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Coniochaeta (*Lecythophora*), *Collophora* gen. nov. and *Phaeomoniella* species associated with wood necroses of *Prunus* trees

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Key words

Collophora
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Abstract Species of the genus *Coniochaeta* (anamorph: *Lecythophora*) are known as pathogens of woody hosts, but can also cause opportunistic human infections. Several fungi with conidial stages resembling *Lecythophora* were isolated from necrotic wood samples of *Prunus* trees in South Africa. In order to reveal their phylogenetic relationships, these fungi were studied on a morphological and molecular (5.8S nrDNA, ITS-1, ITS-2, GAPDH, EF-1α, 28S nrDNA, 18S nrDNA) basis. Some of the isolates were identified as *Coniochaeta* (*Sordariomycetes*), including *C. velutina* and two new species, *C. africana* and *C. prunicola*. The majority of the isolates, however, formed pycnidial or pseudopycnidial synanamorphs and were not closely related to *Coniochaeta*. According to their 28S nrDNA phylogeny, they formed two distinct groups, one of which was closely related to *Helotiales* (*Leotiomycetes*). The new genus *Collophora* is proposed, comprising five species that frequently occur in necrotic peach and nectarine wood, namely *Co. africana*, *Co. capensis*, *Co. paarla*, *Co. pallida* and *Co. rubra*. The second group was closely related to *Phaeomoniella chlamydospora* (*Eurotiomycetes*), occurring mainly in plum wood. Besides *P. zymoides* occurring on *Prunus salicina*, four new species are described, namely *P. dura*, *P. effusa*, *P. prunicola* and *P. tardicola*. In a preliminary inoculation study, pathogenicity was confirmed for some of the new species on apricot, peach or plum wood.

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INTRODUCTION

Gams & McGinnis (1983) reintroduced the genus *Lecythophora* (Melin & Nannfeldt 1934), confining it to anamorphs of *Coniochaeta*, and excluding it from *Phialophora* sensu Schol-Schwarz (1970), who placed these fungi in the *Phialophora hoffmannii* or *Phialophora lignicola* groups. *Lecythophora* is characterised by its hyaline hyphae and its mostly intercalary phialides with very short lateral necks, periclinal wall thickening and flaring collarettes (Gams 2000). Weber studied the morphology and LSU phylogeny of a number of *Lecythophora* species, several of which were linked to species of the ascomycetous genus *Coniochaeta* (Weber 2002, Weber et al. 2002). Currently, 17 *Coniochaeta* species and one *Barrina* species are known to form *Lecythophora* anamorphs, including anamorphs that can be considered as *Lecythophora*, but were described as *Phialophora* or *Hormonema* (Moreau & Moreau 1949, Cain 1961, Minoura et al. 1977, Udagawa & Furuya 1979, Hawksworth & Yip 1981, Mahoney & La Favre 1981, Udagawa & Sugiyama 1982, Yokoyama & Ito 1988, Kamiya et al. 1995, Ramaley 1997, Romero et al. 1999, Weber 2002, Asgari & Zare 2006). Other *Coniochaeta* species form different anamorphs, or have not yet been linked to any anamorph (García et al. 2006, Asgari et al. 2007). The most recent key comprises 54 well-documented *Coniochaeta* species (Asgari et al. 2007). However, only 21 *Coniochaeta* species were included in the latest published DNA phylogeny of the genus (García et al. 2006). *Coniochaeta* and *Barrina polyspora* belong to the *Coniochaetaceae* (Malloch & Cain 1971) within the *Coniochaetales* (Huhndorf et al. 2004, García et al. 2006). *Coniochaeta* is homothallic, and usually produces perithecia in culture (Raju & Perkins 2000). However, in some species/strains these perithecia remain infertile (Weber 2002).

Species of *Coniochaeta* and their *Lecythophora* anamorphs occur on dung of various animals (mainly mammals), in woodpulp, on wood or bark of different trees, in water (even with extremely low pH and high concentrations of heavy metals), in soil, leaves, and leaf litter, and rarely in non-woody host plants like *Gramineae* (Melin & Nannfeldt 1934, Eriksson 1992, López-Archilla et al. 2004, Asgari et al. 2007). *Coniochaeta*/*Lecythophora* species have been isolated from asymptomatic, dormant buds and young plants of *Vitis vinifera* (Dugan et al. 2002, Casieri et al. 2009). *Coniochaeta ligniaria* was isolated from decaying bark of *Prunus avium* in the Netherlands (CBS 178.75). Popushoi (1971) reported several species on fruit trees in Moldavia: *C. ambigua* on dry twigs of apricot and cherry, *C. calva* on twigs of quince, cherry and plum, *C. ligniaria* on dry twigs and wood of pear and plum and *C. velutina* on wood of apple and pear trees.

Some species such as *Lecythophora hoffmannii* (teleomorph: *Coniochaeta ligniaria*) and *L. mutabilis* are also known as human pathogens involved in keratitis, subcutanous abscesses, peritonitis, endocarditis and septic shock (de Hoog et al. 2000, Drees et al. 2007, Taniguchi et al. 2009). They have also been isolated from food, e.g., butter (Samson et al. 2004). On the other hand, some *Coniochaeta* species have been found to exhibit useful biochemical properties. For example, a strain of *Coniochaeta ellipsoidea* forms the newly discovered antibiotic coniosetin, which has a pronounced antibacterial and antifungal action, inhibiting even drug-resistant strains of *Staphylococcus aureus* (Segeth et al. 2003). *Coniochaeta ligniaria* is effective

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in biological detoxification of lignocellulosic biomass and can potentially be used to convert it to fuels and chemicals (López et al. 2004). Colonisation of torrefied grass fibres with the same fungus resulted in reduced phytotoxicity and increased plant growth (Trifonova et al. 2009).

While intercalary phialides with short lateral necks are characteristic for the genus *Lecythophora*, several other genera are known that also commonly form intercalary hyphal cells with conidiogenous protrusions that are not separated from the hyphal cell by a septum, or are even reduced to short necks or openings with collarettes. Examples include *Phialemonium* (Gams & McGinnis 1983), the *Calosphaeriophora* anamorph of *Calosphaeria africana* (Damm et al. 2008a), two newly described *Phaeomoniella* species (Lee et al. 2006), *Phialophora sessilis* and *Phialophora reptans* (de Hoog et al. 1999) and *Neotyphodium* (Morgan-Jones & Gams 1982, Glenn et al. 1996). Also, *Cladorrhinum* almost exclusively produces intercalary phialides with widely flaring collarettes (von Arx & Gams 1967). Weber (2002) described two species, '*Lecythophora*' spp. 1 and 2, that are similar to *Lecythophora*, but not closely related to it (Weber et al. 2002). In the following overview, we will refer to genera with phialidic conidiogenesis that mainly form reduced intercalary phialides. These variant phialides range in form from adelophialides, which are hyphal cells with longer or shorter protrusions or necks, often opening with a collarette and not delimited by a basal septum, to aphanophialides, which are verticillately arranged, reduced, flask-shaped phialides with a narrow neck, often seen in groups of several per hyphal cell, to pleurophialides, which are intercalary hyphal cells with mostly one lateral opening with collarette (Gams 1971) as seen in *Lecythophora*-like fungi (Gams 2000). Many other genera or species form intercalary phialides as well – for example *Acremonium*, *Phaeoacremonium* (where they are called type I phialides), *Lecanicillium dimorphum* and *L. tenuipes*, anamorphs of *Jattaea* species, *Phaeocrella acerosa*, *Calosphaeriophora pulchella* and diverse *Phialophora* species. In these genera and species, though, discrete phialides are usually the predominant conidiogenous structures formed (Gams & McGinnis 1983, Zare & Gams 2001, Réblová et al. 2004, Mostert et al. 2006b, Damm et al. 2008a, b, Essakhi et al. 2008, Marincowitz et al. 2008).

Many fungi resemble *Lecythophora*-like taxa in forming masses of conidia on reduced conidiogenous cells directly on their hyphae, and often undergo microcyclic conidiation, resulting in a yeast-like appearance in culture, e.g. *Aureobasidium*, *Hormonema*, *Exophiala*, and the anamorph of *Tromeropsis microtheca*. Even microscopically they can easily be confused with the species studied here, since the structures are very small and the conidiogenous cells difficult to recognise. However, *Aureobasidium* forms conidia synchronously on minute denticles (Hermanides-Nijhof 1977, Zalar et al. 2008) and *Aureohyphozyma*, *Exophiala*, *Hormonema*, *Hyphozyma*, the hyphozyma-like anamorphs of *Tromeropsis microtheca* and *Valsaria insitiva* produce conidia laterally from hyphae with holoblastic-percurrent conidiogenesis (Hermanides-Nijhof 1977, de Hoog 1977, Glawe 1985, de Hoog & Smith 1986, Hosoya & Otani 1995, Weber 2002). If the conidiogenesis is phialidic as in *Lecythophora*-like fungi, periclinal thickening or collarettes are usually visible, but no denticles or annellides.

During a survey in stone fruit orchards, we isolated various fungi with hyaline, aseptate conidia released from intercalary phialides, mainly reduced to hyphal cells with short necks or small openings with collarettes. The phialides resembled the conidiogenous cells of *Lecythophora* species. Most of them did not form teleomorph structures, but formed pycnidial or pseudopycnidial synanamorphs in culture. The objective of the current study was to investigate the phylogenetic relationships of these different *Lecythophora*-like fungi, as well as to describe the new species and test their pathogenicity on *Prunus*.

MATERIALS AND METHODS

Sampling and fungal isolation

Fungi were isolated from branches of trees with dieback or necrotic symptoms. Samples were taken in stone fruit (*Prunus* spp.) orchards in the Western Cape and the Limpopo Provinces of South Africa according to the method described in Damm et al. (2007). Single-conidial isolates were obtained from the strains for further study. Reference strains are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch (STE-U) in Stellenbosch, South Africa, and the CBS-KNAW Fungal Biodiversity Centre (CBS) Utrecht, The Netherlands. Isolates used for morphological and sequence analyses and in the preliminary pathogenicity test are presented in Table 1.

Morphological analysis

To enhance sporulation, double-autoclaved pine needles or double autoclaved grapevine wood pieces were placed onto the surface of synthetic nutrient-poor agar medium (SNA; Nirenberg 1976), and incubated at 25 °C in the dark for 2 wk (anamorphs) or 2–3 mo (teleomorphs). Measurements, photographs of characteristic structures and vertical sections through ascomata and conidiomata were made according to Damm et al. (2007). Microscopic preparations were made in clear lactic acid or water, with 30 measurements per structure, and observations were made with a Nikon SMZ800 dissecting microscope (DM) or with a Nikon Eclipse E600 microscope using differential interference contrast (DIC) illumination. Colony characters and pigment production were noted after 2 wk of growth on malt extract agar (MEA, 2 % malt extract, Oxoid Ltd., England; 1.5 % agar, Difco, USA) and 2 % potato-dextrose agar (PDA; Crous et al. 2009) incubated at 25 °C. Colony colours were rated according to Rayner (1970). Growth characteristics were studied on MEA plates incubated in the dark at temperatures ranging from 5–35 °C, in 5° intervals.

Phylogenetic analysis

Genomic DNA of the isolates was extracted using the method of Damm et al. (2008b). The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS-1 and ITS-2), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a partial sequence of the translation elongation factor 1α (EF-1α), of the 28S nrDNA (LSU) and of the 18S nrDNA (SSU) were amplified and sequenced using the primer pairs ITS-1F (Gardes & Bruns 1993) + ITS-4 (White et al. 1990), GDF1 + GDR1 (Guerber et al. 2003), EF1-728F + EF1-986R (Carbone & Kohn 1999), NL1 + NL4 (O'Donnell 1993) and NS1 + NS8 (White et al. 1990) or NS1 + NS24 (Gargas & Taylor 1992), respectively. Additional primers were used for sequencing the SSU, NS2–NS5 (White et al. 1990). The LSU sequences were added to the outgroup (*Lipomyces starkeyi* U45824 and *Saccharomyces cerevisiae* J01355) and sequences obtained from GenBank (www.ncbi.nlm.nih.gov). The alignment was assembled and manually adjusted using Sequence Alignment Editor v2.0a11 (Rambaut 2002). Phylogenetic analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) v4.0b10 (Swofford 2000). Alignment gaps were treated as missing 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

Table 1 Species names, accession numbers, isolation details and GenBank accession numbers of isolates studied.

Species	Accession No.[1]	Host	Location	Collector	Patho test[3]	GenBank accessions				
						ITS	LSU	SSU	GAPDH	EF
Collophora africana	STE-U 6113/CBS 120872*	*Prunus salicina*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154570	GQ154609	GQ154630	GQ154648	GQ154643
Collophora capensis	STE-U 6199/CBS 120879*	*P. salicina*	Franschhoek, Western Cape, South Africa	U. Damm	x	GQ154571	GQ154610	GQ154631	GQ154649	GQ154644
	STE-U 6339	*P. salicina*	Franschhoek, Western Cape, South Africa	U. Damm		GQ154572				
	STE-U 6340	*P. salicina*	Franschhoek, Western Cape, South Africa	U. Damm		GQ154573				
	STE-U 6341	*P. salicina*	Franschhoek, Western Cape, South Africa	U. Damm		GQ154574				
Collophora paarla	STE-U 6114/CBS 120877*	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154586	GQ154613	GQ154634	GQ154651	GQ154646
Collophora pallida	STE-U 6197/CBS 120878*	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154575	GQ154611	GQ154632		
	STE-U 6185	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154576				
	STE-U 6115/CBS 121443	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154577	GQ154612	GQ154633		GQ154645
	STE-U 6349	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154578				
	STE-U 6350	*P. salicina*	Modimolle, Limpopo, South Africa	U. Damm		GQ154579			GQ154650	
	STE-U 6116	*P. salicina*	Modimolle, Limpopo, South Africa	U. Damm		GQ154580				
	STE-U 6351	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm		GQ154581				
	STE-U 6352	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm		GQ154582				
	STE-U 6353	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm		GQ154583				
	STE-U 6332	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154584				
	STE-U 6335	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154585				
Collophora rubra	STE-U 6109/CBS 120873*	*P. persica*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154547	GQ154606	GQ154627		
	STE-U 6329	*P. persica*	Paarl, Western Cape, South Africa	U. Damm		GQ154546				
	STE-U 6330	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154548				
	STE-U 6354	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154549				
	STE-U 6355	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154550				
	STE-U 6198/CBS 121441	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154551	GQ154607	GQ154628	GQ154647	GQ154642
	STE-U 6196	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154552				
	STE-U 6331	*P. persica* var. *nucipersica*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154553				
	STE-U 6111/CBS 121442	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154554				
	STE-U 6135	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154555				
	STE-U 6137	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154556				
	STE-U 6136	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154557				
	STE-U 6138	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154558				
	STE-U 6112	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154559				
	STE-U 6333	*P. persica*	Paarl, Western Cape, South Africa	U. Damm		GQ154560				
	STE-U 6110	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154561	GQ154608	GQ154629		
	STE-U 6334	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154562				
	STE-U 6367	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154563				
	STE-U 6336	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154564				
	STE-U 6337	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154565				
	STE-U 6338	*P. persica*	Modimolle, Limpopo, South Africa	U. Damm		GQ154566				
	STE-U 6414	*P. dulcis*	Tulbagh, Western Cape, South Africa	Unknown[2]		GQ154567				
	STE-U 6415	*P. dulcis*	Tulbagh, Western Cape, South Africa	Unknown[2]		GQ154568				
	STE-U 6416	*P. dulcis*	Tulbagh, Western Cape, South Africa	Unknown[2]		GQ154569				
Coniochaeta africana	STE-U 5952/CBS 120868*	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154539	GQ154601	GQ154621		

Coniochaeta prunicola	STE-U 6107/CBS 120875*	*P. armeniaca*	Robertson, Western Cape, South Africa	U. Damm	x	GQ154540	GQ154602	GQ154622		
	STE-U 5953/CBS 121445	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154541	GQ154603	GQ154623		
Coniochaeta velutina	STE-U 5950/CBS 120874	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154542	GQ154604	GQ154624		
	STE-U 5951	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154543		GQ154625		
	STE-U 6105/CBS 121444	*P. armeniaca*	Robertson, Western Cape, South Africa	U. Damm	x	GQ154544	GQ154605	GQ154626		
	STE-U 6106	*P. armeniaca*	Robertson, Western Cape, South Africa	U. Damm		GQ154545				
Phaeomoniella dura	STE-U 6122/CBS 120882*	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154597	GQ154617	GQ154638		
Phaeomoniella effusa	STE-U 6121/CBS 120883*	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154598	GQ154618	GQ154639		
Phaeomoniella prunicola	STE-U 6118/CBS 120876*	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154590	GQ154614	GQ154635		
	STE-U 6342	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154587				
	STE-U 6343	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154588				
	STE-U 6344	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154589				
	STE-U 6345	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154591				
	STE-U 6119	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154592	GQ154615	GQ154636		
	STE-U 6346	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154593				
	STE-U 6347	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154594				
	STE-U 6348	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm		GQ154595				
	STE-U 6117	*P. salicina*	Paarl, Western Cape, South Africa	U. Damm	x	GQ154596	GQ154616	GQ154637		
Phaeomoniella tardicola	STE-U 6123/CBS 121757*	*P. armeniaca*	Robertson, Western Cape, South Africa	U. Damm	x	GQ154599	GQ154619	GQ154640		
Phaeomoniella zymoides	STE-U 6120/CBS 121168	*P. salicina*	Mookgopong, Limpopo, South Africa	U. Damm	x	GQ154600	GQ154620	GQ154641		

[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 received from the Plant Disease Clinic of the Department of Plant Pathology, University of Stellenbosch, South Africa.

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

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. Since we could not assign or differentiate some of the taxa, we used SSU, LSU, ITS and for some taxa EF-1α and GAPDH sequences for sequence comparisons and in BLASTn searches (www.ncbi.nlm.nih.gov). Sequences derived in this study were lodged at GenBank, the alignment in TreeBASE (www.treebase.org/treebase-web/home.html), and taxonomic novelties in MycoBank (www.MycoBank.org; Crous et al. 2004).

Pathogenicity tests

The preliminary pathogenicity test was conducted with 15 taxa on detached apricot (cv. 'Belida'), peach (cv. 'San Pedro') and plum (cv. 'Southern Bell') shoots. Depending on strain availability, one or two isolates per taxon were used and treated as subsamples in statistical analysis. Vegetative shoots were prepared 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). *Eutypa lata* (STE-U 6081) was used as positive (pathogen) control and *Acremonium strictum* (STE-U 6296) and uncolonised PDA plugs as negative (non-pathogen) controls. Shoots were incubated at 25 °C in moist chambers (> 93 % RH) for 2 wk, after which the bark was peeled off and lesions visible on the surface of the xylem tissue 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 v8.1 (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.

RESULTS

Phylogeny

The LSU analyses combined 36 taxa and 644 characters including the alignment gaps, of which 231 characters were parsimony-informative, 69 variable and 344 constant. After a heuristic search, four most parsimonious trees were retained (length = 1 142 steps, CI = 0.452, RI = 0.656, RC = 0.296, HI = 0.548) of which one is shown in Fig. 1. The main clades in the phylogeny represent different classes within the ascomycete subphylum *Pezizomycotina*. Isolates STE-U 6109, 6113, 6199, 6197 and 6114 (GenBank GQ154606, GQ154609–GQ154611, GQ154613) form two groups (100 % bootstrap support) in the *Leotiomycetes* clade, next to *Crinula caliciiformis* AY544680 (*Helotiales*), *Pseudeurotium zonatum* AF096198, *Leuconeurospora pulcherrima* AF096193 (*Pseudeurotiaceae*) and *Erysiphe aquilegiae* AB022405 (*Erysiphales*). Isolates STE-U 5952, 6107 and 5950 (GenBank GQ154601, GQ154602, GQ154604) group with *Coniochaeta ligniaria* AF353586 (100 %, *Coniochaetales*) in the *Sordariomycetes* clade (100 %). Isolates STE-U 6118, 6120–6123 (GenBank GQ154614, GQ154617–GQ154620) group with *Phaeomoniella chlamydospora* AY772237 and *Moristroma japonicum* AY254052 (96 %, *Chaetothyriomycetes*) in the *Eurotiomycetes* clade (66 %). BLASTn searches of LSU, SSU and ITS sequences of the taxa studied confirmed these results or are explained in detail below.

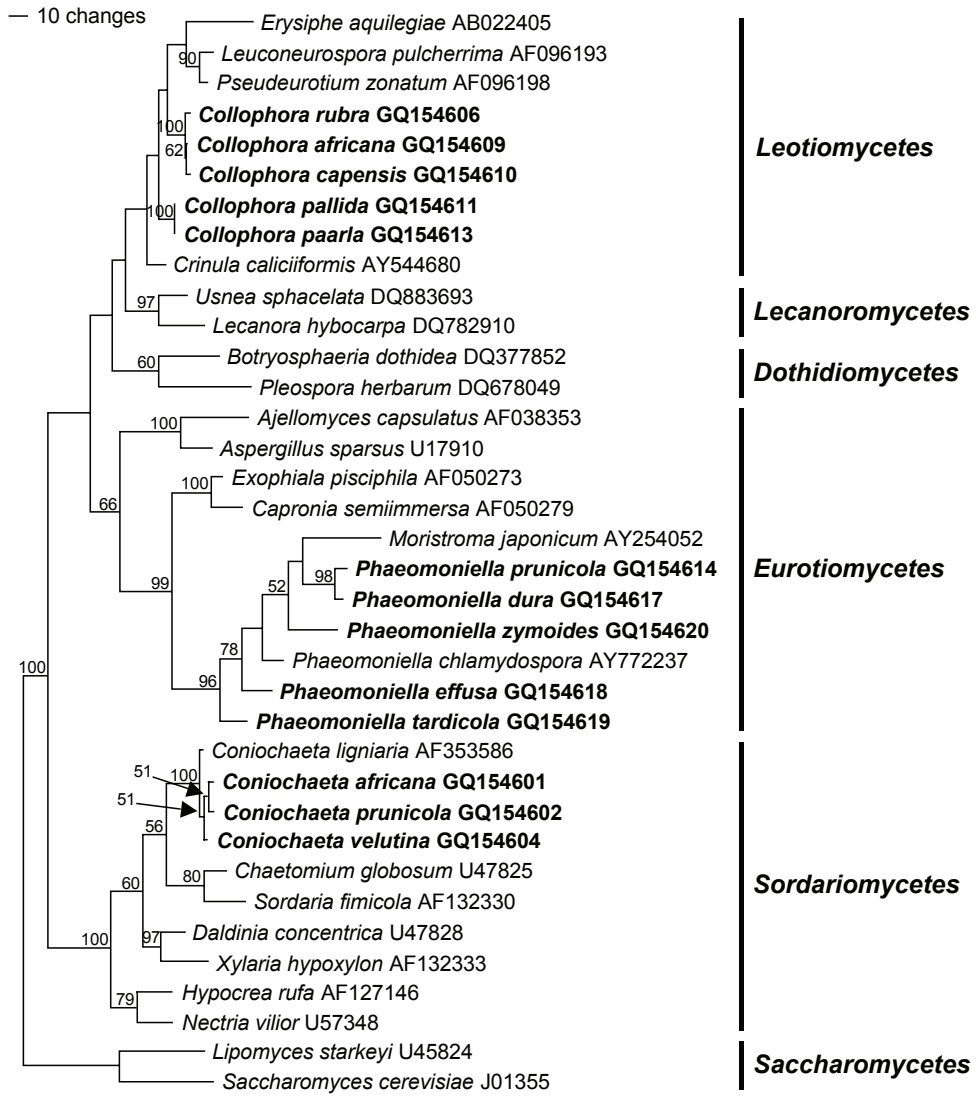

Fig. 1 One of 4 most parsimonious trees obtained from heuristic searches of LSU gene sequences of Pezizomycotina (length = 1 142 steps, CI = 0.452, RI = 0.656, RC = 0.296, HI = 0.548). Bootstrap support values (1 000 replicates) above 50 % are shown at the nodes. *Lipomyces starkeyi* U45824 and *Saccharomyces cerevisiae* J01355 were used as outgroup. Isolates analysed in this study are emphasized in **bold**.

Taxonomy

The *Lecythophora*-like fungi isolated from *Prunus* wood could be assigned to 13 species representing three phylogenetically distinct genera: *Collophora* gen. nov., *Coniochaeta* (anamorph: *Lecythophora*) and *Phaeomoniella*. Five species of *Collophora*, two species of *Coniochaeta* and four species of *Phaeomoniella* proved distinct from known species, and are newly described.

Collophora Damm & Crous, *gen. nov.* — MycoBank MB516622

Teleomorph. unknown.

Coloniis tarde crescentibus, humidis, albidis, cremeis vel rubicundis, mycelio aerio sparse evoluto vel nullo. Conidiophora unicellularia. Cellulae conidiogenae enteroblasticae, intercalares, redactae ad adenophiales breves, saepe cum collarettis sicut in hyphis. Conidia aggregata circum hyphas et in pagina agari. Conidiomata pseudopycnidialia, solitaria vel aggregate, subglobosa, superficialia vel semiimmersa, unilocularia vel multilocularia, pariete ex textura epidermoidea crassitunicata composito, irregulariter dehiscenti. Conidiophora hyalina, ramosa, septata, filiformia. Cellulae conidiogenae enteroblasticae, hyalinae, collis brevibus saepe acropleurogeniter formatis (in quoque cellulis sub septo lateraliter vel terminaliter formatis). Conidia pseudopycnidiarum et cellularum hypharum intercalarium minuta, hyalina, unicellularia, cylindracea vel ellipsoidea.

Type species. *Collophora rubra* Damm & Crous, *sp. nov.*

Etymology. Hyphae carry short necks or, more often, mere collarettes that release conidia (collarette Lat. = neckband, phorus Gr. = carrying).

Colonies slow-growing, moist, white, cream or reddish colours, with sparse or lacking aerial mycelium. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, intercalary, reduced to very short adelophialides or more often with collarettes formed directly on hyphal cells. *Conidia* aggregated in masses around the hyphae and on the agar surface. *Conidiomata* pseudopycnidial, solitary or aggregated, subglobose, superficial or semi-immersed, uni- to multilocular, wall composed of thick-walled textura epidermoidea, dehiscence irregular. *Conidiophores* hyaline, branched, septate, filiform. *Conidiogenous cells* enteroblastic, hyaline, often short necks formed laterally in each cell just below the septum as well as terminally (acropleurogenous). *Conidia* of pseudopycnidia and intercalary hyphal cells small, hyaline, 1-celled, cylindrical to ellipsoidal.

Notes — The genus *Collophora* usually forms conidiomata in culture but no teleomorph, which distinguishes it from *Lecythophora* anamorphs of *Coniochaeta* species (Weber 2002) and the anamorph of *Mycocalicium schefflerae* (Samuels & Buchanan 1983) that form perithecia or apothecia in culture,

respectively. Other *Lecythophora*-like anamorphs that do not form conidiomata may differ in colony colour, which is orange-yellow in *Barrina polyspora* (Ramaley 1997), or in pigmentation of the apical region, as in the *Calosphaeriophora* anamorph of *Calosphaeria africana* (Damm et al. 2008a), or in the shape of the intercalary phialides, as in the anamorph of *Igneocumulus yuccae* (Ramaley 2003), which has short, narrow necks that are often wider than long. '*Lecythophora*' sp. 1 described by Weber (2002) forms discrete, ventricose phialides in old cultures. These phialides are often aggregated on dendroid conidiophores, and the vegetative hyphae are often very narrow (< 1 µm). These features were not observed in *Collophora* species. The anamorph of *Munkovalsaria rubra* produces a red pigment as do some *Collophora* species. However, cultures emit a strong odour of m-cresol and form no conidiomata. Intercalary phialides are arranged in irregularly branched conidiophores (Aptroot 1995).

Conidia of *Phialemonium* are formed on discrete, short-stalked conidial heads (Gams & McGinnis 1983), while conidia of *Collophora* usually emerge from collarettes attached directly to hyphae and become aggregated in masses around those hyphae and on the agar surface. Phialides of *Humicolopsis* are similar to those of *Phialemonium*. Also, *Humicolopsis* colonies turn grey to black due to the presence of dark chlamydospores (Marchand et al. 1976) that are not found in *Collophora.* Conidiogenous necks of *Neotyphodium* anamorphs of *Epichloë* are even longer than those of *Phialemonium*, and are aculeate (Morgan-Jones & Gams 1982, Glenn et al. 1996, Chen et al. 2009).

Some *Phaeomoniella* species and *'Lecythophora*' sp. 2 (Weber 2002) produce conidia in conidiomata as well as from dispersed conidiogenous cells. However, the conidiomata are pycnidial and not pseudopycnidial, which means they are usually not stromatic and unilocular. The conidiomatal wall is composed of textura angularis in *Phaeomoniella* and of textura globulosa in *'Lecythophora*' sp. 2, but not of textura epidermoidea as in *Collophora*. Also, the acropleurogenous conidiogenous cells of *Collophora* are distinctive in this comparison (Crous & Gams 2000, Weber 2002, Lee et al. 2006, this paper). Colonies of most *Phaeomoniella* species and of *'Lecythophora*' sp. 2 produce greenish pigments that do not occur in *Collophora*. *Phialophora sessilis* and *Phialophora reptans* colonies also differ in colour from *Collophora*: they are olivaceous-black due to pigmented hyphae and conidiogenous cells (de Hoog et al. 1999).

In the genus *Cyphellophora*, conidia are septate, often sickle-shaped and pigmented (Decock et al. 2003), while in *Collophora* conidia are aseptate, hyaline to subhyaline and differently shaped. *Cladorrhinum* anamorphs of *Apiosordaria* and *Cercophora* form a reticulate system of hyaline or pale ochraceous, branched conidiophores, each cell with a lateral phialidic opening with widely flaring collarettes. Conidia of most species are dacryoid (Mouchacca & Gams 1993). Conidiogenous cells in *Collophora* differ by not being arranged as conidiophores or parts thereof. *Collophora* does not form aphanophialides as are characteristically seen in *Aphanocladium* (Gams 1971) and some species of *Lecanicillium* (Zare & Gams 2001). Usually, in *Collophora*, collarettes or periclinal wall thickenings are visible and several conidia are formed from each conidiogenous cell in basipetal succession.

There are few other fungal genera known that form conidiomata with filiform, acropleurogenously branched conidiophores (Sutton 1980). Among the genera with these characters is *Catenophora*, which produces conidiomata that are acervular, containing holoblastic conidiogenous cells, and pale brown conidia. *Pyrenochaeta*, *Pleurophoma* and *Sirophoma* have phialides and acropleurogenously branched conidiophores, but these structures are formed in pycnidia composed of thick-walled textura angularis, and have a single central, circular ostiole, while conidiomata of *Collophora* are pseudopycnidial with a wall formed by thick-walled textura epidermoidea and irregular dehiscence. *Sirodothis* (anamorph of *Tympanis*) differs in developing unilocular pycnidium-like structures on a basal stroma. Its conidiomatal wall is composed of textura angularis, while pseudopycnidia of *Collophora* are sessile or semi-immersed, often multilocular, and have a wall formed of thick-walled textura epidermoidea. *Cytonema* develops a characteristic rostrate ostiole, while conidiomata of *Collophora* open by irregular rupture.

Collophora is closely related to *Pseudeurotium* and other *Pseudeurotiaceae* (Fig. 1). However, *Collophora* species have phialidic conidiogenesis, while *Teberdinia*, the anamorph of *Pseudeurotium*, has sympodial conidiogenesis (Sogonov et al. 2005).

Collophora africana Damm & Crous, *sp. nov*. — MycoBank MB516623; Fig. 2

Collophorae rubrae similis, sed in vitro tarde crescentibus, conidiophoris pseudopycnidiarum magis ramosis, ramulis divergentibus, saepe < 2 µm latis, conidiis sicut in hyphis formatis, (2.5–)3.5–5.5(–8) × 1–2(–2.5) µm, conidiis conidiomatum 3–3.5(–4.5) × 1–1.5 µm.

Etymology. Named after its continent of origin, Africa.

Vegetative hyphae hyaline, 1–2 µm wide, smooth-walled, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphae and in pseudopycnidia. *Conidiophores on hyphae* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, reduced to very short adelophialides or more often with collarettes formed directly on hyphal cells; necks cylindrical, 0.5–3 × 0.5–1 µm; collarettes cylindrical to narrowly funnel-shaped, very thin-walled, 0.5–2 long, opening 0.5–1 µm wide, often inconspicuous. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical to obovate, straight, with both ends obtuse or with a papillate apex, smooth-walled, containing small droplets, (2.5–)3.5–5.5(–8) × 1–2(–2.5) µm, mean ± SD = 4.5 ± 1.2 × 1.5 ± 0.3 µm, L/W ratio = 3.1. *Microcyclic conidiation* occurs, with conidia developing into mother cells, becoming > 10 µm long, 2–3 µm wide, and sometimes septate, with minute collarettes at one or both ends. *Conidiomata* pseudopycnidial, produced on pine needles, on SNA and on MEA in 2–4 wk; on pine needles solitary, subglobose, superficial, 50–250 µm wide; on agar medium solitary or within a stroma, dark brown, uni- to multilocular; wall 10–30 µm thick, composed of several layers of reddish brown textura epidermoidea with thick-walled, indistinctly delimited cells; opening by irregular rupture, often appearing cup-shaped when mature, surrounded by hyaline hyphal appendages. *Conidiophores* lining the inner conidiomatal cavity, hyaline, septate, usually not constricted at septa, 10–35 µm long, branched at the base and above, branches diverging, straight, filiform. *Conidiogenous cells* enteroblastic, hyaline, monophialidic, with conidiogenous loci formed intercalary immediately below the septum (acropleurogenously) as well as terminally, cylindrical when terminal, tapering slightly towards the tip, 4–7 × 1–1.5 µm, basal cells up to 2.5 µm wide; collarettes 0.5–1 µm long, opening < 0.5–1 µm, periclinal thickening visible. *Conidia* hyaline, 1-celled, cylindrical to ellipsoidal, straight, with both ends obtuse or with a slightly acute apex, smooth-walled, containing small droplets, 3–3.5(–4.5) × 1–1.5 µm, mean ± SD = 3.3 ± 0.3 × 1.4 ± 0.1 µm, L/W ratio = 2.4.

Culture characteristics — *Colonies on PDA* convex, slimy, with entire margin, white to pale rosy-buff and pale luteous, with thinly white to pale flesh floccose aerial mycelium; reverse white to pale luteous to rosy-vinaceous, turning red with age; entire medium scarlet to red due to diffuse pigment; *on MEA* umbonate, folded towards the centre, with radial growth rings

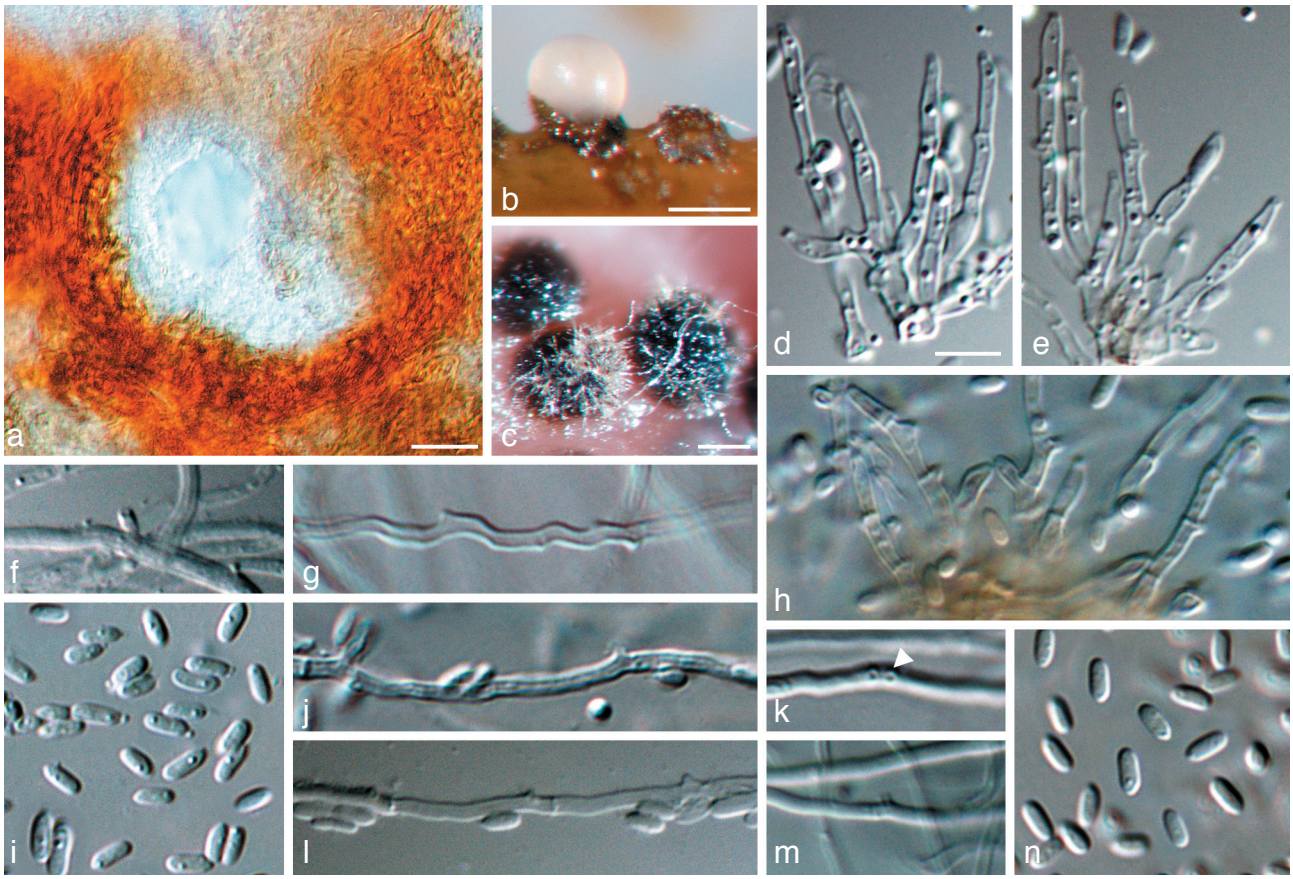

Fig. 2 *Collophora africana*. a. Longitudinal section through a pseudopycnidium; b, c. pseudopycnidia on pine needle (b), and MEA medium (c); d, e, h. conidiophores lining the inner wall of pseudopycnidia; n. conidia formed in pseudopycnidia; f, g, j–m. conidiogenous cells on hyphal cells (arrow head: openings in plan view); i. conidia formed on hyphal cells. All from ex-type culture CBS 120872. a, d–n: DIC; b, c: DM. — Scale bars: a = 10 µm; b, c = 100 µm; d = 5 µm; d applies to d–n.

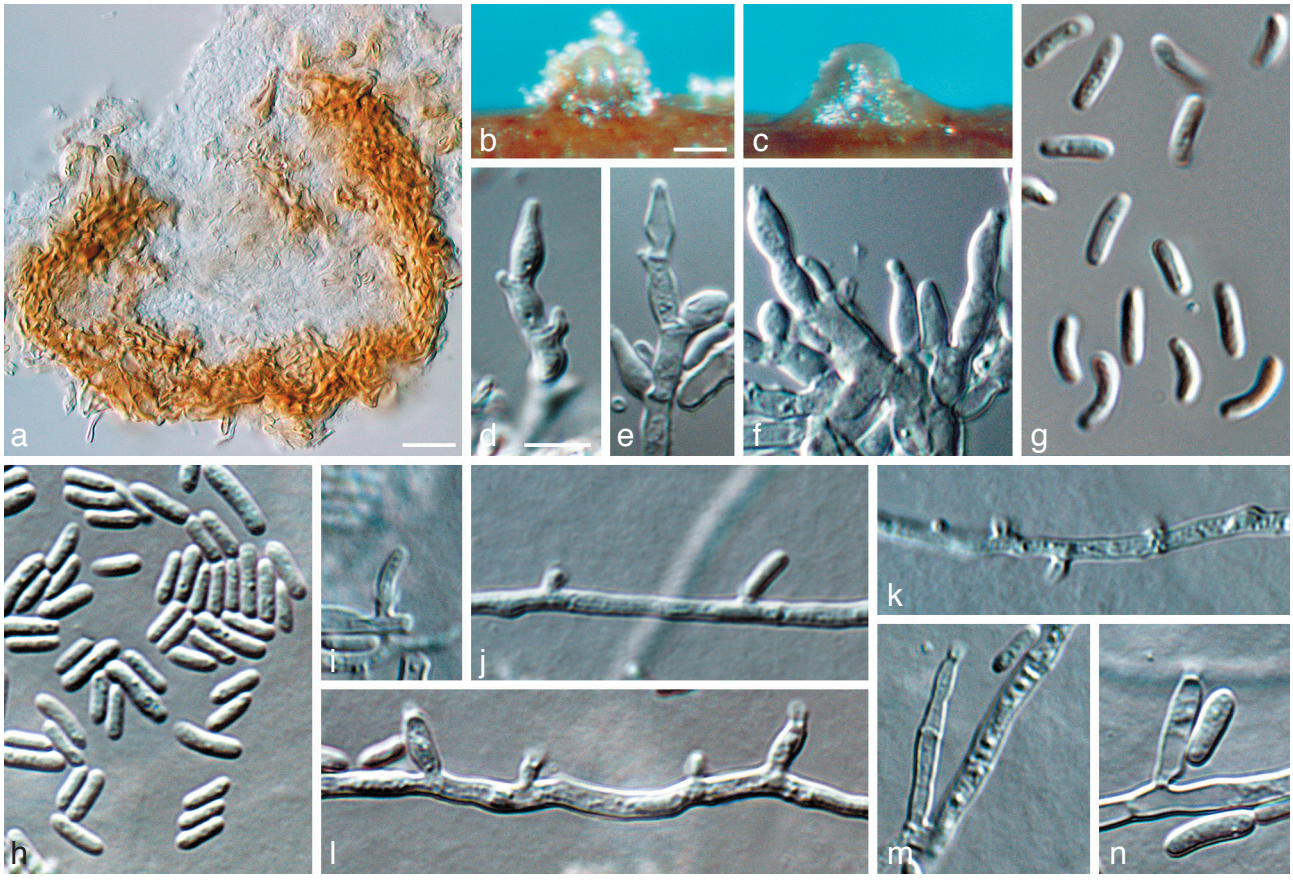

Fig. 3 *Collophora capensis*. a. Longitudinal section through a pseudopycnidium; b, c. conidia oozing from pseudopycnidia on pine needles; d–f. conidiophores lining the inner wall of pseudopycnidia; g. conidia formed in pseudopycnidia; h. conidia formed on hyphal cells; i–n. conidiogenous cells on hyphal cells. All from ex-type culture CBS 120879. a, d–n: DIC; b, c: DM. — Scale bars: a = 20 µm; b = 100 µm; d = 5 µm; b applies to b, c; d applies to d–n.

and undulate margin; surface pale rosy-vinaceous to vinaceous-grey, with black spots and white, floccose-felty aerial mycelium in the centre; reverse peach to coral to dark vinaceous; sparse amounts of a red pigment released into medium; 5 mm diam in 2 wk (25 °C in the dark), min 5 °C, max 30 °C, opt 20 °C.

Specimen examined. South Africa, Western Cape Province, Paarl, from reddish brown necrosis in wood of *P. salicina*, 10 June 2004, *U. Damm*, CBS H-19993 holotype, culture ex-type CBS 120872 = STE-U 6113.

Notes — *Collophora africana* and *Co. rubra* both form red pigments that colour the colony and surrounding medium. The species are distinct in that the conidiomatal conidiophores of *Co. africana* branch basitonously and mesotonously with divergent branches, usually < 2 µm wide, while those of *Co. rubra* are less often branched and almost parallel in arrangement. The growth rate of *Co. rubra* is similar to that of *Co. capensis*, but conidia formed both in conidiomata and on hyphae are shorter.

The closest relative of *Co. africana* (STE-U 6113) is *Co. capensis* (STE-U 6199). The two species show only a few differences in sequence: two substitutions in ITS (99 % identity), three substitutions in LSU, one intron in SSU and five substitutions within the common introns (the ones that all *Collophora* species have), but no differences in GAPDH and EF-1α. Further, the ITS sequences of both *Co. africana* STE-U 6113 (GQ154570) and *Co. capensis* STE-U 6199 (GQ154571) are 96 % identical with those of *Co. rubra* STE-U 6109 (GQ154547) and 93 % identical with those of *Co. pallida* STE-U 6197 (GQ154575) and *Co. paarla* STE-U 6114 (GQ154586).

Collophora capensis Damm & Crous, *sp. nov.* — MycoBank MB516624; Fig. 3

Collophorae africanae similis, sed conidiis in hyphis intercalaribus et in pseudopycnidiis majoribus, (4–)4.5–6.5(–9) × 1–1.5(–2) µm et (4–)4.5–6(–7) × 1–1.5 µm.

Etymology. Name refers to the Cape Province of South Africa, where this fungus was collected.

Vegetative hyphae hyaline, 1.5–3 µm wide, smooth-walled, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells, in hyphae (endoconidia) and in pseudopycnidia. *Conidiophores on hyphae* mostly reduced to conidiogenous cells, 2-celled, hyaline, cylindrical conidiophores rare, 15–20 × 1.5–2 µm. *Conidiogenous cells* enteroblastic, hyaline, mainly intercalary, mostly reduced to mere collarettes formed directly on hyphal cells; necks cylindrical to doliiform, 3–6 × 1–2 µm, discrete phialides cylindrical, constricted at the base or navicular, 5–12 × 1.5–2 µm; collarettes conspicuous, cylindrical or flaring, thin-walled, 1.5–2 µm long, opening 1 µm wide, with periclinal thickening visible. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical, sometimes curved, with both ends obtuse or attenuated to one side, smooth-walled, (4–)4.5–6.5(–9) × 1–1.5(–2) µm, mean ± SD = 5.5 ± 1.2 × 1.5 ± 0.3 µm, L/W ratio = 3.8. *Microcyclic conidiation* occurs, with conidia developing into mother cells, often becoming > 10 µm long, 2–3 µm wide, and sometimes becoming septate, with minute collarettes at one or both ends. *Conidiomata* pseudopycnidial, produced on pine needles, on SNA and on MEA in 2–4 wk, 80–200 µm diam, unilocular to convoluted, wall 15–25 µm, composed of several layers of brown textura epidermoidea with thick-walled, indistinctly delimited cells, opening by irregular rupture. *Conidiophores* lining the inner conidiomatal cavity, hyaline or slightly brown, branched at the base and above, septate, constricted at the septae, branches diverging, 20–40 µm long. *Conidiogenous cells* enteroblastic, hyaline, monophialidic, conidiogenous loci formed intercalary, immediately below the septum (acropleurogenously) as well as terminally, inflated, 4–7 × 2–3 µm, terminal phialides flask-shaped; collarettes inconspicuous, opening 0.5–1 µm, periclinal thickening visible. *Conidia* cylindrical or allantoid, with one end obtuse, the other one obtuse, truncate or attenuated, smooth-walled, (4–)4.5–6(–7) × 1–1.5 µm, mean ± SD = 5.5 ± 0.8 × 1.4 ± 0.1 µm, L/W ratio = 3.9

Culture characteristics — *Colonies on PDA* flat to umbonate, moist to slimy, with sparse, villose, white to apricot or lacking aerial mycelium and undulate margin; surface white, pale luteous, rosy-buff to bay, sometimes with rose or olivaceous-grey spots, white margin; reverse white, pale luteous to rosy-buff to scarlet or dark-vinaceous, sometimes little reddish pigment exuding into the medium; *on MEA* umbonate, with floccose-filty aerial mycelium in the centre and undulate margin; surface rosy-buff; reverse cinnamon, vinaceous to bay in the centre, white margin; 5 mm (25 °C) diam in 2 wk, min 5 °C, max 25 °C, opt 15 °C.

Specimen examined. South Africa, Western Cape Province, Paarl, from necrosis close to pruning wound in wood of *P. salicina*, 10 June 2004, *U. Damm*, CBS H-19994 holotype, culture ex-type CBS 120879 = STE-U 6199.

Notes — *Collophora capensis* is similar to *Co. africana* in growth rate. However, both conidia formed on hyphae as well as those formed in conidiomata are longer than conidia of all other *Collophora* species. Also, the conidia of other species are often allantoid and conidia of *Co. africana* are not. Additionally, minimum and optimum temperatures are lower than those for the other three species. *Collophora africana* is the closest relative of *Co. capensis* and shows only a few differences in DNA sequence (see above).

Collophora paarla Damm & Crous, *sp. nov.* — MycoBank 516625; Fig. 4

Collophorae pallidae similis, sed conidiis majoribus, (3–)4–7.5(–11) × (0.5–)1–2(–3) µm.

Etymology. Name refers to Paarl, a town in the Western Cape Province of South Africa, where this fungus was collected.

Vegetative hyphae hyaline, 1.5–5 µm wide, lacking chlamydospores. Sporulation abundant, conidia formed on hyphal cells and in hyphae (endoconidia). *Conidiophores* mostly reduced to conidiogenous cells, directly formed on hyphae, conidiophores rare, hyaline, septate, 10–20 × 2–3 µm. *Conidiogenous cells* enteroblastic, intercalary, mostly reduced to mere openings with collarettes or short cylindrical necks, 2–4 × 1–2 µm, discrete conidiophores rare, cylindrical, sometimes ampulliform, 5–10 × 2–3 µm; collarettes cylindrical or flaring, 0.5 µm long, opening 0.5–1 µm. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical, with both ends obtuse or a papillate apex, smooth-walled, often biguttulate, (3–)4–7.5(–11) × (0.5–)1–2(–3) µm, mean ± SD = 5.8 ± 1.8 × 1.4 ± 0.4 µm, L/W ratio = 4.2. *Microcyclic conidiation* occurs, with conidia developing into mother cells, often > 10 µm long, 2–3 µm wide and sometimes septate, with minute collarettes at one or both ends. *Endoconidia* formed uni- and biseriately. *Conidiomata* not observed.

Culture characteristics — *Colonies on PDA* flat, moist, irregularly wrinkled in the centre, with undulate or erose margin, lacking aerial mycelium; first very pale rosy-buff, later buff to flesh; reverse first buff, later flesh, sometimes colony turns sulphur-yellow towards the margin or pure yellow in the centre and sulphur-yellow towards the margin and sulphur-yellow pigment released into medium; *on MEA* flat, moist, lacking aerial mycelium, with erose margin; rosy-vinaceous, reddish pigment turns medium apricot to umber; 14 mm diam in 2 wk (25 °C dark), min 5 °C, max 30 °C, opt 20 °C.

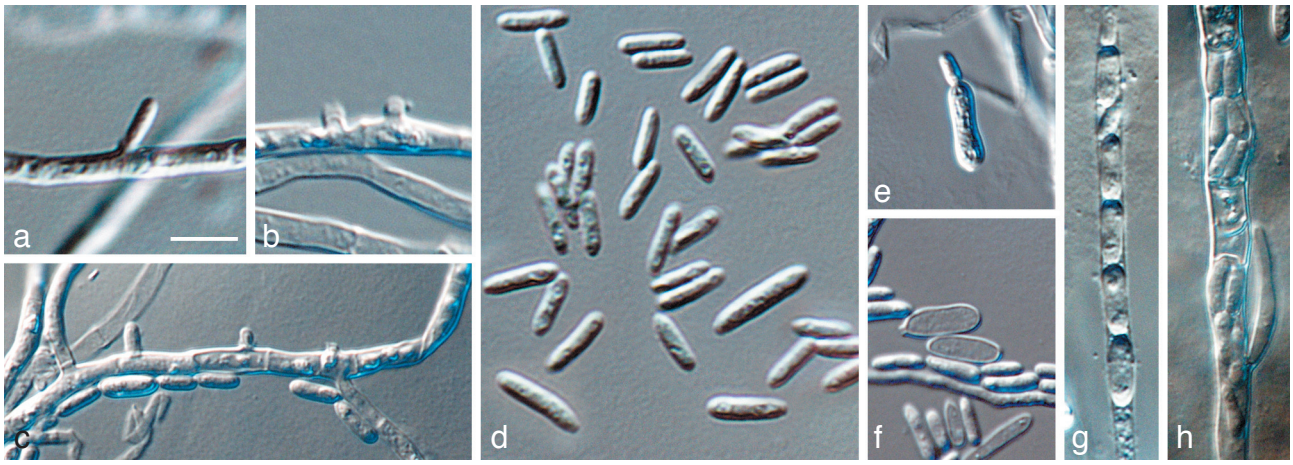

Fig. 4 *Collophora paarla*. a–c. Conidiogenous cells on hyphal cells; d. conidia formed on hyphal cells; e, f. microcyclic conidiation; g, h. endoconidia. All from ex-type culture CBS 120877. a–h: DIC. — Scale bar: a = 5 µm; a applies to a–h.

Specimen examined. South Africa, Western Cape Province, Paarl, from dark brown necrosis in wood of *P. persica*, 10 June 2004, *U. Damm*, CBS H-19996 holotype, culture ex-type CBS 120877 = STE-U 6114.

Notes — *Collophora paarla* is similar to *Co. pallida* in growth rate, initial colony colour and formation of endoconidia, but forms larger conidia (av. 5.8 µm) on intercalary hyphae than *Co. pallida* (av. 3.9 µm). Also, the vegetative hyphae are wider (1.5–5 µm) than those of *Co. pallida* (1–2 µm). *Collophora paarla* sometimes forms yellow pigments on PDA or reddish pigments on MEA; these coloured metabolites are not seen in *Co. pallida*. However, *Co. paarla* cultures do not turn scarlet as do those of *Co. rubra* and *Co. africana*.

There are only a few differences in the DNA sequences between *Co. paarla* (STE-U 6114) and *Co. pallida* (STE-U 6197). Compared to *Co. pallida*, (STE-U 6197) *Co. paarla* (STE-U 6114) has one insertion in ITS, which is 99 % identical. It has four differences in EF-1α (which is 98 % identical), two additional introns in SSU, five substitutions within the introns that all *Collophora* species have, but no differences in LSU and GAPDH sequences. In relation to *Co. africana*, *Co. rubra* and *Co. tardicola*, however, *Co. paarla* and *Co. pallida* show many differences in ITS (≥ 33 bp; 92–93 % identical), LSU (≥ 22 bp, 95 %), SSU (≥ 18 bp, incl. four additional introns and one less intron, 99 % identical), GAPDH (≥ 28 bp, > 70 %) and EF-1α (≥ 96 bp, hardly alignable).

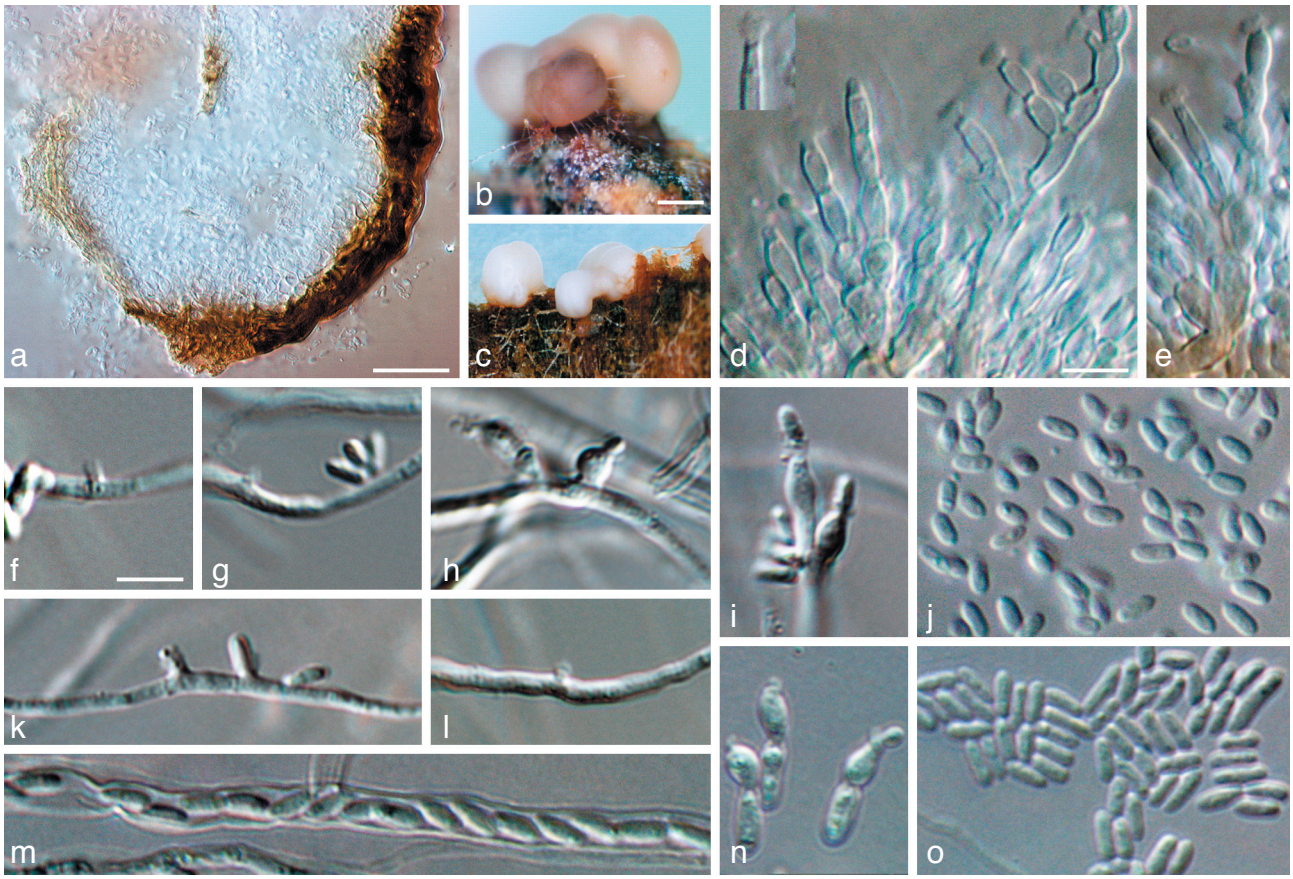

Fig. 5 *Collophora pallida*. a. Longitudinal section through a pseudopycnidium; b, c. conidia oozing from pseudopycnidium on grapevine wood; d, e. conidiophores lining the inner wall of pseudopycnidia; j. conidia formed in pseudopycnidia; f–i, k, l. conidiogenous cells on hyphal cells; m. endoconidia; n. microcyclic conidiation; o. conidia formed on hyphal cells. All from ex-type culture CBS 120878. a, d–o: DIC; b, c: DM. — Scale bars: a = 20 µm; b = 100 µm; d = 5 µm; d applies to d–o.

Collophora pallida Damm & Crous, *sp. nov.* — MycoBank MB516626; Fig. 5

Collophorae rubrae similis, sed in vitro sine pigmento rubro, conidiophoris in pseudopycnidiis cum collarettis longe infundibularibus, conidiis in hyphis intercalaribus (2.5–)3–5(–7) × 1–1.5(–2) µm, conidiis in conidiomatibus hyalinis, aseptatis, cylindraceis, utrinque obtusis, (2.5–)3–3.5(–4) × 1–1.5(–2) µm.

Etymology. Named after the pale colour of the colony (pallidus Lat. = pale).

Vegetative hyphae hyaline, 1–2 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphal cells, in hyphae (endospores) and in pseudopycnidia. *Conidiophores on hyphae* mostly reduced to conidiogenous cells, directly formed on hyphae; conidiophores rare, hyaline, septate, branched, 6–12 × 2–3 µm. *Conidiogenous cells* enteroblastic, mostly reduced to mere openings with collarettes formed directly on hyphal cells, adelophialides or discrete phialides rare; necks of adelophialides 1–3.5 × 1–2 µm, discrete phialides doliiform or ampulliform, often constricted at the base, 4–6 × 2–2.5 µm; collarettes cylindrical or flaring, frayed, < 0.5–1(–2) µm long, opening ≤ 0.5 µm. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical, with both ends obtuse or a papillate apex, smooth-walled, (2.5–)3–5(–7) × 1–1.5(–2) µm, mean ± SD = 3.9 ± 1 × 1.1 ± 0.2 µm, L/W ratio = 3.4. *Microcyclic conidiation* occurs, with conidia developing into mother cells often becoming > 10 µm long, 2–2.5 µm wide and sometimes septate and possessing minute collarettes at one or both ends. *Endoconidia* uniseriate within hyphae, hyaline, 1-celled, cylindrical to obovate, with both ends obtuse, smooth-walled, 3–4.5(–5) × 1–1.5 µm, mean ± SD = 3.3 ± 0.7 × 1.5 ± 0.1 µm, L/W ratio = 2.6. *Conidiomata* pseudopycnidial, produced on pine needles, on SNA and on MEA in 2–4 wk, subglobose, pale to dark brown, 100–900 µm diam, uni- to multilocular, wall composed of several layers of brown textura epidermoidea with thick-walled, indistinctly delimited cells, glabrous, opening by irregular rupture. *Conidiophores* lining the inner conidiomatal cavity, hyaline, smooth-walled, filiform, septate, constricted at the septa, branched (3–4 ×) at the base and above, 20–50 µm long, basal cell pigmented and 3.5–4.5 µm wide. *Conidiogenous cells* enteroblastic, hyaline, monophialidic, conidiogenous loci terminally, sometimes intercalary immediately below the transverse septae, 3.5–8 × 1.5–2.5 µm; collarette very long, tuft-like/funnel-shaped, 1–3 × 1–3 µm, openings 1–1.5 µm, periclinal wall thickening conspicuous. *Conidia* hyaline, aseptate, smooth-walled, cylindrical with obtuse ends, (2.5–)3–3.5(–4) × 1–1.5(–2) µm, mean ± SD = 3.2 ± 0.3 × 1.4 ± 0.2 µm, L/W ratio = 2.2

Culture characteristics — *Colonies on PDA* flat, moist, none or sparse floccose or villose aerial mycelium, surrounded by appressed funiculose mycelium, with undulate margin; white to very pale rosy-buff; *on MEA*: flat to umbonate, moist, mycelium appressed funiculose, with sparse, villose or lacking aerial mycelium and lobate margin; rosy-buff; 16 mm diam in 2 wk on PDA (25 °C dark), min 5 °C, max 30 °C, opt 20 °C.

Specimens examined. South Africa, Western Cape Province, Paarl, from necrosis close to pruning wound in wood of *P. persica*, 10 June 2004, *U. Damm*, CBS H-19995 holotype, culture ex-type CBS 120878 = STE-U 6197; Limpopo Province, Mookgopong, from necrosis in wood of *P. salicina*, 31 Aug. 2004, *U. Damm*, CBS 121443 = STE-U 6115.

Notes — *Collophora paarla* and *Co. pallida* are the only *Collophora* species for which endoconidia have been observed. Conidia formed on intercalary hyphae of *Co. pallida* are shorter (av. 3.9 µm) than those of *Co. rubra* (av. 4.8 µm), *Co. africana* (av. 4.5 µm), *Co. capensis* (av. 5.5 µm) and *Co. paarla* (av. 5.8 µm). Conidiophores in conidiomata differ from those of *Co. rubra*, *Co. africana* and *Co. capensis* in having very long collarettes (often 3 µm long). Unlike the four other species, *Co. pallida* has not observed to release any pigments into the surrounding medium. The colonies stay white to very pale rosy-buff. *Collophora pallida* is the closest relative of *Co. paarla* and shows only a few differences from it in the DNA sequences examined (see above).

Collophora rubra Damm & Crous, *sp. nov*. — MycoBank 516627; Fig. 6

Conidia enteroblastice formata, in conidiophoris redactis ad orificia cum collarettis sicut in hyphis et conidia acropleurogena vel terminalia, in conidiophoris filiformibus, ramosis, septatis, in pseudopycnidiis formatis. Conidia in hyphis (3.5–)4–5.5(–8) × 1–2(–3.5) µm et in conidiomatibus (3–)3.5–4(–4.5) × (1–)2 µm. In vitro cum pigmento rubro.

Etymology. Named after the red colour of the colony and the pigment exuded by the fungus (ruber Lat. = red).

Vegetative hyphae hyaline, 1–3 µm wide, smooth-walled, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphae directly and in pseudopycnidia. *Conidiophores on hyphae* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, reduced to very short adelophialides or more often with collarettes formed directly on hyphal cells; necks cylindrical, 1–4 × 0.5–2 µm; collarettes cylindrical or narrowly funnel-shaped, very thin-walled, 0.5–3 µm long, opening 0.5–1(–2) µm wide. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical to obovate, often slightly bent, with both ends obtuse or with a papillate apex, smooth to verruculose, (3.5–)4–5.5(–8) × 1–2(–3.5) µm, mean ± SD = 4.8 ± 0.9 × 1.5 ± 0.5 µm, L/W ratio = 3.25, containing small droplets. *Microcyclic conidiation* occurs, conidia developing into mother cells, often becoming > 10 µm long, 2–3 µm wide, and sometimes septate, with minute collarettes at one or both ends. *Conidiomata* pseudopycnidial, produced on pine needles, on SNA and on MEA in 2–4 wk; on pine needles solitary, subglobose, with irregular surface, superficial or semi-immersed, 90–600 µm wide; on agar medium solitary or within a stroma, uni- to multilocular; wall up to 60 µm thick, composed of several layers of reddish brown textura epidermoidea with thick-walled, indistinctly delimited cells, opening with an irregular rupture, mature conidiomata often appearing cup-shaped, surrounded by hyaline to brown hyphal appendages, nearly glabrous to completely covered with hairs. *Conidiophores* lining the inner conidiomatal cavity, hyaline, septate, slightly constricted at the base, sometimes branched at the base, more rarely above, filiform, straight or slightly zigzag-shaped, in almost parallel arrangement, 20–60 µm long. *Conidiogenous cells* enteroblastic, hyaline, monophialidic, conidiogenous loci formed intercalary, immediately below the septum (acropleurogenously) as well as terminally, 4–8 × 2–2.5 µm; collarettes cylindrical, short, often inconspicuous, 0.5–1 µm long, opening 0.5–1 µm. *Conidia* hyaline, cylindrical to ellipsoidal, with both ends obtuse or one end slightly acute, sometimes slightly curved, smooth-walled, containing small droplets, (3–)3.5–4(–4.5) × (1–)2 µm, mean ± SD = 3.7 ± 0.4 × 1.5 ± 0.2 µm, L/W ratio = 2.5.

Culture characteristics — *Colonies on PDA* flat, moist, undulate margin, rust to apricot in the centre, pale luteous or saffron towards margin, with black spots and little white to rust floccose aerial mycelium in middle, turning red to blood colour with age; reverse same colours, entire medium scarlet to red due to diffuse pigment; *on MEA* flat, undulate margin, colony centre vinaceous to dark vinaceous with black spots and white to pale flesh aerial mycelium, margin and entire medium red to scarlet due to diffuse pigment; reverse blood colour in the centre, red to scarlet at margin; 10 mm diam in 2 wk (25 °C dark), min 5 °C, max 30 °C, opt 20 °C.

Specimens examined. South Africa, Western Cape Province, Paarl, from reddish brown V-shaped necrosis close to several pruning wounds in wood of *P. persica*, 10 June 2004, *U. Damm*, CBS H-19992 holotype, culture ex-type

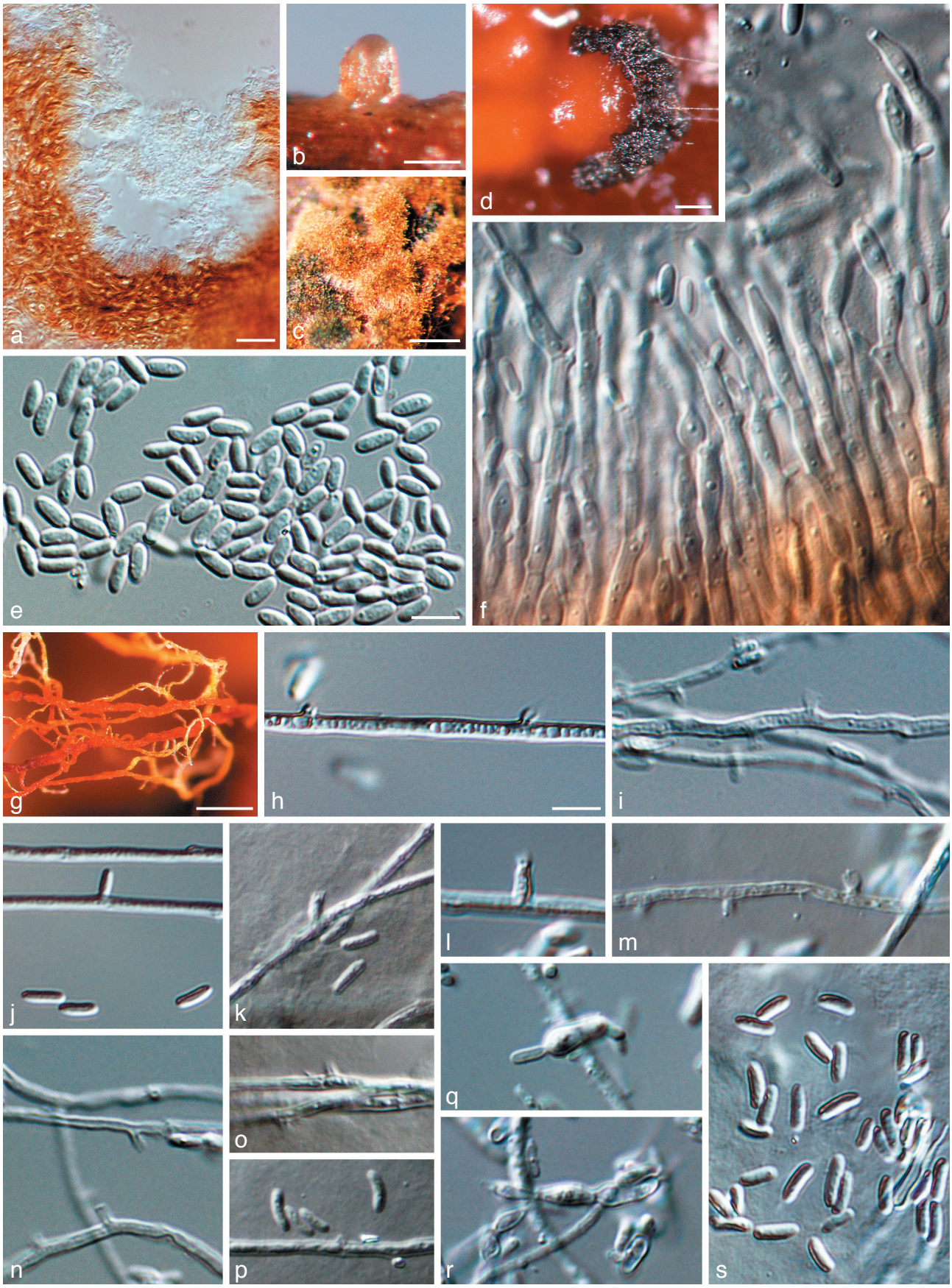

Fig. 6 *Collophora rubra*. a. Longitudinal section through a pseudopycnidium; b–d. pseudopycnidia on pine needle (b), grapevine wood (c) and MEA medium (d); e. conidia formed in pseudopycnidia; f. conidiophores lining the inner wall of a pseudopycnidium; g. hyphae on PDA medium; h–p. conidiogenous cells on hyphal cells; q–r. microcyclic conidiation; s. conidia formed on hyphal cells. All from ex-type culture CBS 120873. a, e, f, h–s: DIC; b–d, g: DM. — Scale bars: a = 20 µm; b, c = 100 µm; d, g = 200 µm; e, h = 5 µm; e applies to e, f; h applies to h–s.

CBS 120873 = STE-U 6109; Limpopo Province, Mookgopong, from brown necrosis close to pruning wound in wood of *P. persica* var. *nucipersica*, 10 June 2004, *U. Damm*, CBS 121441 = STE-U 6198.

Notes — The formation of a red pigment is the most striking character of *Co. rubra*. It shares this character with *Co. africana*, but the latter differs in growth rate and in producing more strongly branched conidiophores formed within conidiomata. *Collophora capensis*, which occasionally exudes small amounts of a reddish pigment, produces larger conidia both on hyphae and in conidiomata.

The ITS sequence of *Co. rubra* STE-U 6109 (GQ154547) is 96 % identical with that of *Co. africana* STE-U 6113 (GQ154570) and *Co. capensis* STE-U 6199 (GQ154571) and 92 % identical with ITS sequences of *Co. pallida* STE-U 6197 (GQ154575) and *Co. paarla* STE-U 6114 (GQ154586).

Coniochaeta africana Damm & Crous, *sp. nov.* — MycoBank MB516628; Fig. 7

Anamorph. Lecythophora sp.

Lecythophorae statui anamorpho *Coniochaetae lignariae* similis, sed collis bifurcate ramosis cum polyphialidibus, conidiis cylindraceis, leniter curvatis, 3.5–5.5(–7) × 1.5–2 µm.

Etymology. Named after its continent of origin, Africa.

Ascomata perithecial, solitary, superficial on pine needles, and superficial or immersed in SNA, subglobose; outer wall consists of dark brown textura angularis, setose, with a central ostiole, up to 140 µm diam, remaining immature (no asci or ascospores). *Setae* brown, cylindrical, tapering to a round tip, generally straight, aseptate, smooth-walled or verruculose, 2–3 µm wide, up to 40 µm long. *Vegetative hyphae* hyaline, 1.5–3 µm wide, lacking chlamydospores. *Conidiophores* mainly reduced to conidiogenous cells; discrete conidiophores rare, cylindrical to ventricose. *Conidiogenous cells* enteroblastic, hyaline, often polyphialidic, mainly intercalary; discrete phialides cylindrical to ventricose, 10–13 × 2 µm; necks of intercalary phialides may be short cylindrical, with or without a broad base tapering to the tip, or bifurcately branched with two openings, or ampulliform, with necks 1–7 µm long (including collarette), 1–3.5 µm wide; collarettes short, cylindrical, 0.5–1 µm long, opening 0.5–1 µm wide, with indistinct periclinal wall thickening. *Sporulation* abundant. *Conidia* aggregated in heads, hyaline, 1-celled, smooth-walled, cylindrical with round ends or with one end slightly acute, sometimes slightly curved, occasionally biguttulate, 3.5–5.5(–7) × 1.5–2 µm, mean ± SD = 4.5 ± 0.9 × 1.7 ± 0.2 µm. *Microcyclic conidiation* occurs.

Culture characteristics — *Colonies on PDA* flat, with felt-like aerial mycelium and fimbriate margin; ochraceous to luteous in middle, white to amber at margin; 15 mm diam in 2 wk (25 °C dark), min 5 °C, max > 35 °C, opt ≥ 35 °C.

Specimen examined. South Africa, Limpopo Province, Mookgopong, from necrosis in wood of *P. salicina*, 31 Aug. 2004, *U. Damm*, CBS H-19990 holotype, culture ex-type CBS 120868 = STE-U 5952.

Notes — The key in Weber (2002) leads to *C. ligniaria*; however, *C. africana* forms polyphialides on bifurcately branched necks, while *C. ligniaria* does not. Additionally, cultures of *C. africana* are slower growing than those of *C. ligniaria*, reaching only 15 mm in 14 d, and remain in shades of yellow, while cultures of *C. ligniaria* reach 25–40 mm in 14 d, and become salmon with age. These characters also do not apply to any other *Coniochaeta* species with a *Lecythophora* anamorph.

A BLASTn search showed that the LSU sequence of *C. africana* isolate STE-U 5952 (GQ154601) differed in ≥ 5 bp (99 % identity) from LSU sequences of *L. mutabilis* (e.g. EF517490, AB100628), 8 bp (99 % identity) from *C. ligniaria* AY198388 and 9 bp (98 % identity) from *L. lignicola* AB261978. The ITS sequence of *C. africana* (GQ154539) differs from that of *C. velutina* isolates STE-U 5950 and 6105 (GQ154542,

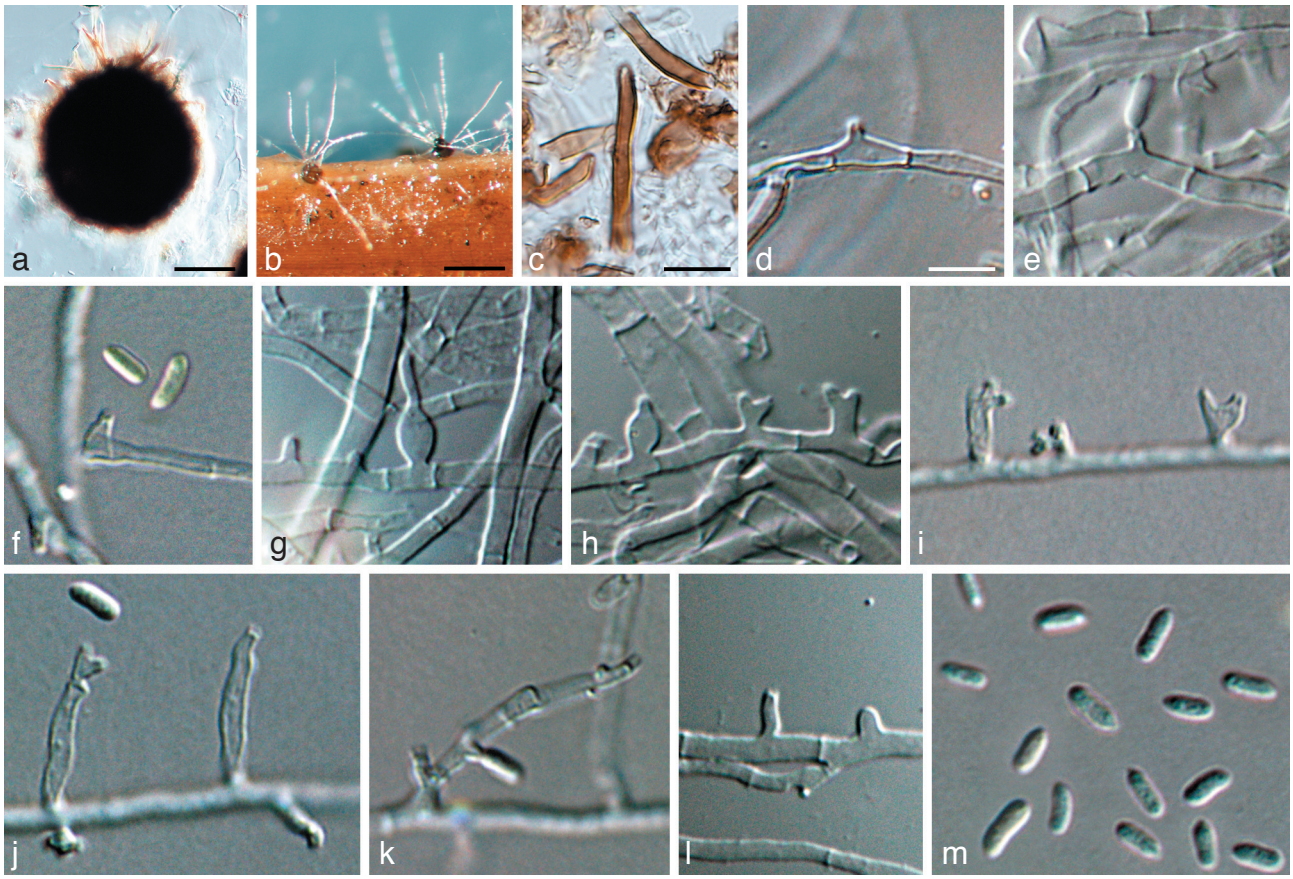

Fig. 7 *Coniochaeta africana*. a, b. Immature perithecia; c. setae; d–l. conidiogenous cells; m. conidia. All from ex-type culture CBS 120868. a, c–m: DIC; b: DM. — Scale bars: a = 50 µm; b = 100 µm; c = 10 µm; d = 5 µm; d applies to d–m.

Fig. 8 *Coniochaeta prunicola*. a. Longitudinal section through a perithecium; b. perithecium; c. perithecium with asci; d. peridium; e. setae; f–h. asci with ascospores; i, j. ascospores; k–r. conidiogenous cells; s. conidia. All from ex-type culture CBS 120875. a, c–s: DIC; b: DM. — Scale bars: a, c = 50 µm; d, f = 10 µm; e = 20 µm; i, k = 5 µm; a applies to a, b; f applies to f–h; i applies to i, j; k applies to k–s.

GQ154544) in 26 bp (95 % identical). The ITS sequence of *C. africana* strain STE-U 5952 is similar to sequences of *C. nepalica* (DQ093664, 95 % identical), *C. ligniaria* (AY198390, 94 % identical), *L. luteoviridis* (DQ404354, 94 % identical) and *L. hoffannii* (AY781227, 94 % identical).

Coniochaeta prunicola Damm & Crous, *sp. nov.* — MycoBank MB516629; Fig. 8

Anamorph. Lecythophora sp.

Coniochaetae velutinae similis, sed coloniis in vitro non atro-brunescentibus. Ascosporae uniseriatae, unicellulares, brunneae, laeves, late amygdaliformes, cum rima germinali longitudinali, (7.5–)8.5–10(–11) × (5–)6–7.5(–8) × (3–)4–5 µm.

Etymology. Named after the host from which it was isolated, *Prunus*.

Ascomata perithecial, solitary, superficial on pine needles, immersed or superficial on PDA, subglobose to pyriform, with a central ostiole, 200–250 µm diam, neck 50–60 µm long; peridium pseudoparenchymatous, 20–25 µm (5–8 layers), outer wall consists of dark brown textura angularis, setose. *Setae* brown (or hyaline), straight, cylindrical, tapering to a round tip, smooth-walled or granulate, 2–3.5 µm wide, up to 80 µm long. *Paraphyses* hyaline, septate, 2–3 × 60–100 µm. *Asci* grow from the bottom of the perithecium between paraphyses, unitunicate, cylindrical, apedicillate, 8 ascospores/ascus, 65–73 × 8–10 µm (av. 69 × 9.5 µm). *Ascospores* uniseriate, 1-celled, brown, smooth-walled, first 'saucer-shaped' (broadly-ellipsoidal in top view and reniform from the side) with granular contents; mature ascospores broadly almond-shaped, ellipsoidal with a longitudinal germ slit (7.5–)8.5–10(–11) × (5–)6–7.5(–8) × (3–)4–5 µm, mean ± SD = 9.2 ± 0.6 × 6.7 ± 0.6 × 4.5 ± 0.6 µm. *Vegetative hyphae* hyaline, 1–4 µm wide, lacking chlamydospores. *Conidiophores* formed directly on hyphae, mostly reduced to conidiogenous cells, rarely 2-celled. *Conidiogenous cells* enteroblastic, adelophialides either short cylindrical or with a broader base tapering to the tip or ampulliform, 3–6 × 1–3 µm; discrete phialides ampulliform, often constricted at the basal septum and sometimes attenuated at the base, 4–15 × 2.5–3.5 µm, generally monophialides, but sometimes polyphialides; collarette distinct, cylindrical, 1–2(–4) µm long, opening 0.5–1 µm wide, periclinal wall thickening indistinct. *Sporulation* abundant. *Conidia* aggregated in heads, hyaline, 1-celled, smooth-walled, mainly allantoid, sometimes cylindrical or ovoid, (2.5–)3.5–6(–8) × 1–2(–3.0) µm, mean ± SD = 4.8 ± 1.2 × 1.3 ± 0.5 µm. *Microcyclic conidiation* not observed.

Culture characteristics — *Colonies on PDA* flat, with sparse aerial mycelium; pale saffron, pale buff to white; 28 mm diam in 2 wk (25 °C dark), min 5 °C, max > 35 °C, opt ≥ 35 °C.

Specimens examined. South Africa, Western Cape Province, Robertson, from olivaceous V-shaped necrosis in wood of *P. armeniaca*, 23 Aug. 2005, *U. Damm*, CBS H-19991 holotype, culture ex-type CBS 120875 = STE-U 6107; Limpopo Province, Mookgopong, from necrosis in wood of *P. salicina*, 31 Aug. 2004, *U. Damm*, CBS 121445 = STE-U 5953.

Notes — The key in Asgari et al. (2007) leads to *C. velutina*, except that the ascospores of that species have guttules. However, cultures of *C. prunicola* do not turn dark as those of *C. velutina* (Weber 2002) do; the latter was also isolated in this study. *Coniochaeta prunicola* isolates produce larger ascospores than *C. velutina*, which produced ascospores measuring 5.5–8 × 4–4.5 × 3–4 µm. These dimensions correspond to those provided by Munk (1957), (6–8 × 4–6 × 3–4 µm). The anamorph of *C. prunicola* is also similar to that of *C. velutina*, but the collarette in the latter is shorter, up to 1 µm long, and the conidia are wider and not regularly allantoid. All former *Coniochaetidium*, *Ephemeroascus* and *Poroconiochaeta* species transferred into *Coniochaeta* by García et al. (2006) differed from *C. prunicola* by having ornamented or broadly umbonate ascospores, or by lacking *Lecythophora* anamorphs. Most of the remaining *Coniochaeta* species have different ascospore sizes, except for *C. calligoni*, *C. pilifera* and *C. trivialis* (syn.: *Hypocopra pilosella*). Asci of these three exceptional species are longer and narrower than those of *C. prunicola*, respectively measuring 70–110 × 6–9 µm, 96–139 × 7–8 µm and 80–90 × 7–8 µm (Saccardo 1891, Bayer 1924, Byzova & Vasyagina 1981).

While LSU sequences of isolates STE-U 6105 and 5950 (GQ154605, GQ154604) are identical to that of *C. velutina* AF353594 (CBS 110474), confirming these isolates as *C. velutina*, the sequences of *C. prunicola* isolates STE-U 5953 and 6107 (GQ154603, GQ154602) differ from *C. velutina* (EU999180, AF353594) sequences (98 % identity). Other closely related species, *C. mutabilis* (e.g. AY219880) and *C. ligniaria* (AY198388), have 99 % and 98 % sequence identity, respectively, with the LSU sequences of *C. prunicola*. The most similar ITS sequences derived from identified strains and found in BLASTn searches and in our own comparisons were those accessed in GenBank as DQ404354 (*L. luteoviridis*), AY198390 (*C. ligniaria*), AY781227 (*L. hoffmannii*), GQ154544 and GQ154542 (*C. velutina*) and GQ154539 (*C. africana*) are 93 % identical.

Phaeomoniella dura Damm & Crous, *sp. nov*. — MycoBank MB516630; Fig. 9

Phaeomoniellae prunicolae similis, sed culturis albidis vel bubalinis, solidis et phialidibus in pycnidiis aggregatis in conidiophoris ramosis, conidiis in pycnidiis hyalinis, unicellularibus, cylindraceis, interdum leniter curvatis, utrinque obtusis, (2.5–)3–3.5(–4) × 1(–1.5) µm, conidiis in hyphis formatis hyalinis, unicellularibus, interdum septatis, cylindraceis, extremo unico obtuso, extremo altero attenuato, 3–6(–10) × 1–2(–3) µm.

Etymology. Named after the tough mycelium (durus Lat. = tough).

Vegetative hyphae hyaline, 1–2.5 µm wide, smooth-walled, lacking chlamydospores; mycelium on PDA tough, leathery. *Sporulation* abundant; conidia formed on hyphal cells and in pycnidia. *Conidiophores on hyphae* often reduced to conidiogenous cells; if not, then 2–3-celled, unbranched, 10–20 × 1.5 µm. *Conidiogenous cells* enteroblastic, rarely occurring as discrete phialides, mostly reduced to adelophialides or more often with collarettes formed directly on hyphal cells; collarettes distinct, cylindrical, 0.5–1 long, opening 0.5 µm wide, with cylindrical to conical necks, 1–2 × 1–2 µm; discrete phialides cylindrical to subcylindrical, sometimes constricted at the base, 5–7 × 1–1.5 µm. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, sometimes septate when very large, cylindrical, with one end obtuse and the other end attenuated; smooth-walled, sometimes biguttulate with tiny droplets, 3–6(–10) × 1–2(–3) µm, mean ± SD = 4.5 ± 1.3 × 1.3 ± 0.5 µm, L/W ratio = 3.5. *Microcyclic conidial* formation rare. *Conidiomata* pycnidial, produced on pine needles on SNA and on MEA in 2–4 wk; on pine needles solitary, subglobose, superficial, 50–240 µm wide, unilocular, opening by irregular rupture, with wall composed of brown textura angularis. *Conidiophores* hyaline, branched and septate. *Conidiogenous cells* enteroblastic, hyaline, consisting of discrete phialides that are ampulliform to conical, 3–6 × 2–4 µm; with cylindrical collarettes, 0.5–1 µm long, opening 0.5–1 µm. *Conidia* hyaline, 1-celled, cylindrical, sometimes slightly curved, with both ends obtuse, smooth-walled, sometimes biguttulate with tiny droplets, (2.5–)3–3.5(–4) × 1(–1.5) µm, mean ± SD = 3.1 ± 0.3 × 1.1 ± 0.1 µm, L/W ratio = 2.9.

Culture characteristics — *Colonies on PDA* flat, moist to slimy, folded towards the centre, with entire margin and sparse, villose, white, aerial mycelium; surface white to pale buff with tiny black spots, sometimes pale honey at centre; *on MEA* flat, folded towards the centre, with undulate margin, grey-

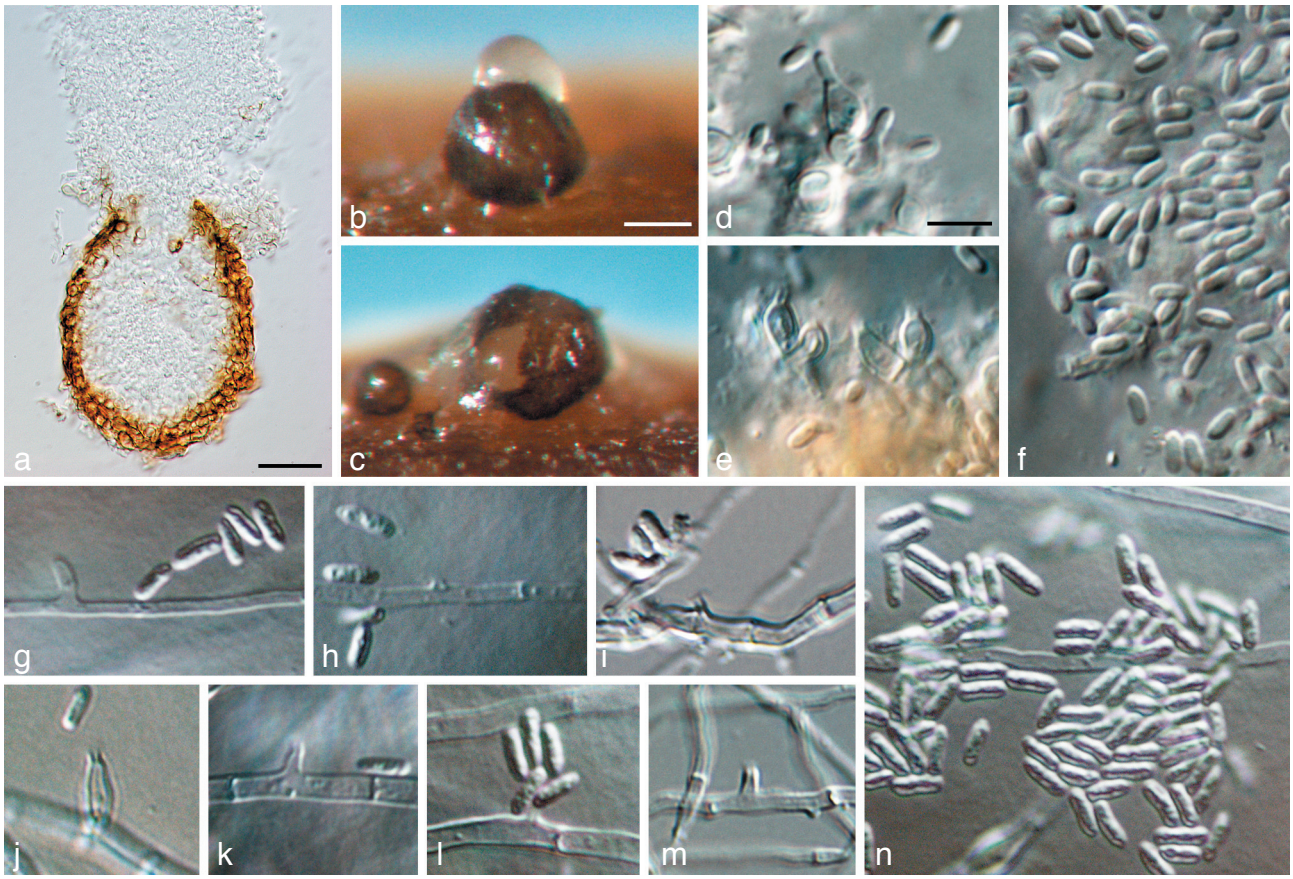

Fig. 9 *Phaeomoniella dura*. a. Longitudinal section through a pycnidium; b, c. conidia oozing from pycnidium on pine needle; d, e. conidiogenous cells lining the inner wall of pycnidia; f. conidia formed in pycnidia; g–m. conidiogenous cells on hyphal cells; n. conidia formed on hyphal cells. All from ex-type culture CBS 120882. a, d–n: DIC; b, c: DM. — Scale bars: a = 20 µm; b = 100 µm; d = 5 µm; b applies to b, c; d applies to d–n.

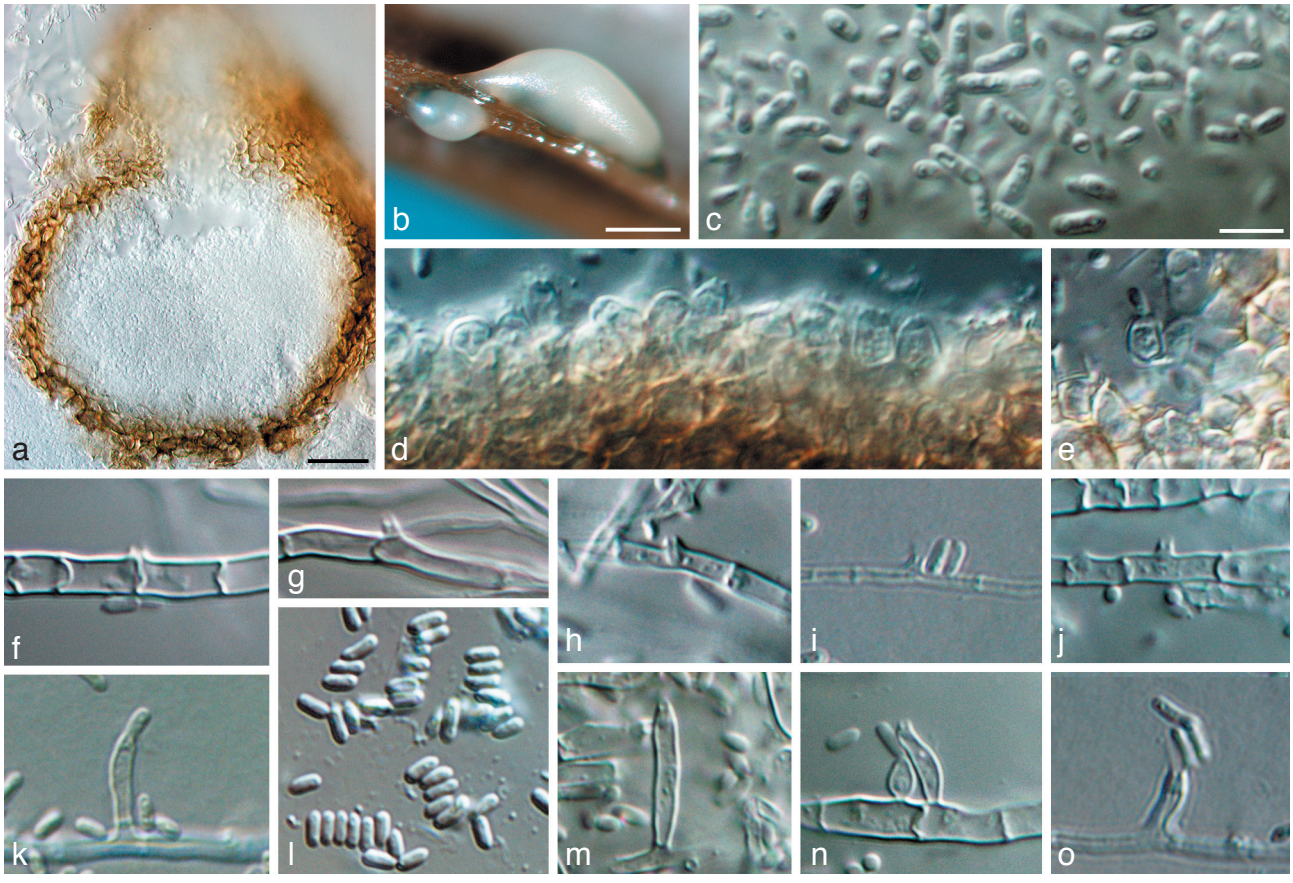

Fig. 10 *Phaeomoniella effusa*. a. Longitudinal section through a pycnidium; b. conidia oozing from pycnidium on pine needle; c. conidia formed in pycnidia; d, e. conidiogenous cells lining the inner wall of pycnidia; f–k, m–o. conidiogenous cells on hyphal cells; l. conidia formed on hyphal cells. All from ex-type culture CBS 120883. a, c–o: DIC; b: DM. — Scale bars: a = 20 µm; b = 500 µm; c = 5 µm; c applies to c–o.

olivaceous with tiny black spots towards the centre, buff at the margin; 20 mm in diam after 2 wk (25 °C dark), min 5 °C, max 30 °C, opt 20 °C.

Specimen examined. South Africa, Limpopo Province, Mookgopong, from necrosis in wood of *P. salicina*, 31 Aug. 2004, *U. Damm*, CBS H-19999 holotype, culture ex-type CBS 120882 = STE-U 6122.

Notes — Conidia formed in the mycelium are longer than those of most species, except *P. zymoides* (mainly 3.5–6 × 0.8–1.9 µm, Lee et al. 2006). Colonies of *P. dura* are, however, much faster growing than *P. zymoides* and lack any greenish or greyish colours.

BLASTn results show that the ITS sequence of *P. dura* strain STE-U 6122 (GQ154597) displays differences from sequences of *P. pinifoliorum* (DQ270240, 88 % identical), *P. chlamydospora* (e.g. AF197973, 86 % identical), *P. zymoides* (e.g. DQ270242, 85 % identical) and *P. capensis* (FJ37239, 84 % identical). ITS sequences of *P. prunicola* (GQ154590), *P. effusa* (GQ154598), *P. tardicola* (GQ154599) are 88 %, 86 % and 86 % identical.

Phaeomoniella effusa Damm & Crous, *sp. nov*. — MycoBank MB516631; Fig. 10

Phaeomoniellae prunicolae similis, sed in vitro pigmentis viridibus uniformiter dispersis. Differt ab omnibus speciebus generis phialidibus in pycnidiis late ellipsoidibus, leniter angularibus, periclinaliter distincte incrassatis, sed sine collarettis distinctis, conidiis in pycnidiis hyalinis, unicellularibus, cylindraceis, interdum leniter curvatis, utrinque obtusis, (2–)2.5–5(–6) × 1–2 µm, et conidiis in hyphis formatis hyalinis, unicellularibus, cylindraceis vel obovatis, interdum leniter curvatis, utrinque obtusis, (2–)2.5–3.5(–4.5) × 1–1.5 µm.

Etymology. Named after the effuse growth of the colonies (effusus Lat. = effuse).

Vegetative hyphae hyaline, 1–3 µm wide, smooth-walled, lacking chlamydospores. *Sporulation* abundant; conidia formed on hyphae and in pycnidia. *Conidiophores on hyphae* mainly reduced to conidiogenous cells, few 2-celled conidiophores, subcylindrical to navicular, 12–22 × 2 µm. *Conidiogenous cells* enteroblastic, discrete phialides rare, mostly reduced to very short adelophialides or more often with collarettes formed directly on hyphal cells; with cylindrical to conical necks, 1.5–3 × 1–2 µm, distinct phialides navicular or elongate-ampulliform and attenuated at the base, 7–12 × 2 µm; collarettes and periclinal thickening conspicuous, collarettes cylindrical to narrowly funnel-shaped, thin-walled, 0.5–2 long, opening 0.5–1 µm wide. *Conidia* aggregated in masses around the hyphae, hyaline, 1-celled, cylindrical to obovate, sometimes slightly curved, both ends obtuse, smooth-walled, containing small droplets, (2–)2.5–3.5(–4.5) × 1–1.5 µm, mean ± SD = 3.0 ± 0.6 × 1.2 ± 0.1 µm, L/W ratio = 2.4. *Microcyclic conidiation* not observed. *Conidiomata* pycnidial, produced on pine needles on SNA and on MEA in 2–4 wk; on pine needles solitary, subglobose, superficial, 100–350 µm wide, unilocular, opening by irregular rupture, wall composed of brown textura angularis. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline, broadly ellipsoidal, somewhat angular, resembling wall cells, 3–5 × 4–6 µm; opening 0.5 µm, periclinal thickening as a broad ring around opening, collarette very short or inconspicuous. *Conidia* hyaline, 1-celled, cylindrical, sometimes slightly curved, both ends obtuse, smooth-walled, containing small droplets, (2–)2.5–5(–6) × 1–2 µm, mean ± SD = 3.5 ± 0.7 × 1.5 ± 0.2 µm, L/W ratio = 2.3.

Culture characteristics — *Colonies on PDA* flat, moist, with sparse aerial mycelium in the centre and undulate to lobate margin; herbage-green, dark herbage-green to olivaceous, white at the margin and sometimes in the centre; *on MEA* flat, moist, with radial growth rings, very little villose, olivaceous-grey aerial mycelium, with entire margin; olivaceous-grey to pale olivaceous-grey; 32 mm diam in 2 wk (25 °C dark), min 5 °C, max 35 °C, opt 30 °C.

Specimen examined. South Africa, Western Cape Province, Paarl, from necrosis in wood of *P. persica*, 10 June 2004, *U. Damm*, CBS H-19998 holotype, culture ex-type CBS 120883 = STE-U 6121.

Notes — *Phaeomoniella effusa* is similar to *P. prunicola*, but greenish pigments are more uniformly distributed in the culture or emerge in radial growth rings. Phialides in pycnidia of *P. effusa* are broadly ellipsoidal, somewhat angular, with pronounced periclinal thickening, but with inconspicuous collarette, while those of *P. prunicola* are ampulliform with a cylindrical collarette.

BLASTn results of the ITS sequence of *P. effusa* strain STE-U 6121 (GQ154598) display differences to sequences of *P. pinifoliorum* (DQ270240, 88 % identical), *P. capensis* (FJ37239, 87 % identical), *P. zymoides* (e.g. DQ270242, 86 % identical) and *P. chlamydospora* (e.g. AB278179, 84 % identical). ITS sequences of *P. prunicola* (GQ154590), *P. tardicola* (GQ154599), *P. dura* (GQ154597) are 89 %, 88 % and 86 % identical.

Phaeomoniella prunicola Damm & Crous, *sp. nov.* — MycoBank MB516632; Fig. 11

Phaeomoniellae chlamydosporae similis, sed conidiis in mycelio persaepe enteroblastice formatis ad orificia lateralia hypharum, unicellularibus, cylindraceis vel ellipsoidibus, utrinque obtusis vel basi obtusa et apice papillato, (2–)2.5–4(–4.5) × 1–1.5(–2) µm, et conidiis in phialidibus simplicibus in pycnidiis hyalinis, cylindraceis vel ellipsoidibus, (2–)2.5–4 × 1–1.5 µm.

Etymology. Named after the host genus, *Prunus*.

Vegetative hyphae hyaline to pale yellow-green, 1.5–2.5 µm wide, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphae and in pycnidia. *Conidiophores on hyphae* hyaline, mainly reduced to conidiogenous cells, few 2-celled cylindrical conidiophores present, 11 × 2.5 µm. *Conidiogenous cells* enteroblastic, discrete phialides rare, intercalary phialides dominating, often collarettes directly on openings in hyphal cells, necks short cylindrical or with a broader base tapering to the tip, 0.5–5 × 0.5–2 µm; collarettes cylindrical, 0.5–2 µm long, opening 0.5–1(–2) µm wide, periclinal thickening inconspicuous, discrete phialides cylindrical, 3–7 × 1.5–2 µm. *Conidia* aggregated in heads or in masses around the hyphae, hyaline, 1-celled, cylindrical to ellipsoidal, with both ends obtuse or obtuse base and papillate apex, smooth-walled, (2–)2.5–4(–4.5) × 1–1.5(–2) µm, mean ± SD = 3.2 ± 0.6 × 1.2 ± 0.2 µm, L/W ratio = 2.6, a few exceptionally large conidia occur that are obovate, up to 6.5 × 3 µm, some biguttulate (very small droplets). *Microcyclic conidiation* observed. *Conidiomata* pycnidial, produced on pine needles on SNA and on MEA in 2–4 wk; on pine needles solitary, globose, superficial, up to 150 µm wide, on agar medium inside a stroma, brown, unilocular, opening by irregular rupture, wall 2–4 cell-layers thick, composed of brown thick-walled textura angularis. *Conidiophores* hyaline, mainly reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, hyaline to pale brown, ampulliform tapering towards a neck with a cylindrical collarette, 3–5 × 2–2.5 µm; collarettes 0.5–1.5 µm long, opening 0.5–1 µm, periclinal thickening sometimes visible. *Conidia* hyaline, cylindrical to ellipsoidal, (2–)2.5–4 × 1–1.5 µm, mean ± SD = 3.1 ± 0.5 × 1.2 ± 0.2 µm, L/W ratio = 2.6.

Culture characteristics — *Colonies on PDA* flat, moist, surface folded towards the centre, lacking aerial mycelium, with fimbriate margin; pale luteous to pale buff, centre, margin or sectors of the colony turn dark greenish to grey-olivaceous with age; *on MEA* flat, moist, olivaceous-black in the centre and at the margin, zone between smoke-grey and olivaceous-black, mottled, fimbriate margin; 22 mm diam after 14 d (25 °C, dark), min 5 °C, max 35 °C, opt 20 °C.

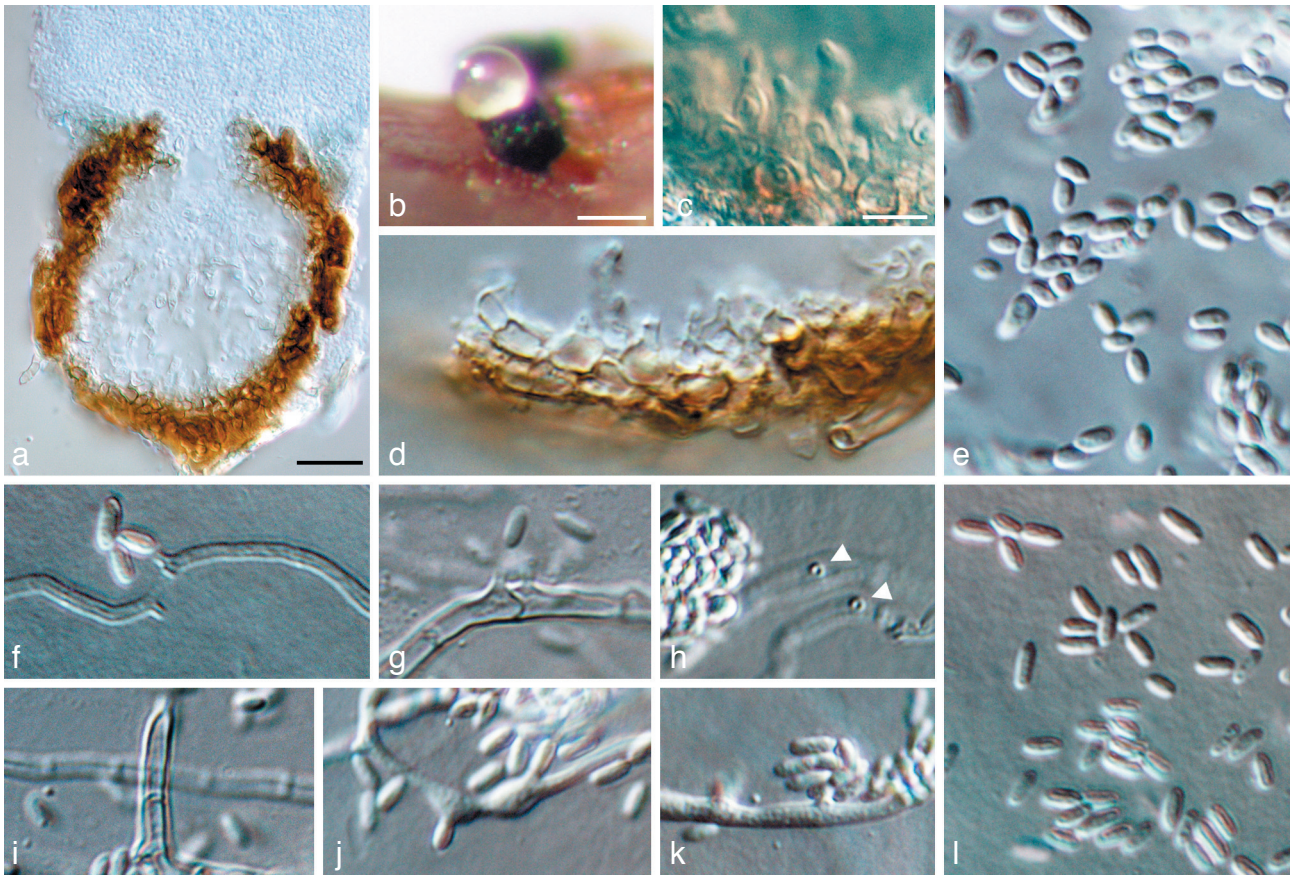

Fig. 11 *Phaeomoniella prunicola*. a. Longitudinal section through a pycnidium; b. conidia oozing from pycnidium on pine needle; c, d. conidiogenous cells lining the inner wall of pycnidia; e. conidia formed in pycnidia; f–k. conidiogenous cells on hyphal cells (arrow heads: openings in plan view); l. conidia formed on hyphal cells. All from ex-type culture CBS 120876. a, c–l: DIC; b: DM. — Scale bars: a = 20 µm; b = 50 µm; c = 5 µm; c applies to c–l.

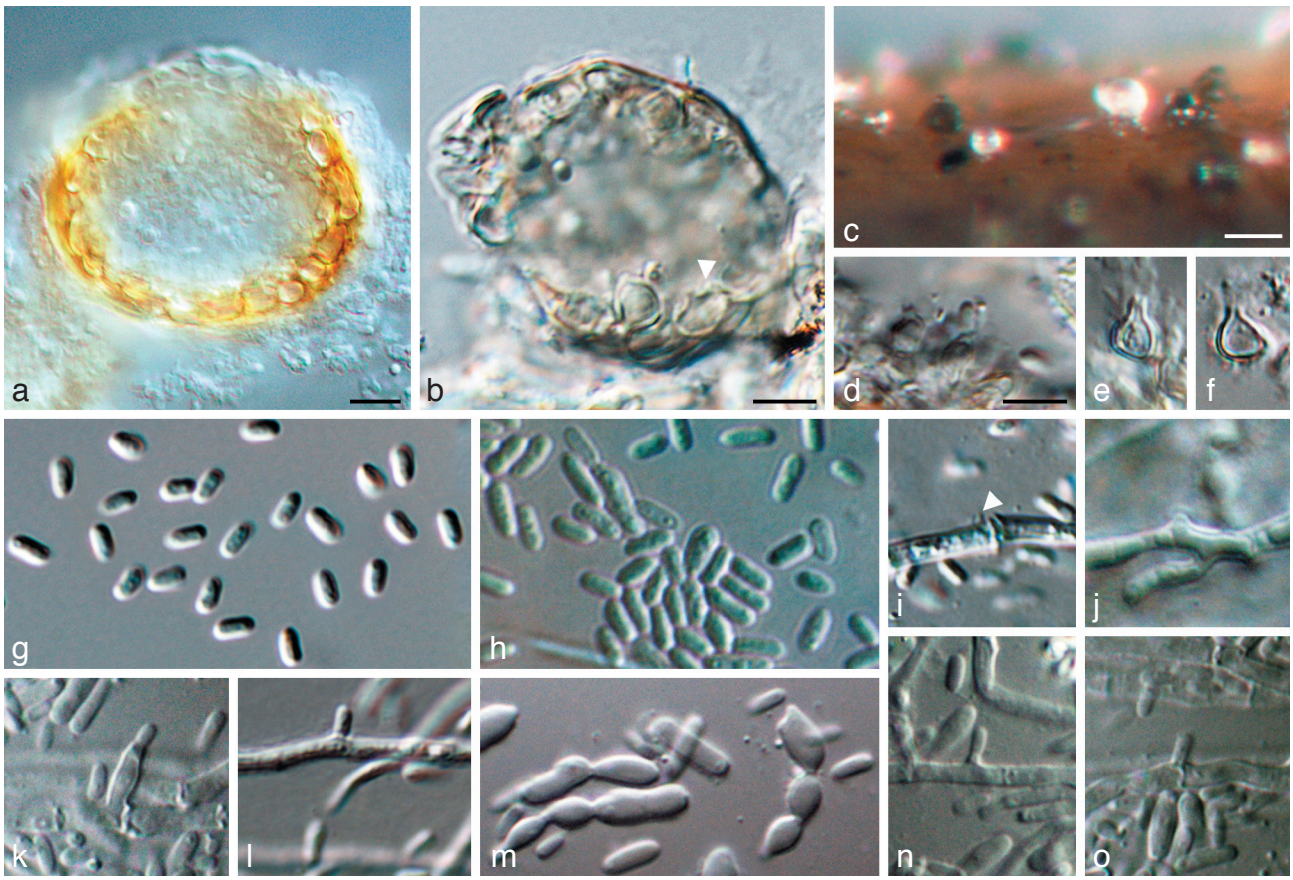

Fig. 12 *Phaeomoniella tardicola*. a. Longitudinal section through a pycnidium; b. pycnidium ruptured by slide preparation showing one-cell layered wall cells acting as conidiogenous cells (arrow head); c. conidia oozing from pycnidia on pine needle; d–f. conidiogenous cells lining the inner wall of pycnidia; g. conidia formed in pycnidia; h. conidia formed on hyphal cells; i–l, n–o. conidiogenous cells on hyphal cells (arrow head: opening in hyphae with collarette); m. microcyclic conidiation. All from ex-type culture CBS 121757. a, b, d–o: DIC; c: DM. — Scale bars: a = 10 µm; b, d = 5 µm; c = 50 µm; d applies to d–o.

Specimens examined. South Africa, Limpopo Province, Mookgopong, from necrosis in wood of *P. persica*, 31 Aug. 2004, *U. Damm*, CBS H-19997 holotype, culture ex-type CBS 120876 = STE-U 6118; Western Cape Province, Paarl, from necrosis in wood of *P. salicina*, 10 June 2004, *U. Damm*, STE-U 6117.

Notes — Conidia formed in the mycelium are narrower than those of *P. pinifoliorum*, and shorter than conidia of *P. dura* and *P. zymoides* (Lee et al. 2006). Unlike in *P. chlamydospora*, discrete phialides or conidiophores are rare (Crous et al. 1996, Crous & Gams 2000). Colonies are much faster-growing than those of *P. tardicola.* The species differs from *P. effusa* by the irregular emergence of defined areas of dark greenish pigment in cultures, either in the centre or margin of the colony or in patches, spots or sectors.

BLASTn results of the ITS sequence of *P. prunicola* strain STE-U 6118 (GQ154590) display differences to sequences of *P. pinifoliorum* (DQ270240, 90 % identical), *P. zymoides* (e.g. DQ270242, 88 % identical), *P. chlamydospora* (e.g. EU018414, 86 % identical) and *P. capensis* (FJ37239, 85 % identical). ITS sequences of *P. effusa* (GQ154598), *P. dura* (GQ154597), *P. tardicola* (GQ154599) are 89 %, 88 % and 85 % identical.

Phaeomoniella tardicola Damm & Crous, *sp. nov.* — MycoBank MB516633; Fig. 12

Phaeomoniellae prunicolae similis, sed culturis tarde crescentibus, albidis vel bubalinis et pycnidiis 15–80 µm diam, cum conidiis unicellularibus, hyalinis, cylindraceis vel obovatis, 2–4.5(–7) × 1–1.5(–2) µm, conidiis in hyphis hyalinis, unicellularibus, leniter curvatis, 3–3.5(–4) × 1–1.5 µm.

Etymology. Named after the slow growth of the fungus (tardus Lat. = slow, -cola Lat. = growing).

Vegetative hyphae hyaline, 1–2 µm wide, septate, lacking chlamydospores. *Sporulation* abundant, conidia formed on hyphae and in pycnidia. *Conidiophores on hyphae* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, reduced to mere openings formed directly on hyphal cells, rarely to short necks, discrete phialides very rare; necks 0.5–1(–5) µm long, 0.5–1 µm wide; collarettes mostly inconspicuous, opening ≤ 0.5 µm wide. *Conidia* 1-celled, hyaline, cylindrical to obovate, smooth-walled, 2–4.5(–7) × 1–1.5(–2) µm, mean ± SD = 3.2 ± 1.2 × 1.3 ± 0.3 µm, L/W ratio = 2.5. *Microcyclic conidiation* rarely observed. *Conidiomata* pycnidial, produced on pine needles on SNA, and on MEA in 2–4 wk, solitary, subglobose, superficial, pale to dark brown, globose to subglobose, 15–80 µm diam, unilocular, opening by irregular rupture, wall 1–2 cell layers thick, composed of pale brown textura angularis. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* enteroblastic, in very small pycnidia wall only one layer, these wall cells also act as conidiogenous cells, in bigger pycnidia discrete phialides, hyaline or brown, ampulliform to angular, 4–5 × 3–5 µm; collarettes 0.5–1 µm long, opening 0.5–1 µm. *Conidia* hyaline, 1-celled, cylindrical, sometimes slightly curved, smooth, 3–3.5(–4) × 1–1.5 µm, mean ± SD = 3.2 ± 0.3 × 1.3 ± 0.2 µm, L/W ratio = 2.5.

Culture characteristics — *Colonies on PDA* umbonate or raised, moist, lacking aerial mycelium, with a folded surface, and fine dentate margin; white to pale buff; *on MEA* dome-shaped, moist, none or very little short, villose aerial mycelium, strongly folded surface, sometimes bursting at the edges, with lobate margin; white, pale rosy-buff to buff; 4 mm in 14 d (25 °C), min 15 °C, max 30 °C, opt 25 °C.

Specimen examined. South Africa, Western Cape Province, Robertson, from pale brown necrosis in wood of *P. armeniaca*, 23 Aug. 2005, *U. Damm*, CBS H-20000 holotype, culture ex-type CBS 121757 = STE-U 6123.

Notes — The typical feature of this species is the extremely slow growth of the umbonate, raised or dome-shaped, white to buff cultures and the very small pycnidia. These features distinguish this species from the two other species with pale colony colours, *P. dura* and *P. pinifoliorum*. While most of the species grow at 5 °C, the minimum growth temperature of *P. tardicola* is 15 °C, a trait it shares only with *P. chlamydospora*.

BLASTn results of the ITS sequence of *P. tardicola* strain STE-U 6123 (GQ154599) display differences from sequences of *P. pinifoliorum* (DQ270240, 91 % identical), *P. capensis* (FJ37239, 86 % identical), *P. chlamydospora* (e.g. AF266656, 85 % identical) and *P. zymoides* (e.g. DQ270242, 84 % identical). ITS sequences of *P. effusa* (GQ154598), *P. dura* (GQ154597), *P. prunicola* (GQ154590) are 88 %, 86 % and 85 % identical.

Pathogenicity

All the isolates studied were isolated from brownish wood necroses or discolorations inside tree branches of various *Prunus* species. In cross-section, the lesions were irregular or round. The bark was often bulging and cracked laterally along the branch above the necrotic areas. *Lecythophora*-like fungi were isolated from these lesions mostly in combination with other fungi, for example *Alternaria*, *Cytospora*, *Diplodia* and *Phaeoacremonium* species. There were, however, many specimens (mostly peach and nectarine) where these fungi, especially *Co. rubra*, were isolated as the only fungus.

Analyses of variance of the lesion length data on apricot, peach and plum cane sections indicate a significant treatment effect ($P < 0.0001$; Anova tables not shown). *Collophora africana* (mean 82.9 mm), *Co. rubra* (69.3 mm), *C. prunicola* (64.3 mm) and *P. dura* (57.2 mm) caused lesions on the xylem of apricot shoots that were significantly longer than the negative controls (27.3–28.7 mm), but also significantly shorter than lesions caused by the pathogen control, *Eutypa lata* (120.0 mm; Table 2). On the xylem of peach shoots, *Co. pallida* (82.8 mm), *C. africana* (77.9 mm) and *P. zymoides* (72.2 mm) caused lesions that were significantly longer than the negative controls (18.4–37.8 mm), although lesions caused by the last two taxa were significantly shorter than those caused by *E. lata* (112.8 mm). Only *Co. paarla* (47.9 mm) and *Co. pallida* (43.9 mm) caused lesions on the xylem of plum shoots that were significantly longer than those caused by the negative controls (15.5–24.9 mm). Again, these lesions were significantly shorter than those caused by *E. lata* (115.0 mm). The *Lecythophora*-like species that caused the longest lesions on peach, plum and apricot shoots were, respectively, *Co. pallida*, *Co. paarla* and *Co. africana*.

Table 2 Means of lesion lengths caused by different *Lecythophora*-like species on detached green apricot, peach and plum shoots.

Fungal species	Mean of lesion length (mm)[3]		
	Apricot	Peach	Plum
Eutypa lata[1]	**120.0 a**	**112.8 a**	**115.0 a**
Collophora africana	**82.9 b**	49.7 cde	26.1 de
Collophora capensis	50.2 cdef	45.1 def	29.1 d
Collophora paarla	22.4 g	51.7 cde	**47.9 b**
Collophora pallida	48.0 cdefg	**82.8 ab**	**43.9 bc**
Collophora rubra	**69.3 bc**	60.9 bcde	33.2 cd
Coniochaeta africana	47.8 cdefg	**77.9 bc**	25.8 de
Coniochaeta prunicola	**64.3 bcd**	43.0 def	33.4 cd
Coniochaeta velutina	42.6 defg	52.7 bcde	29.3 d
Phaeomoniella dura	**57.2 bcde**	46.2 def	23.6 de
Phaeomoniella effusa	50.3 cdef	47.1 def	30.4 d
Phaeomoniella prunicola	25.0 fg	60.9 bcde	32.9 cd
Phaeomoniella tardicola	35.5 efg	62.7 bcde	25.7 de
Phaeomoniella zymoides	42.7 defg	**72.2 bcd**	27.6 d
Acremonium strictum[2]	28.7 fg	18.4 f	24.9 de
Agar plug[2]	27.3 fg	37.8 ef	15.5 e
LSD ($P < 0.05$)	26.6	30.7	11.8

[1] Pathogen control.
[2] Non-pathogen controls.
[3] Means followed by the same letter are not significantly different ($P < 0.05$), means significantly different from the non-pathogen controls are emphasized in **bold**.

Additionally to the lesions in xylem, we also frequently observed lesions on the bark surface of apricot and especially of peach canes, mostly in the form of narrow, dark brown rings around the inoculation site. Such lesions were frequently observed on peach canes inoculated with *Co. pallida*, *C. africana*, *P. zymoides*, *Co. africana*, *Co. rubra*, *P. effusa* and *P. prunicola*, and also on apricot shoots inoculated with *Co. capensis*. Other species formed surface lesions on apricot and peach canes less frequently, while on plum, lesions on the bark surface were rarely observed. Bark lesions were also observed on apricot and peach canes inoculated with *Eutypa lata* (positive control), but never in negative controls.

The fungi could be reisolated, except in the case of *P. zymoides*, *P. dura*, *P. tardicola* and *A. strictum*. None of the *Lecythophora*-like species was isolated from the negative controls.

DISCUSSION

Wood of *Prunus* species showing necrosis symptoms is often colonised by different species of fungi with reduced phialides, resembling *Lecythophora*, the anamorph of *Coniochaeta*. In spite of their similar anamorphs, the fungi studied here belong to three genera that are not closely related to each other. In fact, they belong to three different classes within the *Pezizomycotina*, namely *Sordariomycetes*, (order *Coniochaetales*, genus *Coniochaeta*), *Eurotiomycetes*, (order *Chaetothyriales*, genus *Phaeomoniella*) and *Leotiomycetes* (order uncertain, genus *Collophora*).

Some of these fungi were identified as *Coniochaeta*. This is the first report of *C. velutina* on *Prunus* and the first report of the genus *Coniochaeta* in South Africa. *Coniochaeta velutina* had been found on many different substrates and hosts (Mahoney & La Favre 1981). Endophytic strains have been grown from birch (*Betula* spp.) leaves in Finland (Helander et al. 2007). ITS sequences of *C. prunicola* show high similarities (one substitution in EF420012, EF420005, and additionally one deletion in EF419915) to those of a fungal endophyte of asymptomatic photosynthetic tissue of *Platycladus orientalis* from Arizona, USA (Hoffman & Arnold 2008). This finding suggests that this species also occurs in cupressaceous trees, at least in that area. According to Mahoney & La Favre (1981), *Coniochaeta* species are of low virulence on most hosts, usually appearing on dead tissue or as opportunistic invaders of previously infected, wounded or senescent tissue. Isolates from stained and decayed wood of *Acer saccarum* were always associated with trunk wounds, but also with other fungi (Basham et al. 1969). In the present study, *Coniochaeta* species were isolated from wood samples that rarely showed necrosis, and were always found in combination with other fungi, like *P. prunicola*, *Phaeoacremonium* spp., other *Coniochaeta* spp. and basidiomycetes. According to the preliminary pathogenicity test, *C. velutina* is not pathogenic to any of the host plants tested, while *C. prunicola* is pathogenic to apricot and *C. africana* to peach.

The second group of fungi we obtained, the *Collophora* species, belong phylogenetically to the class *Leotiomycetes* (Wang et al. 2006). However, there is a diversity of fungi closely related to it in the orders *Helotiales* and *Erysiphales*, as well as the family *Pseudeurotiaceae*; most of these fungi form either apothecia or cleistothecia. The lack of teleomorph formation in *Collophora* species makes it difficult to suggest phylogenetic affiliation with a specific order of *Leotiomycetes*. Although these species form two clades in the LSU phylogeny, they are placed in one genus, because of their similar morphological features and the lack of morphological characters distinguishing the two clades. Schol-Schwarz (1970) mentioned some pale, cream-coloured strains in the *Phialophora hoffmannii* group that developed either apothecia or a pycnidial or sporodochial state, often containing branched conidiophores. They could not be identified at that time. It is possible that some of these strains belong to *Collophora*.

The third group of fungi is closely related to *Phaeomoniella chlamydospora* (Crous & Gams 2000), *P. zymoides*, *P. pinifoliorum* (Lee et al. 2006), *P. capensis* (Crous et al. 2008), *Moristroma quercinum* and *M. japonicum* (Nordén et al. 2005). *Phaeomoniella chlamydospora* and *Moristroma* have both been shown to belong to the *Chaetothyriales* (Groenewald et al. 2001, Nordén et al. 2005). Colonies of *Chaetothyriales* (black yeasts) are typically very dark-olivaceous, compact, yeast-like and slow-growing (Gams 2000, Badali et al. 2008, Li et al. 2008). The newly described *Phaeomoniella* species have a yeast-like appearance, are more or less slow-growing; just two of them turn dark-olivaceous. However, while *Moristroma* species develop ascostroma and pycnidia on *Quercus* wood (Nordén et al. 2005), none of the *Phaeomoniella* species studied here or elsewhere formed any teleomorph structures. While *Moristroma* forms only holoblastic hyphomycetous conidia, *Phaeomoniella* species produce enteroblastic conidia in pycnidia and usually in the mycelium as well.

There are some characters that most of the known and newly described *Phaeomoniella* species share: white to greenish, moist to slimy colonies with little aerial mycelium and the production of phialoconidia in pycnidia and in the mycelium. The type species, *P. chlamydospora*, is the only species of the genus that mainly produces distinct conidiophores and dark chlamydospores (Crous & Gams 2000). Other species, described in this paper and by Lee et al. (2006), produce conidia only on short phialides or necks of intercalary phialides or on mere openings in hyphal cells. They have no or only hyaline chlamydospores. Colonies of *P. capensis* do not form conidia in the mycelium at all and are salmon, apricot or flesh coloured (Crous et al. 2008).

In *Phaeomoniella*, conidiogenous cells and pycnidial conidiophores vary, ranging from simple short cells that are hardly distinguishable from wall cells to branched conidiophores with ampulliform or cylindrical phialides. Crous et al. (2008) considered this complex to represent more than one genus and placed *P. capensis* here mainly because of its phylogenetic relationship to *P. chlamydospora*. In spite of the morphological and molecular variability and the fact that they do not form a monophyletic clade apart from *Moristroma*, the new species have been described as *Phaeomoniella* species in this paper, because at this stage we are hesitant to introduce more new genera in this complex. The species are all closely related to *P. chlamydospora* and share some of the characters with this species or with some of the other species presently accommodated in this genus.

One of the five *Phaeomoniella* species could be identified as *P. zymoides*. *Phaeomoniella zymoides* was originally isolated from needles of *Pinus densiflora* in Korea, but also as an endophyte of *Cornus sanguinea* and on lichens on *Pinus sylvestris* in Spain (CBS 122753, CBS 122752). This is the first report of *P. zymoides* from *Prunus* as well as from South Africa. The other four *Phaeomoniella* species were described as new species, *P. dura*, *P. effusa*, *P. prunicola* and *P. tardicola*. *Phaeomoniella prunicola* was frequently isolated from wood of *P. salicina* and occurs in two provinces in South Africa. The other species were isolated only from plum (*P. zymoides*, *P. dura*), peach (*P. effusa*) or apricot wood (*P. tardicola*).

Most of the species newly described in this study were probably undiscovered because of the lack of investigations on the fungal flora of *Prunus* wood in South Africa. The slow, yeast-like growth of most of the species in culture may have also caused these species to be overlooked, as may the difficulties involved in identifying such relatively nondescript cultures.

The fungi might have been overlooked or overgrown or even considered as yeasts and discarded as miscellaneous unknown fungi by persons looking for familiar pathogens. The isolation technique can also play a role. Lee et al. (2006) considered the low pH (3.7) of their media to be an aid in detecting the slow-growing acid-tolerant *P. zymoides* and *P. pinifoliorum.* In this study, however, we isolated species of all three genera, including *P. zymoides*, on common media (SNA, PDA) with a pH between 6 and 7. The more frequent species, *Co. rubra*, *Co. pallida* and *P. prunicola* did not show preference for one of the two media used.

While *P. chlamydospora* is a well-known grapevine trunk disease pathogen (Petri grapevine decline, Crous & Gams 2000, Fourie & Halleen 2004, Mostert et al. 2006a,c), *P. zymoides* and *P. pinifoliorum* were isolated from healthy looking pine needles (Lee et al. 2006) and *P. capensis* from leaf blight symptoms on *Encephalartos* (Crous et al. 2008). However, all species studied in this paper have been isolated from *Prunus* wood with necrosis symptoms. Some of the new fungi even occurred abundantly on peach and nectarine (*Co. rubra*) or on plum (*P. prunicola*, *Co. pallida*). All three fungi were found both in the Western Cape and the Limpopo Province of South Africa. *Collophora rubra*, originally isolated from 35 wood specimens, was often (on 10 specimens) the only fungus isolated from the specimen with necrotic symptoms. Because of the origin of the isolates and the results of the preliminary pathogenicity tests we consider some of the new fungi as potential pathogens on *Prunus* wood. However, of the three more common species, only *Co. pallida* caused lesions on the *Prunus* species from which it had been isolated, that is, peach and plum. *Collophora rubra* caused lesions only on apricot shoots and *P. prunicola* did not cause lesions at all. Further studies are necessary to discover the role of the newly described fungi in wood, and estimate their economic impact on fruit production.

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