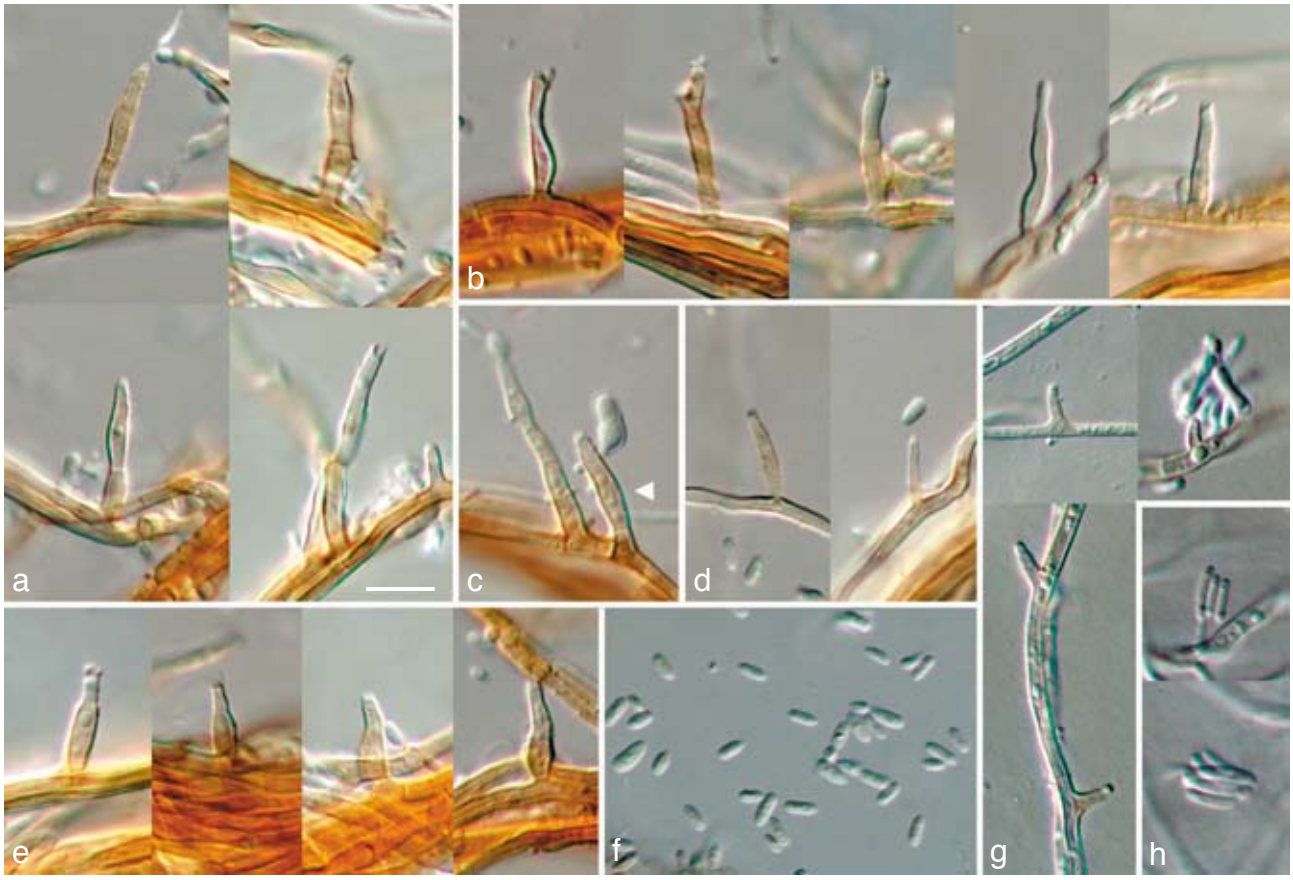

Fig. 3 *Phaeoacremonium fuscum*. a–f. Aerial structures on MEA; a. conidiophores; b. type III phialides; c. conidiophore and type II phialide (indicated by arrow head) ; d. type I phialides; e. type II phialides; f. conidia. — g, h. Structures on the surface of and in MEA; g. adelophialides; h. conidia; all from CBS H-19944 (holotype); a–h: DIC. — Scale bar: a = 10 µm, applies to a–h.

set 1, 298 in data set 2). A selection of 40 isolates was used for the phylogenetic analysis, with further 25 sequences being added from GenBank. The data set contained 931 characters including the gaps, of which 431 were parsimony-informative, 107 were variable and parsimony-uninformative, and 393 were constant. After a heuristic search, 312 most parsimonious trees with the same overall topology (differences only within species) were retained (Length = 1807 steps, CI = 0.522, RI = 0.833, RC = 0.435, HI = 0.478), of which one is shown in Fig. 2. The majority of the isolates grouped with known species of Togniniaceae: 10 of the isolates with *Pm. scolyti* (97 % bootstrap support), two with *Pm. griseorubrum* (99 %), one with *Pm. subulatum* (100 %), five with *Pm. australiense* (99 %), one with *T. parasitica* (100 %), one with *T. fraxinopennsylvanica* (100 %), two with *T. viticola* (97 %), seven with *T. minima* (100 %) and three with *Pm. iranianum* (100 %). A further eight isolates did not group with any known species. Isolates STE-U 6177 and 6364, as well as 5967 and 5968, formed two clades with 100 % bootstrap support that formed a sister group (90 %) to *T. novae-zealandiae*, *T. fraxinopennsylvanica* and *T. argentinensis* (94 %). Isolate STE-U 5966 grouped with these species (98 %) but formed a separate lineage. Isolate STE-U 6104 also formed a separate lineage in a clade (97 %) with *Pm. angustius*, *T. viticola*, *T. austroafricana* and *Pm. theobromatis*. Two isolates, STE-U 5969 and 6366, formed a sister group next to *Pm. venezuelense* (100 %).

Taxonomy

The 42 strains of *Phaeoacremonium* isolated from stone fruit wood (Table 1) could be assigned to 14 species based on the DNA sequence data generated and their morphology. Five species proved distinct from known species and are newly described below. Mostert et al. (2006a) developed a polyphasic, online identification system for species recognition (www.cbs.knaw.nl/phaeoacremonium/biolomics.aspx). The latter key has been updated to include all taxonomic novelties described in this study.

Phaeoacremonium fuscum L. Mostert, Damm & Crous, *sp. nov.* — MycoBank 505140; Fig. 3

Phaeoacremonio venezuelensi simile, sed coloniis in cultura (OA) fuscis-nigris vel isabellinis, conidiophoris brevioribus et phialibus typorum I et II.

Etymology. Named after its dark brown colonies (*fuscus* Lat. = dark brown).

Aerial structures — *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 10; hyphae tuberculate with warts up to 2 µm diam, verruculose, orange-brown and 1–2 µm wide. *Conidiophores* short and usually unbranched, up to 3-septate, bearing 1–2 terminal phialides, sometimes showing percurrent rejuvenation, (14–)17–28(–40) (av. 23) µm long and (1.5–)2(–2.5) (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sometimes polyphialidic, sparsely tuberculate to verruculose, orange-brown, sometimes hyaline; collarettes, slightly flaring, 1–2 µm long and 2–2.5 µm wide; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, (2–)4–7(–8) × 1–1.5(–2) (av. 5 × 1.5) µm; type II phialides subcylindrical or navicular, some elongate-ampulliform and attenuated at the base, tapering towards the apex, (7–)9–11(–12) × 1.5–2(–2.5) (av. 10 × 2) µm; type III phialides mostly subcylindrical, some navicular, 13–17(–20) × (1.5–)2(–2.5) (av. 15 × 2) µm, gradually tapering towards the apex. Type I and II phialides most common. *Conidia* hyaline, oblong-ellipsoidal some reniform, (3.5–)4–5 × (1–)1.5(–2) (av. 4 × 1.5) µm, L/W = 3.4.

On surface or submerged in the agar — *Phialides* pale orange-brown or hyaline, cylindrical, 1–4(–7) × 1–1.5(–2) (av. 3 × 1) µm. *Conidia* hyaline, cylindrical or allantoid, (4–)5–7(–8) × (1–)1.5–2 (av. 6 × 2) µm, L/W = 3.3.

Cultural characteristics — Colonies reaching a radius of 13.5–14 mm after 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 30 °C, maximum 37 °C. Colonies on MEA flat, mostly felty with a few woolly tufts, with entire margin; after 8 d and 16 d colonies dark mouse-grey (13''''k) to greyish sepia (15''''l) becoming buff (19''d) towards the margin above, reverse same. Colonies on PDA flat, felty to powdery, with entire margin; after 8 d and 16 d fawn (13'''i) to vinaceous-buff (17''i), similar in reverse, becoming sepia (15''m) to fawn (13'''i) after 16 d. Colonies on OA flat, felty to woolly, with entire margin; after 8 d brown-vinaceous (5'''m) to isabelline (17''i), after 16 d fuscous-black (7''''k) to isabelline (17''i) above. A pale brown pigment produced after 16 d on PDA.

Specimen examined. South Africa, Limpopo province, Mookgopong, from small dark brown central V-shaped necrosis close to canker developing from old pruning wound in wood of *Prunus salicina*, 31 Aug. 2004, *U. Damm*, CBS H-19944 holotype, culture ex-type CBS 120856 = STE-U 5969.

Notes — The various species that have grey-brown colonies and a growth rate that falls in the range of *Pm. fuscum* include *Pm. inflatipes*, *Pm. iranianum*, *Pm. krajdenii*, *Pm. sphinctrophorum* and *Pm. venezuelense* (Mostert et al. 2006a). Of these, *Pm. venezuelense* also has orange-brown mycelium, but can be distinguished by the predominance of type III phialides and hyaline phialides on and in the agar, in comparison with the predominance of phialide type I and II and often pale orange-brown phialides of *Pm. fuscum*. Furthermore, the maximum growth temperature of *Pm. fuscum* was at 37 °C, in comparison with 40 °C in the case of *Pm. venezuelense*.

Phaeoacremonium pallidum Damm, L. Mostert & Crous, *sp. nov.* — MycoBank 505141; Fig. 4

Phaeoacremonio angustio simile, sed conidiis latioribus, in cultura (OA) coloniis albis, ad 20 °C optime crescentibus.

Etymology. Named after its pale colonies (*pallidus* Lat. = pale).

Aerial structures — *Aerial mycelium* sparse, consisting of branched, septate hyphae that occur singly or in bundles of up to 21; hyphae tuberculate with warts up to 2 µm diam, verruculose to smooth, hyaline and 1–2.5 µm wide. *Conidiophores* short and usually unbranched, up to 3-septate, bearing one terminal phialide, (14–)17–36(–40) (av. 27) µm long and 1.5–2 (av. 2) µm wide. *Phialides* terminal or lateral, mostly monophialidic, sometimes polyphialidic, sparsely tuberculate to verruculose, hyaline; collarettes slightly flaring, 1–1.5 µm long and 1 µm wide; type I phialides most predominant, cylindrical, occasionally widened at the base, tapering towards the apex, (1.5–)3–6(–7) ×

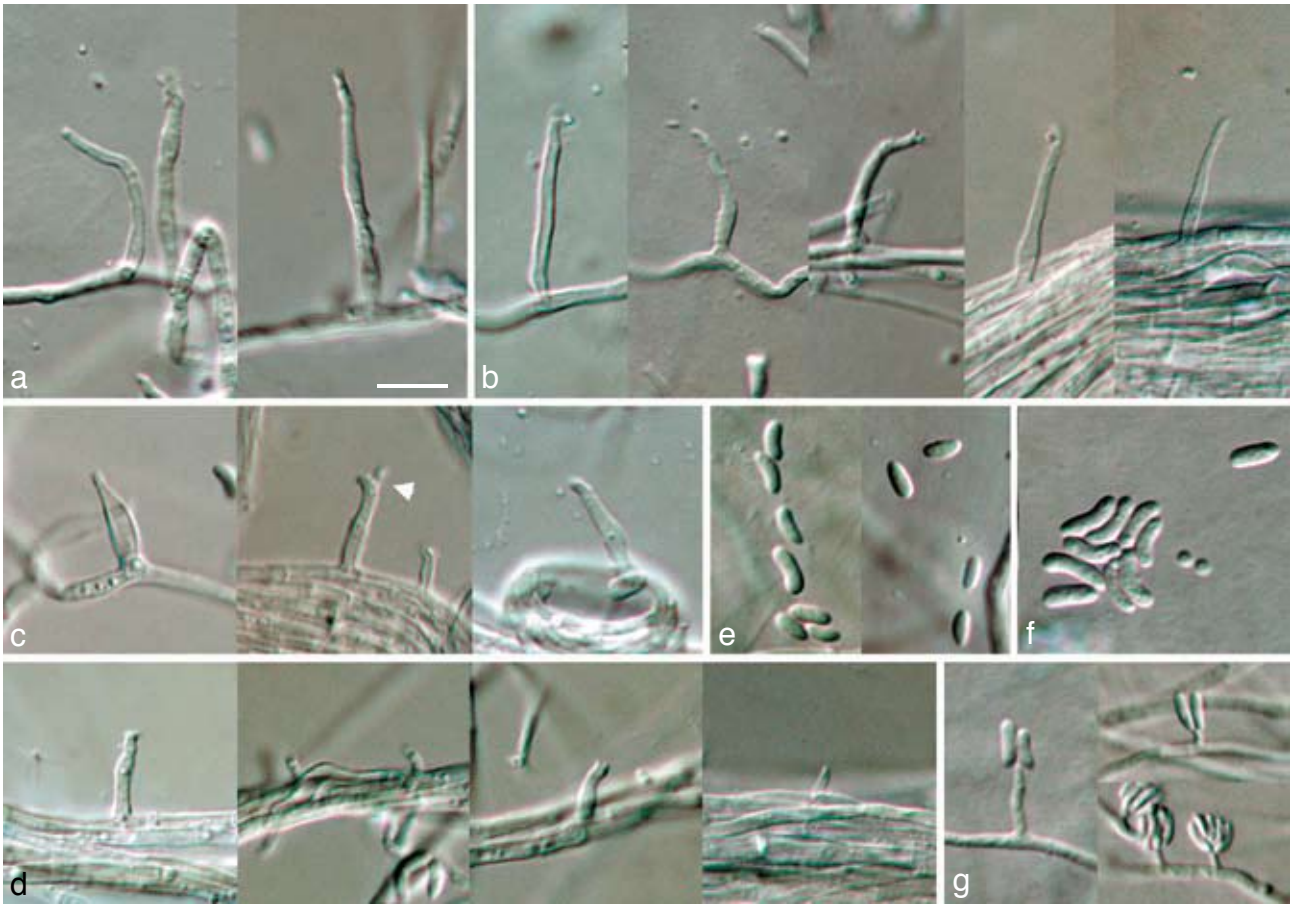

Fig. 4 *Phaeoacremonium pallidum*. a–e. Aerial structures on MEA; a. conidiophores; b. type III phialides; c. type II phialides (arrow head indicates polyphialide); d. type I phialides; e. conidia. — f, g. Structures on the surface of and in MEA; f. conidia; g. adelophialides with conidia; all from CBS H-19945 (holotype). a–g: DIC. — Scale bar: a = 10 µm, applies to a–g.

1–1.5 (av. 4 × 1) µm; type II phialides, subcylindrical or navicular, tapering towards the apex, (7–)9–11(–12) × 1.5–2 (av. 10 × 1.5) µm; type III phialides cylindrical or subcylindrical, 16–19 × 1 (av. 18 × 1) µm, gradually tapering towards the apex. *Conidia* hyaline, oblong-ellipsoidal or allantoid to reniform, (3.5–)4–6(–7) × 1.5–2 (av. 5 × 2) µm, L/W = 2.6.

On surface or submerged in the agar — *Phialides* hyaline, cylindrical to subcylindrical, (1–)2–6(–10) × 1–1.5 (av. 4 × 1) µm. *Conidia* hyaline, allantoid or cylindrical, (4–)6–8(–11) × (1.5–)2(–2.5) (av. 7 × 2) µm, L/W = 3.6.

Cultural characteristics — Colonies reaching a radius of 7.5–8.5 mm after 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 20 °C, maximum 30 °C. Colonies on MEA flat, mostly felty with very little aerial mycelium, appearing yeast-like, with entire margin; after 8 d and 16 d colonies buff (19''d), similar in reverse. Colonies on PDA flat, felty, with entire to lobate margin; after 8 d buff (19''d), reverse same. Colonies on OA flat, felty with entire margin; after 8 d and 16 d white above.

Specimen examined. South Africa, Western Cape province, Bonnievale, from irregular necrosis with dark brown annual rings close to pruning wound in wood of *Prunus armeniaca*, 23 Aug. 2005, *U. Damm*, CBS H-19945 holotype, culture ex-type CBS 120862 = STE-U 6104.

Notes — Colonies do not have a distinct colour ranging from buff (on MEA and PDA) to white (on OA). Of the various pale-coloured species, *Pm. pallidum* resembles *Pm. angustius*, especially in the predominance of the type I phialide and the shape of the type II phialides that are subcylindrical or navicular (Mostert et al. 2006a). *Phaeoacremonium pallidum* and *T. vibratilis* can be distinguished by their optimum growth temperature at 20 °C (Réblová & Mostert 2007). *Phaeoacremonium pallidum* can be distinguished by the presence of all three phialide types in comparison with *Phaeoacremonium vibratilis* having type I and type II phialides.

Phaeoacremonium prunicolum L. Mostert, Damm & Crous, *sp. nov.* — MycoBank 505139; Fig. 5

Phaeoacremonio novae-zealandiae simile, sed conidiis longioribus, in cultura (OA) pigmento flavido nullo.

Etymology. Named after its host, *Prunus*.

Aerial structures — *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 11; hyphae tuberculate with warts up to 2 µm diam, verruculose, medium brown and 1–2 µm wide. *Conidiophores* short and usually

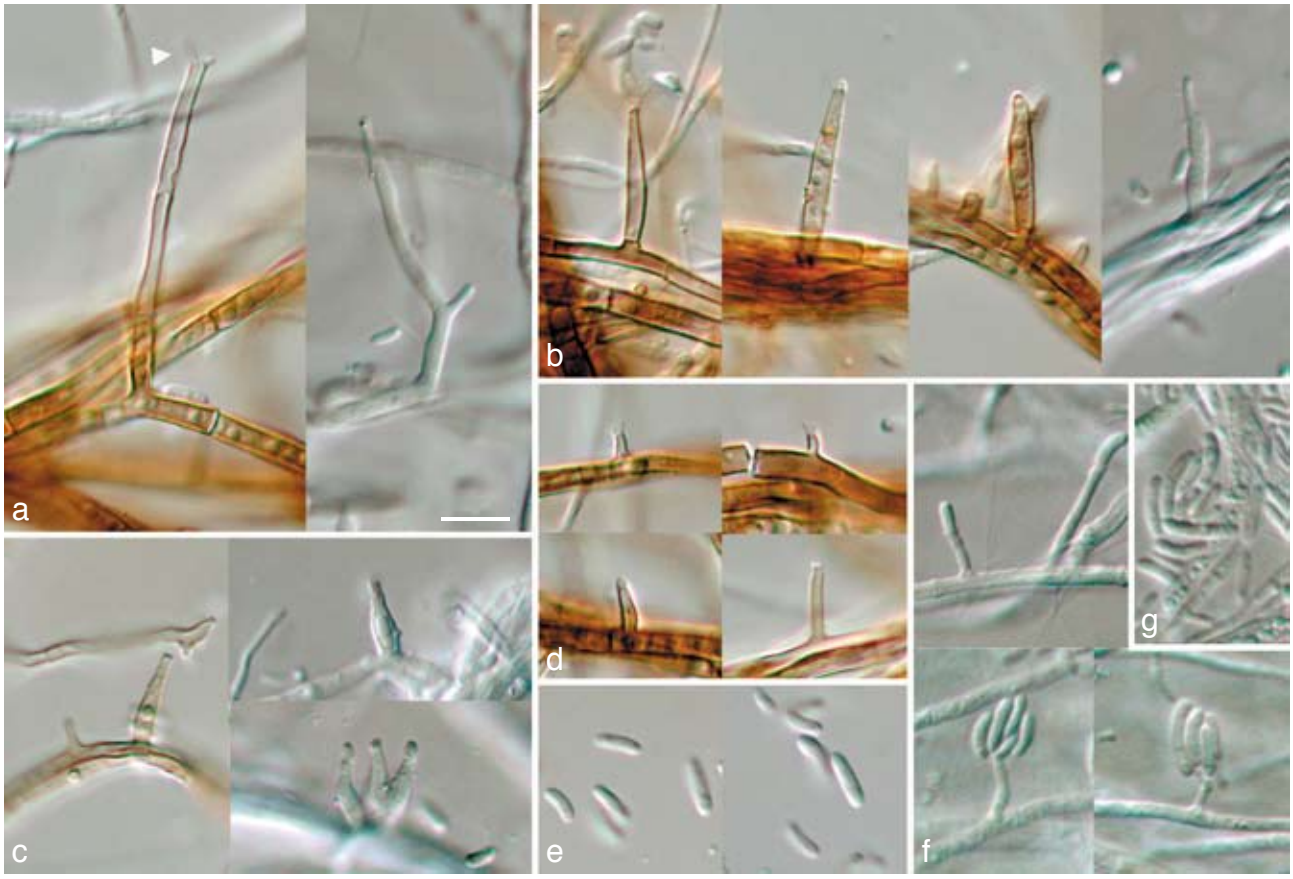

Fig. 5 *Phaeoacremonium prunicolum*. a–e. Aerial structures on MEA; a. conidiophores (arrow head indicates polyphialide); b. type III phialides; c. type II phialides; d. type I phialides; e. conidia. — f, g. Structures on the surface of and in MEA; f. adelophialides with conidia; g. conidia; all from CBS H-19943 (holotype); a–g: DIC. — Scale bar: a = 10 µm, applies to a–g.

unbranched, up to 2-septate, mostly bearing one terminal phialide, 14–37(–70) (av. 26) µm long and 1.5–3 (av. 2) µm wide. *Phialides* terminal or lateral, sometimes polyphialidic, sparsely tuberculate to verruculose or smooth, pale brown to subhyaline; collarettes slightly flaring, 1–1.5 µm long and 1–1.5 µm wide; type I phialides cylindrical, occasionally widened at the base, tapering towards the apex, (2–)3–6(–9) × 1–1.5 (av. 5 × 1) µm; type II phialides elongate-ampulliform and attenuated at the base, or navicular, tapering towards the apex, (7–)9–12(–13) × 1.5–2(–2.5) (av. 11 × 2) µm; type III phialides subcylindrical to navicular, (13–)14–18(–21) × (1.5–)2(–3) (av. 16 × 2) µm, gradually tapering towards the apex. Type I and III phialides most common. *Conidia* hyaline, oblong-ellipsoidal, cylindrical or allantoid, 5–7(–8) × 1–1.5(–2) (av. 6 × 1.5) µm, L/W = 3.9.

On surface or submerged in the agar — *Phialides* hyaline, cylindrical, (1–)2–6(–10) × 1(–1.5) (av. 4 × 1) µm. *Conidia* hyaline, cylindrical or allantoid, 6–8(–10) × 1.5(–2) (av. 7 × 1.5) µm, L/W = 4.6.

Cultural characteristics — Colonies reaching a radius of 8.5–10 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, mostly felty to short woolly, with entire margin; after 8 d and 16 d colonies olivaceous grey (21''''i) to buff (19''d), similar in reverse. Colony characters on PDA similar to those on MEA. Colonies on OA flat, felty with woolly tufts, with entire margin; after 8 d pale buff (19''f), after 16 d olivaceous green (23''') to pale buff (19''f).

Specimens examined. South Africa, Limpopo province, Mookgopong, from irregularly roundish, reddish to greenish brown necrosis in wood of *Prunus salicina* close to pruning wound, 31 Aug. 2004, *U. Damm*, CBS H-19943 holotype, culture ex-type CBS 120858 = STE-U 5967; Mookgopong, from reddish brown V-shaped necrosis in wood of *Prunus salicina* close to pruning wound, 31 Aug. 2004, *U. Damm*, STE-U 5968.

Notes — *Phaeoacremonium prunicolum* can be distinguished by having olive-grey colonies on MEA, PDA as well as OA. Similar species such as *Pm. novae-zealandiae* also have olive-grey colonies on MEA, but can be differentiated by its ability to produce a yellow pigment on OA (Mostert et al. 2006a), whereas this is absent in *Pm. prunicolum*.

Togninia africana Damm, L. Mostert & Crous, *sp. nov.* — MycoBank 505138; Fig. 6

Anamorph. *Phaeoacremonium* sp.

Togniniae viticolae similis, sed peritheciis majoribus et ascosporis guttulatis.

Etymology. Named after the continent of origin, Africa.

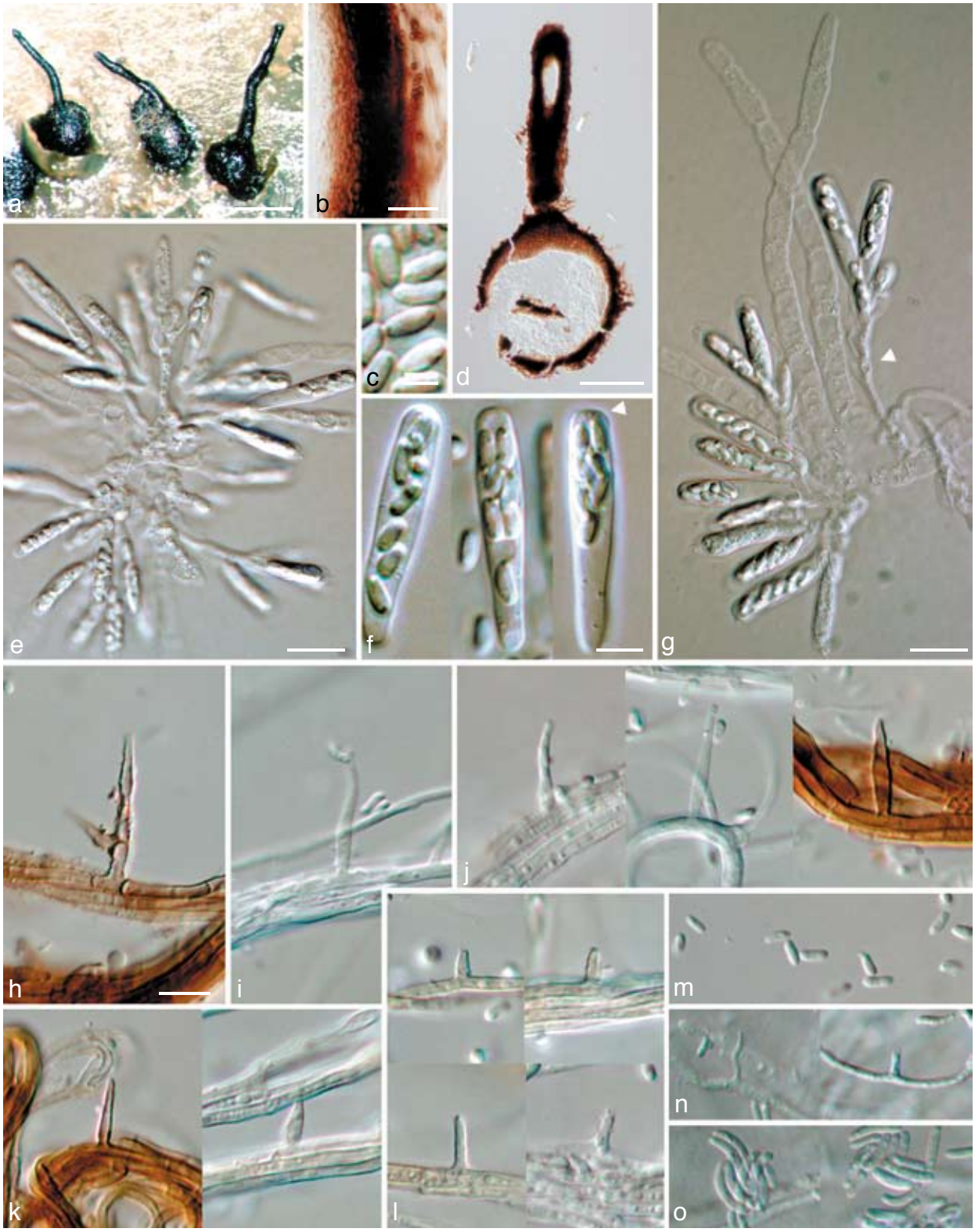

Fig. 6 *Togninia africana* teleomorph and anamorph states. a. Perithecia on SNA; b. peridium; c. ascospores; d. longitudinal section through perithecium; e. asci attached to ascogenous hyphae and paraphyses; f. asci; g. asci attached to ascogenous hyphae and paraphyses (remnant bases indicated by arrow head). — h–m. Aerial structures on MEA; h, i. conidiophores; j. type III phialides; k. type II phialides; l. type I phialides; m. conidia. — n, o. Structures on the surface of and in MEA; n. adelophialides; o. conidia; all from CBS H-19942 (holotype); a: DM, b–o: DIC. — Scale bars: a = 500 µm; b = 20 µm; c = 2.5 µm; d = 200 µm; f = 5 µm; e, g, h = 10 µm; h applies to h–o.

Ascomata — *Perithecia* formed on SNA containing the pieces of necrotic wood after 2 mo of incubation; non-stromatic, solitary, superficial to semi-immersed, subglobose to obpyriform, (215–)270–395(–440) µm diam, basal part (270–)315–440(–460) µm tall. Wall consisting of two regions of *textura angularis*: outer region dark brown, individual cells hardly visible, 15–25 µm thick; inner region pale brown becoming hyaline towards the centre, 5–7 cell layers and 10–15 µm thick. Surface covered with brown, septate hyphal appendages that become hyaline towards the tip. *Perithecial neck* curved, 1 per perithecium, 550–1000 (av. 720) µm long, 70–130 µm wide at the base, 35–65 µm wide at the tip. *Paraphyses* hyaline, septate, unbranched, cylindrical with round tips, slightly constricted at septa, 30–130 (av. 80) µm long, narrowing from 3–6.5 µm at the base to 1.5–4 µm at the apex, persistent. *Asci* arising in acropetal succession from sympodially proliferating ascogenous hyphae that appear spicate when mature, hyaline, clavate, with bluntly rounded apex and base, (16–)20–25.5(–26) × (3.5–)4–5(–5.5) (av. 22.5 × 4.5) µm. *Ascogenous hyphae* hyaline, branched, smooth-walled, 15–28 × 1.5–2.5 µm, remnant bases 4–6.5 × 2–3.5 µm. *Ascospores* aseptate, hyaline, smooth-walled, ellipsoidal to subcylindrical with rounded ends, sometimes slightly bent, containing small guttules at the ends, biseriate, (2.5–)3.5–4.5(–5.5) × 1.5–2(–2.5) (av. 4 × 1.8) µm.

Aerial structures — *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 22; hyphae tuberculate, with warts up to 2 µm diam, verruculose to smooth, mostly hyaline, some pale brown, 1–3 µm wide. *Conidiophores* short and usually unbranched, up to 2-septate, sometimes constricted at septa, bearing one terminal phialide, sometimes showing percurrent rejuvenation, (13–)15–23(–24) (av. 19) µm long and 2 µm wide. *Phialides* terminal or lateral, mostly monophialidic, sometimes polyphialidic, sparsely tuberculate to verruculose, hyaline, sometimes subhyaline; collarettes slightly flaring, 1–2 µm long and 1 µm wide; type I phialides most predominant, cylindrical, occasionally widened at the base, tapering towards the apex, (2–)3–5(–7) × 1–1.5(–2) (av. 4 × 1) µm; type II phialides subcylindrical, some elongate-ampulliform and attenuated at the base, (4–)7–11(–12) × (1–)1.5–2(–3) (av. 9 × 2) µm; type III phialides subcylindrical or navicular, 13–20(–28) × (1.5–)2(–2.5) (av. 16 × 2) µm. Type II and III phialides often strongly tapered towards the apex. *Conidia* hyaline, cylindrical or allantoid, (4.5–)5–8(–12) × 1.5–2 (av. 7 × 1.5) µm, L/W = 4.

On surface or submerged in the agar — *Phialides* hyaline, cylindrical, 1–7(–18) × 1–1.5(–2) (av. 4 × 1) µm. *Conidia* hyaline, cylindrical or allantoid, (5–)6–9(–12) × 1.5–2 (av. 8 × 2) µm, L/W = 4.3.

Cultural characteristics — Colonies reaching a radius of 9–9.5 mm after 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, mostly felty with a few woolly tufts, with entire margin; after 8 d and 16 d colonies buff (19"d), similar in reverse. Colonies on PDA flat, felty, with entire margin; after 8 d buff (19"d), similar in reverse; after 16 d vinaceous-buff (15'"d) becoming buff (19"d) with straw undertone, reverse honey (19"b) to buff (19"d). Colonies on OA flat, felty to woolly, with entire margin; after 8 d and 16 d olivaceous-buff (21'"d) to buff (19"d) with straw (21'd) undertone above. Prominent yellow pigment produced on MEA, OA and after 16 d also on PDA.

Specimen examined. South Africa, Western Cape province, Montagu, from greenish brown V-shaped necrosis in wood under canker developing from a broken-off twig of *Prunus armeniaca*, 23 Aug. 2005, *U. Damm*, CBS H-19942 holotype, culture ex-type CBS 120863 = STE-U 6177.

Notes — *Togninia africana* can be distinguished by its buff coloured colonies together with the production of a yellow pigment on MEA. *Phaeoacremonium angustius* is similar to the *Phaeoacremonium* anamorph of *T. africana* in the pale coloured colonies, predominance of the type I phialide and production of yellow pigment on OA. However, *Phaeoacremonium angustius* forms yellow-white to grey-red colonies on OA (Mostert et al. 2006a) in comparison with olivaceous-buff colonies of *T. africana*. The type II and III phialides taper sharply towards the apex, similar to the subulate phialides found in *Pm. subulatum*. Only *T. viticola* has both perithecia that often exceed 300 µm diam and mainly ellipsoidal ascospores as found in *T. africana*. However, *T. viticola* has one to three necks per perithecium and ascospores that can also be curved, whereas *T. africana* has one neck per perithecium and ascospores that are predominantly straight.

Togninia griseo-olivacea Damm, L. Mostert & Crous, *sp. nov.* — MycoBank 505137; Fig. 7

Anamorph. *Phaeoacremonium* sp.

Togniniae fraxinopennsylvanicae similis, sed peritheciis minoribus cum collo breviore.

Etymology. Named after its greyish olivaceous colonies (*griseolus-olivaceus* Lat. = greyish olivaceous).

Ascomata — Single-conidial isolates gave rise to perithecia on PDA after 3 mo; non-stromatic, solitary, superficial, globose to subglobose, dark brown, (150–)225 µm diam, (150–)200 µm tall (dimensions of only one mature perithecium available; measurements of immature perithecia in brackets). Wall consisting of two regions of *textura angularis*: outer region dark brown, 4–7 cells and 5–15 µm thick; inner region pale brown becoming hyaline towards the centrum, 5–6 cell layers and 10–20 µm thick. Surface covered with brown, septate hyphal appendages. *Perithecial neck* black, straight, 1 per perithecium, (200–)460 µm long, (40–)50 µm wide at the base, (25–)37 µm wide at the tip, dividing into two near the tip. *Paraphyses* hyaline, septate, unbranched, cylindrical with rounded tips, slightly constricted at septa, 30–70 (av. 45) µm long, 2–4.5 µm wide at the base. *Asci* arising in acropetal succession from sympodially proliferating ascogenous hyphae that appear spicate when mature, hyaline, clavate, rounded apex and base, 15–16 × 3–4 (av. 15.5 × 3.5) µm. *Ascogenous hyphae* hyaline, branched, smooth-walled, 13–23 × 2–3 µm, remnant bases only occasionally observed, 6–9(–12) × 3–4(–5) µm. *Ascospores* aseptate, hyaline, smooth-walled, ellipsoidal to reniform with rounded ends, sometimes containing small guttules at the ends, biseriate, 3–5(–6) × 1.5–2 (av. 3.5 × 1.5) µm.

Aerial structures — *Mycelium* consisting of branched, septate hyphae that occur singly or in bundles of up to 17; hyphae tuberculate with warts up to 2 µm diam, verruculose to verrucose, medium brown, 1–2 µm wide. *Conidiophores* short and usually unbranched, up to 2-septate, bearing one terminal phialide, (20–)22–35(–42) (av. 29) µm long and 1.5–2 (av. 2) µm wide.

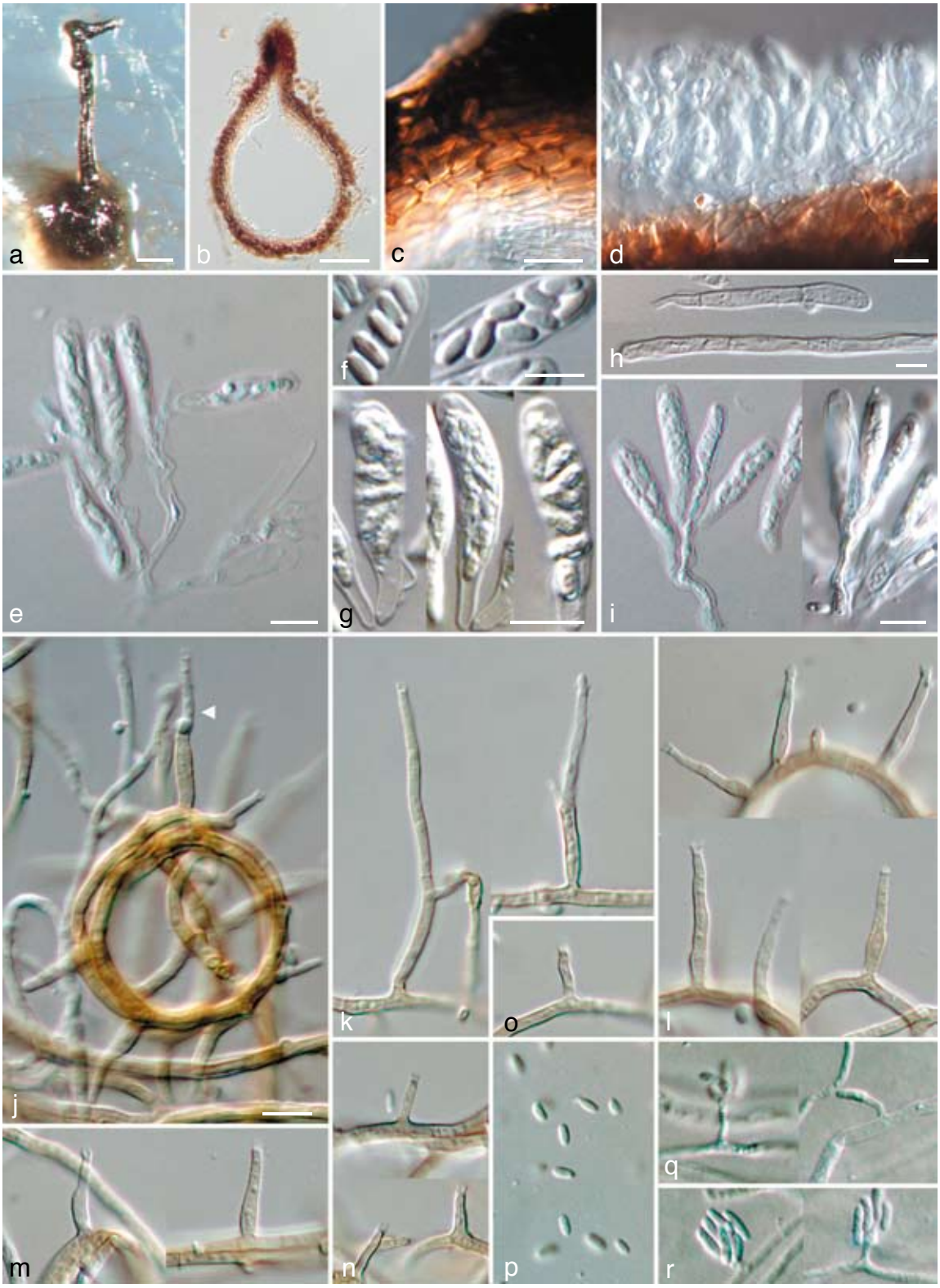

Fig. 7 *Togninia griseo-olivacea* teleomorph and anamorph states. a. Perithecium on PDA; b. longitudinal section through perithecium; c. peridium; d, e. asci attached to ascogenous hyphae; f. ascospores; g. asci; h. paraphyses; i. asci attached to ascogenous hyphae. — j–p. Aerial structures on MEA; j. ring-like growth of mycelium with conidiophore (indicated by arrow head); k. conidiophores; l. type III phialides; m. type II phialides; n, o. type I phialides; p. conidia. — q, r. Structures on the surface of and in MEA; q. adelophialides with conidia; r. conidia; all from CBS H-19941 (holotype). a: DM, b–r: DIC. — Scale bars: a = 100 µm; b = 50 µm; c–i = 5 µm; j = 10 µm; j applies to j–r.

Phialides terminal or lateral, mostly monophialidic, sometimes polyphialidic, sparsely tuberculate to verruculose, medium to pale brown, sometimes hyaline; collarettes slightly flaring, 1 µm long and 1–2 µm wide; type I phialides predominant, cylindrical, occasionally widened at the base, tapering towards the apex, (2–)3–6(–9) × 1–1.5(–2) (av. 4 × 1) µm; type II phialides, elongate-ampulliform and attenuated at the base, or subcylindrical, tapering towards the apex, (7–)9–12(–13) × 1.5–2(–2.5) (av. 11 × 2) µm; type III phialides mostly subcylindrical, some navicular, (13–)14–18(–20) × (1.5–)2 (av. 16 × 2) µm, gradually tapering towards the apex. *Conidia* subhyaline, oblong-ellipsoidal or reniform, (3.5–)4–5(–6) × 1.5–2 (av. 5 × 1.5) µm, L/W = 2.8.

On surface or submerged in the agar — *Phialides* hyaline, cylindrical, (1.5–)2–6(–10) × 1(–1.5) (av. 4 × 1) µm. *Conidia* hyaline, cylindrical, reniform or allantoid, (5–)6–7(–8) × 1.5–2 (av. 6 × 2) µm, L/W = 3.6.

Cultural characteristics — Colonies reaching a radius of 9–9.5 mm in 8 d at 25 °C. Minimum temperature for growth 10 °C, optimum 25 °C, maximum 30 °C. Colonies on MEA flat, mostly felty, becoming woolly with age, with entire margin; after 8 d colonies buff (19"d) above, in reverse olivaceous (21"k) to buff (19"d); after 16 d mouse-grey (13""i) to buff (19"d) above, similar in reverse. Colonies on PDA flat, felty with a few woolly tufts, with entire margin; after 8 d buff (19"d) with a few olivaceous (21"k) spots above, similar in reverse; after 16 d olivaceous (21"k) ring in centre becoming buff (19"d) towards margin, similar in reverse. Colonies on OA flat, felty with woolly tufts and entire margin; after 8 d and 16 d isabelline (17"i) to buff (19"d) above.

Specimen examined. South Africa, Limpopo province, Mookgopong, from reddish brown to greenish irregular necrosis with darker discoloured annual rings in wood of *Prunus armeniaca* close to pruning wound with resin exudation, 31 Aug. 2004, *U. Damm*, CBS H-19941 holotype, culture ex-type CBS 120857 = STE-U 5966.

Notes — The various *Togninia* and *Phaeoacremonium* species that have brownish coloured colony centres with a broad buff ring towards the margin on MEA include *Pm. australiense*, *T. novae-zealandiae* and *T. parasitica*. *Togninia griseo-olivacea* can be distinguished from *Pm. australiense* and *T. novae-zealandiae* by not producing a yellow pigment in OA and, additionally, from *T. novae-zealandiae* by forming shorter asci. *Togninia griseo-olivacea* differs from *T. parasitica* in the shape of the ascospores that are usually allantoid in *T. parasitica* and ellipsoidal to reniform in *T. griseo-olivacea*. The *Phaeoacremonium* anamorph of *T. griseo-olivacea* does not have such long conidiophores as *T. parasitica* (av. length 47 µm), prominent warts (up to 3 µm diam) or the predominant type III phialides found in *T. parasitica* (Mostert et al. 2006a). The *Togninia* species that have relatively short asci and short oblong-ellipsoidal ascospores with ranges that overlap with that of *T. griseo-olivacea* include *T. argentinensis*, *T. austro-africana*, *T. fraxinopennsylvanica* and *T. novae-zealandiae*. The size ranges of the asci and ascospores mostly resemble that of *T. fraxinopennsylvanica*. *Togninia griseo-olivacea* can be distinguished by having on average smaller perithecia and a shorter neck length.

Pathogenicity

All the isolates had been obtained from discoloured wood inside living branches of trees of different *Prunus* species. In cross-section, the symptomatic wood had either irregularly shaped or V-shaped necrotic lesions and were situated close to old pruning wounds and/or cankers, sometimes also associated with gummosis. In plum wood, such lesions were often reddish brown in the centre and greenish towards the margin. *Phaeoacremonium* species were mostly isolated from these lesions in combination with other fungi, for example Botryosphaeriaceae and *Schizophyllum commune.*

Table 2 Means of lesion lengths caused by different *Phaeoacremonium* species on detached green plum and apricot shoots, and mean re-isolation frequencies of these species from observed lesions.

Fungal species	Mean of lesion length (mm)[1]		Mean of re-isolation frequency (%)	
	Plum	Apricot	Plum	Apricot
Togninia parasitica	55.0 a	63.2 a	75	8
Phaeoacremonium iranianum	36.1 bc	56.5 ab	80	38
Pm. subulatum	33.5 bc	57.7 ab	83	8
Pm. griseorubrum	32.8 bc	57.2 ab	70	55
T. africana	33.1 bc	53.9 ab	75	50
T. griseo-olivacea	39.4 b	38.8 bcd	92	50
T. minima	37.8 bc	45.7 abc	75	29
T. viticola	35.8 bc	47.6 abc	84	8
Pm. prunicolum	35.2 bc	33.4 cd	84	25
T. fraxinopennsylvanica	33.9 bc	45.7 abc	80	13
Pm. scolyti	33.2 bc	50.6 abc	80	17
Pm. australiense	33.0 bc	44.2 abcd	54	38
Pm. pallidum	31.5 bcd	38.7 bcd	25	0
Pm. fuscum	25.1 cd	39.3 bcd	100	8
Acremonium strictum	19.8 de	32.4 cd	25	0
Agar plug	12.6 e	24.8 d	–	–
LSD ($P < 0.05$)	12.0	19.9		

[1] Means followed by the same letter are not significantly different ($P < 0.05$).

Analyses of variance of the lesion length data on apricot and plum cane sections indicated a significant treatment effect ($P < 0.0001$; Anova tables not shown). All species, except *Pm. fuscum* and *Pm. pallidum*, caused lesions in the xylem of plum shoots that were significantly longer than the controls (Table 2). The re-isolation frequencies from plum were between 70 % and 100 % for all fungi, except *Pm. australiense* and *Pm. pallidum*. Five species caused lesions on apricot shoots that were significantly longer than the controls: *T. parasitica*, *Pm. iranianum*, *Pm. subulatum*, *Pm. griseorubrum* and *T. africana*. Additionally, *T. parasitica*, *Pm. iranianum* and *Pm. griseorubrum* also caused lesions that were visible on the bark surface of more than half of the apricot canes; mostly dark brown rings around the inoculation site. Other species formed surface lesions on apricot canes less frequently. On plum, surface lesions on the bark were only occasionally observed. Most of the species were re-isolated from apricot wood in frequencies below 50 %. No *Phaeoacremonium* species were isolated from the negative controls. *Togninia parasitica* was the species that induced the longest lesions on plum and apricot wood.

DISCUSSION

Although *Phaeoacremonium* species have previously been relatively unknown from stone fruit trees, this study reveals these hosts to harbour a broad diversity (14 species) and abundance (present in 33 of the 257 specimens, with 3 specimens occupied by more than one *Phaeoacremonium* species) of this genus from *Prunus* trees in South Africa. Most species were found in plum (8 species) and apricot (8 species) wood, while peach and nectarine were rarely colonised by *Phaeoacremonium* (2 species). However, there were no *Phaeoacremonium* species known on *Prunus salicina*, *P. persica* and *P. persica* var. *nucipersica* before the onset of this study. Most species found on *P. armeniaca* also represent new reports on *Prunus*, except for *T. parasitica* and *T. minima* (Hawksworth et al. 1976, Mostert et al. 2006a). We also observed regional differences. While the species found in the Cape Winelands (Paarl, Stellenbosch, Franschhoek) comprised only known species, three of the four species found in the Limpopo province were new to science. Reasons for this could be the relative remoteness of the area compared to the Western Cape province (hosts not previously sampled for microfungi), and the different climate (summer-rainfall area vs winter-rainfall area).

The dominant species on stone fruit trees were *Pm. scolyti* and *T. minima*. *Togninia minima* is known as one of the causal organisms of Petri disease and esca on grapevines, and has previously been found on *V. vinifera* in South Africa (Groenewald et al. 2001, Mostert et al. 2003). In this study, *T. minima* was found on three *Prunus* species in the Western Cape province of South Africa. *Phaeoacremonium scolyti* is also known on *V. vinifera* in South Africa (Mostert et al. 2003). In our study, the fungus had the broadest host range and was found on all *Prunus* species sampled. According to Mostert et al. (2006b), *Pm. scolyti* could be dispersed between woody hosts by bark beetles, as it has previously been isolated from beetles (Kubátová et al. 2004). Rumbos (1986) assumed *Pm. parasiticum* to be spread throughout a cherry orchard by bark and wood-boring beetles. *Phaeoacremonium scolyti* was also the only species that occurred in different orchards in the Western Cape and Limpopo provinces of South Africa.

Notwithstanding the new taxa, several known species were also found on *Prunus*, four of which comprise new reports for South Africa. *Phaeoacremonium australiense*, *Pm. iranianum* and *T. fraxinopennsylvanica* had been previously reported from grapevines in other countries (Groenewald et al. 2001, Mostert et al. 2006a, Gramaje et al. 2007). This study shows, however, that these fungi also occur on *Prunus* species in South Africa. *Phaeoacremonium australiense* was even quite common in one orchard in the Western Cape. *Phaeoacremonium griseorubrum*, which was previously known only from human infections in Japan and the United States (Mostert et al. 2005), was found here to also occur in wood of *Prunus salicina* in South Africa.

All *Phaeoacremonium* spp. were associated with wood decay symptoms on *Prunus* trees. According to the pathogenicity test, most species were shown to be potentially pathogenic to plum, while only a few species were shown to be potentially pathogenic to apricot. The species most commonly isolated from *Prunus* wood, *Pm. scolyti*, was not the most virulent species. Three of the species, *Pm. subulatum*, *T. parasitica* and *T. viticola*, had been tested on grapevines in greenhouse and field experiments (Halleen et al. 2007). While in our study, *T. parasitica* was the most virulent *Phaeoacremonium* species on apricot and plum wood, it was less virulent on grapevines trunks than *T. viticola* and *Pm. subulatum* (Halleen et al. 2007). The relevance of *T. parasitica* in die-back disease on *Prunus* species is uncertain, since only one isolate was obtained. Rumbos (1986) showed *Pm. parasiticum* to be pathogenic on cherry, apricot, olive and peach. *Togninia minima* and *T. parasitica* also caused discolorations in wood of potted kiwifruit vines (Di Marco et al. 2004). Associated field symptoms and the pathogenicity test indicate a possible pathogenic relationship of these *Phaeoacremonium* species and *Prunus* trees.

Only one of the five new *Phaeoacremonium* species, namely *Pm. fuscum*, had a maximum growth temperature of 37 °C in comparison with 30 °C for the other species. The ability to grow at 37 °C suggests that it has the potential to survive at human body temperature, while the other species appear to be strictly plant-associated taxa.

Phaeoacremonium species are commonly isolated from healthy (Halleen et al. 2003) and symptomatic grapevines (Mostert et al. 2006a). In grapevines they occur in association with other fungi, namely *Phaeomoniella chlamydospora*, *Fomitiporia* species and to a lesser extent, *Stereum hirsutum* (Larignon & Dubos 1997, Mugnai et al. 1999, Fischer 2002). In this study, *Phaeoacremonium* species have mostly been found in combination with other fungi. Because *Phaeoacremonium* species that had frequently been isolated from diseased vines only gave a weak host response in pathogenicity trials on grapevines, Halleen et al. (2007) assumed that they might not be able to cause disease on their own, but required synergism with other fungi of this disease complex. Some of these fungi have been shown to be associated with stress-related diseases (Ferreira et al. 1999), and Halleen et al. (2007) only observed a clear disease expression in a field-trial monitored over a longer period.

Except for *Pm. griseorubrum*, all known *Phaeoacremonium* species found on *Prunus*, had previously been isolated from *Vitis vinifera* (Crous et al. 1996, Groenewald et al. 2001, Mostert et al. 2005, 2006a). *Phaeoacremonium* species are known as causal organisms of Petri disease, destructive grapevine trunk disease (decline, die-back), and young grapevine decline (Scheck et al. 1998). Petri disease is considered as a major reason for the death of vines in nurseries and young vineyards in the Western Cape province of South Africa (Halleen et al. 2003). *Togninia minima* is well-known on *Vitis* from South Africa (Mostert et al. 2003), and *T. minima*, *T. parasitica*, *T. viticola* and *Pm. subulatum* have been shown to be true wood colonisers and vascular pathogens of grapevines (Sparapano et al. 2001, Halleen et al. 2007). Based on the results obtained in the present study on different *Prunus* species, *Phaeoacremonium* species seem to lack host-specificity. Since a number of fungi, which have previously been reported to be pathogenic to grapevines were isolated from the wood of *Prunus* spp., stone fruit orchards should be considered as potential inoculum sources of grapevine trunk disease pathogens. Pathogenic or saprobic survival of these grapevine trunk disease pathogens in stone fruit orchards could have serious implications for disease management practices employed on farms where vineyards are planted adjacent to fruit tree orchards.

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