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REGULAR ARTICLE

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Novel *Paraconiothyrium* species on stone fruit trees and other woody hosts

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Key words

Coniothyrium
Microdiplodia
Paraphaeosphaeria
phylogeny
Pleosporales
systematics

Abstract Coniothyrium-like fungi are common wood and soil inhabitants and hyperparasites on other fungi. They belong to different fungal genera within the Pleosporales. Several isolates were obtained on wood of different *Prunus* species (plum, peach and nectarine) from South Africa, on *Actinidia* species from Italy and on *Laurus nobilis* from Turkey. Morphological and cultural characteristics as well as DNA sequence data (5.8S nrDNA, ITS1, ITS2, partial SSU nrDNA) were used to characterise them. The isolates belonged to three species of the recently established genus *Paraconiothyrium*. This is the first report of *Paraconiothyrium brasiliense* on *Prunus* spp. from South Africa. Two new species are described, namely *Paraconiothyrium variabile* sp. nov. on *Prunus persica* and *Prunus salicina* from South Africa, on *Actinidia* spp. from Italy and on *Laurus nobilis* from Turkey, and *Paraconiothyrium africanum* sp. nov. on *Prunus persica* from South Africa. Although other known species of *Paraconiothyrium* commonly produce aseptate conidia, those of *P. africanum* and *P. hawaiiense* comb. nov. are predominantly two-celled.

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INTRODUCTION

Coniothyrium-like fungi are known as biological control agents (Finch-Savage et al. 2003), potential bioremediators (Sasaki et al. 2006), producers of metabolites inhibiting influenza virus replication (Fukami et al. 2000), and as producers of substances with potential anticancer activity (Turbyville et al. 2006). Tsuda et al. (2003) described metabolites of a *Paraconiothyrium* isolate from a marine horse mussel with antagonistic and antifungal abilities. On the other hand, *Coniothyrium* species are involved in human skin infections (Guarro et al. 1999, Miele et al. 2002). Ascomycetous fungi with coniothyrium-like anamorphs are common colonisers of wood and leaves of woody host plants, for example *Leptosphaeria coniothyrium* (anamorph: *Coniothyrium fuckelii*) on stems of *Rubus* spp., *Microsphaeropsis olivacea* on twigs and branches of *Cytisus*, *Hedera*, *Laurus*, *Lycium* and *Sambucus* and *Thyridaria rubronotata* (anamorph: *Cyclothyrium juglandis*) as plurivorous species (Ellis & Ellis 1985). *Paraconiothyrium sporulosum* (≡ *C. sporulosum*) is common in soil and *P. minitans* (≡ *C. minitans*) is almost exclusively known from fungal sclerotia (Whipps & Gerlagh 1992). However, *P. minitans* can also cause wood rot of birch and pine (Nilsson 1973), while *P. sporulosum* can cause core rot of apples in California (Michailides et al. 1994). *Coniothyrium cerealis* and *P. sporulosum* are able to degrade wood (Haider & Domsch 1969, Nilsson 1973). *Readeriella zuluensis* (≡ *C. zuluense*; Crous et al. 2007) causes cankers on *Eucalyptus* (Cortinas et al. 2006), while *Coniothyrium* species were also associated with stem necroses of *Fraxinus excelsior* (Przybyl 2002).

The delimitation of genera and species of these fungi is hampered by the variability in characters such as mode of conidiogenesis and conidial morphology. Fungi with brown, 1(–2)-celled conidia that are formed on simple conidiogenous cells in brown pycnidia are generally referred to as *Coniothyrium* species. Since the type species of the genus, *C. palmarum*, forms annellidic conidiogenous cells, Sutton (1980) included in *Coniothyrium* only species with annellides and brown, thick-walled, 0–1-septate, verruculose conidia, species with thin-walled, 1-celled conidia and phialides were referred to as *Microsphaeropsis*. Other coniothyrium-like species separated from *Coniothyrium* s.s. by Sutton (1980) are *Cyclothyrium* and *Cytoplea*. Verkley et al. (2004) established a new genus, *Paraconiothyrium*, to accommodate some species with *Coniothyrium* anamorphs, including *C. minitans* and *C. sporulosum,* and described four new species. Within this genus, they observed both phialidic and percurrent (annellidic) conidiogenesis. Only a few species with coniothyrium-like anamorphs have known teleomorphs, belonging in the ascomycete genera *Cucurbidothis*, *Leptosphaeria*, *Massarina*, *Paraphaeosphaeria, Pleospora, Thyridaria* (anamorph: *Cyclothyrium*) (Sivanesan 1984), *Neophaeosphaeria, Phaeosphaeriopsis* (Camara et al. 2001, 2003), and *Readeriella* (*Teratosphaeria*) (Crous et al. 2007). *Paraconiothyrium* spp., decribed by Verkley et al. (2004), did not produce teleomorph states, but were shown to belong to *Paraphaeosphaeria* s.s. (Pleosporales) based on their SSU phylogeny. *Coniothyrium palmarum*, *Microsphaeropsis olivacea* and *Cyclothyrium juglandis*, the type species of *Coniothyrium*, *Microsphaeropsis* and *Cyclothyrium*, respectively, as well as *Cytoplea* spp. also grouped in the Pleosporales, but were distant from each other and from *Paraphaeosphaeria*/*Paraconiothyrium* spp.

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

Species	Accession no.[1]	Host	Location	Collector	Isolated from	GenBank accessions ITS	SSU
Paraconiothyrium africanum	STE-U 6316/CBS 121166*	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295650	EU295654
Paraconiothyrium brasiliense	STE-U 6300, CBS 121426	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295632	EU295651
	STE-U 6301	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295638	
	STE-U 6303, CBS 121165	*Prunus persica* var. *nucipersica*	Mookgopong, South Africa	U. Damm	Necrotic wood	EU295635	
	STE-U 6302, CBS 121427	*Prunus salicina*	Paarl, South Africa	U. Damm	Necrotic wood	EU295633	
	STE-U 6304	*Prunus salicina*	Mookgopong, South Africa	U. Damm	Necrotic wood	EU295637	
	STE-U 6305	*Prunus salicina*	Mookgopong, South Africa	U. Damm	Necrotic wood	EU295636	
	STE-U 6306	*Prunus salicina*	Mookgopong, South Africa	U. Damm	Necrotic wood	EU295634	
Paraconiothyrium hawaiiense	CPC 12265/CBS 120025*	*Sophora chrysophylla*	Hawaii, USA	W. Gams & Y. Degawa	Stems	DQ885897	EU295655
	CPC 12268	*Sophora chrysophylla*	Hawaii, USA	W. Gams & Y. Degawa	Stems	DQ885896	EU295656
Paraconiothyrium variabile	ER 1380, CBS 119486	*Actinidia chinensis*	Latina, Italy	L. Riccioni	Necrotic wood	EF055359	
	ER 1386, CBS 120014	*Actinidia chinensis*	Latina, Italy	L. Riccioni	Necrotic wood	EF055360	
	CBS 119633, Det 303-2005 nr 41	*Laurus nobilis*	Günlüce/Marmaris, Turkey	M.E. Göre & C. Bucak	Leaves	EU295649	
	STE-U 5848	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295645	
	STE-U 6308	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295646	
	STE-U 6309, CBS 121164	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295640	
	STE-U 6310	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295647	
	STE-U 6311, CBS 121163*	*Prunus persica*	Paarl, South Africa	U. Damm	Necrotic wood	EU295639	EU295653
	STE-U 6312	*Prunus persica*	Paarl, South Africa	U. Damm	Pycnidia on bark	EU295642	
	STE-U 6315	*Prunus persica*	Paarl, South Africa	U. Damm	Necrotic wood	EU295644	
	STE-U 6307	*Prunus salicina*	Stellenbosch, South Africa	U. Damm	Necrotic wood	EU295641	
	STE-U 6313, CBS121754	*Prunus salicina*	Paarl, South Africa	U. Damm	Necrotic wood	EU295648	EU295652
	STE-U 6314	*Prunus salicina*	Paarl, South Africa	U. Damm	Necrotic wood	EU295643	

[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; ER: Culture collection of CRA – Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Rome, Italy, * ex-type cultures.

Microsphaeropsis olivacea (≡ *C. olivaceum*) has been isolated from many woody hosts including *Prunus persica* (Sutton 1980) and *Laurus* spp. (Ellis & Ellis 1985). In a study of Buck et al. (1998), fungi in the form genus *Coniothyrium* belonged to the most prevalent epiphytic fungi from peach bark and were found more often on young bark than on scaffold bark, and more often on smooth bark than on lenticels. Buck & Traquair (1998) showed some of these isolates (identified as *C. olivaceum*) to produce siderophores and to have antagonistic abilities against other fungi. Additional coniothyrium-like species described from *Prunus* spp. include: *C. cerasi* on branches of *Prunus cerasi* in Italy, *C. insitivum* f. *syringae* on branches of *Prunus padi* in Italy and France (Saccardo 1884) and *C. pruni* on leaves of *Prunus armeniaca* and *Prunus domestica* and in mature fruits of *Prunus armeniaca* in Australia (Saccardo 1906). However, these species have not been recollected since their original description and are presently not known from culture. Therefore, it remains uncertain which coniothyrium-like genus they belong to. *Paraconiothyrium sporulosum* has been reported from *Actinidia* sp. in New Zealand, identified by CBS in 1999 (PDD 70683; http://nzfungi.landcareresearch.co.nz/html/search_collections.asp). In South Africa, several coniothyrium-like species are known as causal organisms of leaf spot diseases of *Eucalyptus* spp. and Proteaceae (Crous et al. 2000), though several have been shown to belong to *Readeriella* (Crous et al. 2007). Although *C. fuckelii* is associated with stem cankers of apple trees (Doidge et al. 1953) and roses (Pole-Evans 1928) in South Africa, no coniothyrium-like fungi have been reported from *Prunus* spp.

During a survey of *Prunus* wood from South Africa, several fungal strains forming coniothyrium-like anamorphs were isolated. These isolates included three different *Paraconiothyrium* species. Two of these species could not be assigned to described species. One of them proved to be conspecific with isolates obtained from *Actinidia* spp. in Italy and from *Laurus nobilis* in Turkey, which had earlier been identified as undescribed species of *Paraconiothyrium*. The aim of the present study is to describe these taxa morphologically and to elucidate their phylogenetic relationships.

MATERIAL AND METHODS

Isolates

Branches of trees with dieback or necrotic symptoms, as well as pruning debris, were sampled from stone fruit (*Prunus* spp.) orchards in the Western Cape and the Limpopo Provinces of South Africa. Strains from necrotic *Prunus* tissue were isolated according to the protocols of Damm et al. (2007). Single-conidial isolates were obtained from sporulating pycnidia in these cultures and on the bark of pruning debris by transferring germinating conidia from 2 % tap water agar onto potato-dextrose agar (2 % PDA, Biolab, Midrand, South Africa). The isolate from *Laurus nobilis* was obtained as described by Göre & Bucak (2007). Isolates from *Actinidia chinensis* in Italy were obtained from necrotic wood tissue of plants with leader die-back disease (Riccioni et al. 2007). Reference strains are maintained in the culture collections of the Department of Plant Pathology, University of Stellenbosch (STE-U), Stellenbosch, South Africa, the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, and the CRA - Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Rome, Italy. Isolates used for morphological and sequence analyses are presented in Table 1.

Morphology

To enhance sporulation, autoclaved filter paper and double-autoclaved pine needles were placed onto the surface of synthetic nutrient agar medium (SNA, Nirenberg 1976), and incubated for 2–4 wk at 25 °C under near-ultraviolet (nuv) light. Measurements, photographs of characteristic structures and vertical sections through conidiomata were made according to Damm et al. (2007). Radial growth rates and cultural characteristics were determined on oatmeal (OA, Gams et al. 2007), cornmeal (CMA, Gams et al. 2007) and 3 % malt extract (MEA, Oxoid) agars. Plates were incubated in the laboratory under diffuse daylight at 20 °C, or in an incubator under nuv light (12 h light, 12 h dark) at 15 °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 °C intervals.

DNA isolation, amplification and analyses

Genomic DNA of all isolates was extracted from fungal mycelium grown on PDA plates following the protocol of Damm et al. (in press). The 5.8S ribosomal gene with the two flanking internal transcribed spacers (ITS1 and ITS2) and the 18S rDNA gene (SSU) were amplified and sequenced using the primer pairs ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) or ITS5 (White et al. 1990) and ITS4, NS1 and NS8 (White et al. 1990), as well as the primers NS2, NS3, NS4, NS5 (White et al. 1990) and NS24 (Gargas & Taylor 1992). The sequences were added to the outgroup (ITS: *Helminthosporium velutinum* AF145704 and *Helminthosporium solani* AF163089, SSU: *Peziza echinospora* AF006309) and sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov). GenBank accession numbers and corresponding taxon names are given in Fig. 1 & 2. The alignments were assembled and manually adjusted using Sequence Alignment Editor v. 2.0a11 (Rambaut 2002). Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003). All characters were unordered and of equal weight. Characters with insertions/deletions, ambiguous position homology as well as constant characters were excluded from the ITS analyses. Gaps, uninformative and constant characters were excluded from the SSU analyses. Maximum parsimony analyses were 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 (Hillis & Bull 1993) with 100 random sequence additions. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for the resulting trees. Sequences derived in this study were lodged at GenBank (Table 1), and the alignments in TreeBASE (TreeBASE: 12345).

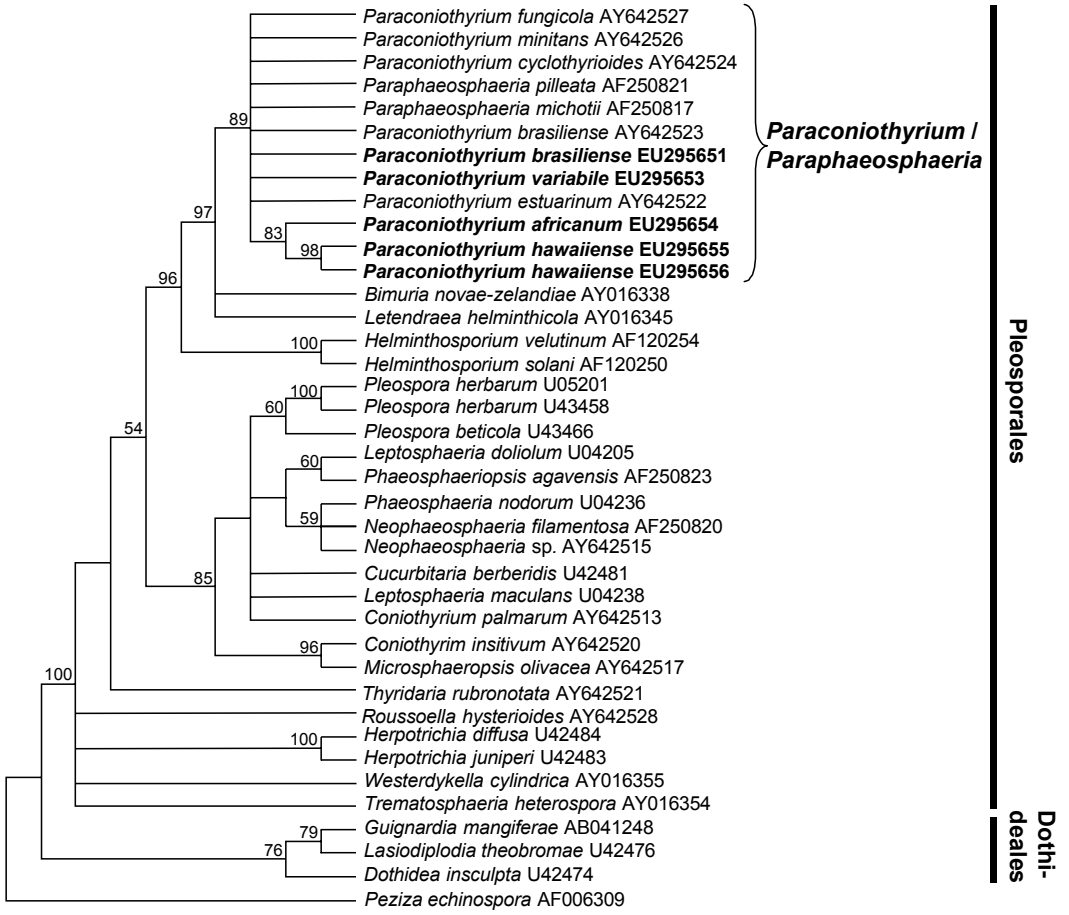

Fig. 1 Strict consensus tree obtained from SSU sequence data of Pleosporales and Dothideales (Length: 353 steps, CI: 0.552, RI: 0.819, RC: 0.452, HI: 0.448). Bootstrap support values (1 000 replicates) above 50 % are shown at the nodes. *Peziza echinospora* AF006309 was used as outgroup. Isolates analysed in this study are emphasized in bold.

RESULTS

Phylogenetic analysis

The SSU alignment of 39 taxa contained 2088 characters, including the alignment gaps and the 350 bp intron of isolate STE-U 6316, of which 160 parsimony-informative characters were included in the maximum parsimony analysis. After a heuristic search 112 most parsimonious trees were retained (Length: 353 steps, CI: 0.552, RI: 0.819, RC: 0.452, HI: 0.448), of which the strict consensus tree is shown in Fig. 1. There were two main clades in the SSU phylogeny, one formed by members of the Pleosporales (100 % bootstrap) and another by members of the Dothideales (76 %). All isolates analysed in this study are situated within the Pleosporales clade. They formed a cluster with *Paraphaeosphaeria/Paraconiothyrium* isolates (89 %). Within this clade, only *P. africanum* and *P. hawaiiense* formed a well-supported group (83 %).

The ITS dataset contained 38 taxa and 639 characters, including the gaps, of which 105 characters were parsimony-informative and included in the maximum parsimony analysis. After a heuristic search, three most parsimonious trees were retained (Length: 164 steps, CI: 0.768, RI: 0.877, RC: 0.674, HI: 0.232), of which the strict consensus tree is shown in Fig. 2. All isolates analysed in this study clustered with the majority of the taxa within the phylogeny (60 % bootstrap), representing different species of the genus *Paraphaeosphaeria*/*Paraconiothyrium*. Seven isolates grouped with the type strain of *P. brasiliense* AY642531 (95 %). The 13 isolates of *P. variabile* from *Prunus*, *Actinidia* and *Laurus* formed a well-supported sister group (89 %) to *P. brasiliense*. Their ITS sequences showed variations in only a single nucleotide. Both *P. africanum* EU295650 and *P. hawaiiense* DQ885896 and DQ885897 (94 %) did not group with any other *Paraconiothyrium* species.

Taxonomy

The 21 strains isolated from wood of *Prunus*, *Actinidia* and *Laurus*, could be assigned to three *Paraconiothyrium* spp. based on the DNA sequence data and their morphology. Two species proved distinct from known species and are newly described below, and one species, formerly belonging to *Microdiplodia*, is newly assigned to the genus *Paraconiothyrium*.

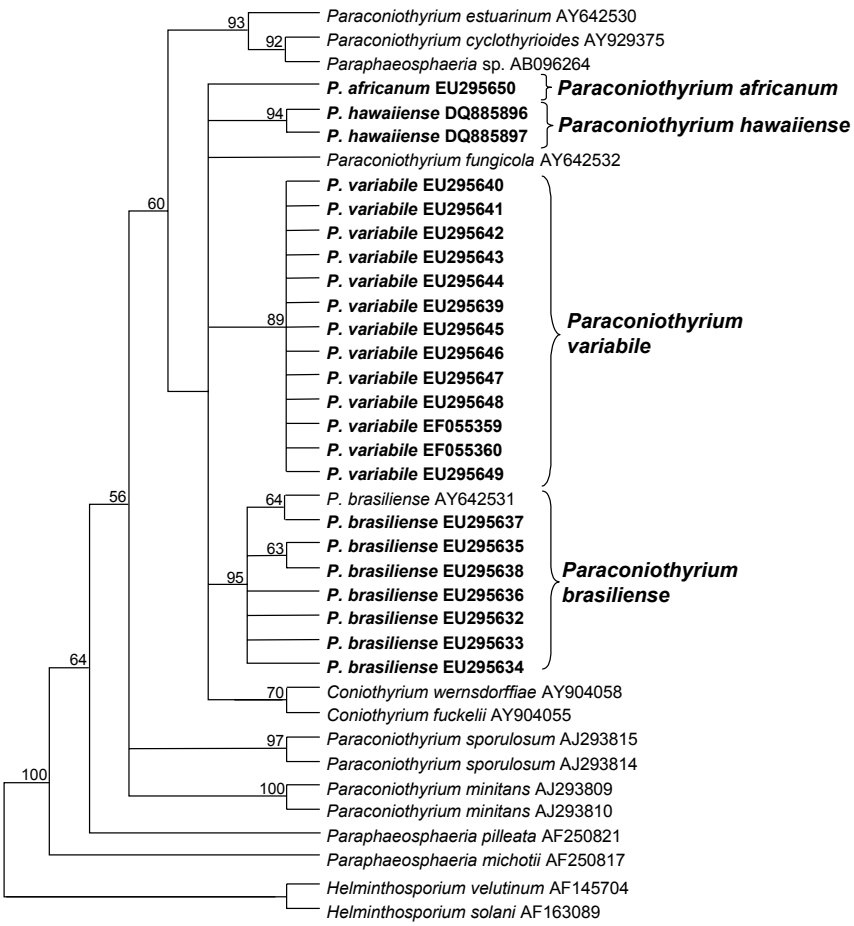

Fig. 2 Strict consensus tree obtained from ITS sequence data of *Parasphaeosphaeria*/*Paraconiothyrium* species (Length: 164 steps, CI: 0.768, RI: 0.877, RC: 0.674, HI: 0.232). Bootstrap support values (1 000 replicates) above 50 % are shown at the nodes. *Helminthosporium velutinum* AF145704 and *Helminthosporium solani* AF163089 were used as outgroup. Isolates analysed in this study are emphasized in bold.

Paraconiothyrium variabile Riccioni, Damm, Verkley & Crous, *sp. nov.* — MycoBank MB511290; Fig. 3

Conidiomata pycnidialia 300–600 µm diam, conidiophora uni- ad tricellularia, 3–15 × 2.5–6 µm. Cellulae conidiogenae conicae ad subulatae vel subcylindricae, doliiformes, late vel elongate ampulliformes variantes, phialidicae tunica sursum periclinaliter incrassata, vel semel ad repetite percurrentes, 2.5–5 × 3–7 µm. Conidia primum hyalina demum pallide brunnea, subcylindrica usque ellipsoidea, utrinque obtusa, continua, pariete tenui glabro vel minute verruculoso, (2.5–)3–4(–5) × 1–2(–2.5) µm, 2.2 × longiora quam lata.

Etymology. Named after the variable shape of the conidiogenous cells (*variabilis* Lat. = variable).

Conidiomata pycnidial, produced on pine needles on SNA in 2–4 wk, solitary, subglobose, 1–3 ostioles, dark brown, superficial to semi-immersed, 300–600 µm diam, wall consisting of 5–8 cell-layers (25–50 µm) of thick-walled dark brown *textura angularis*, becoming hyaline and thin-walled towards the inside of the pycnidium, that is surrounded by brown hyphal appendages. *Conidiophores* lining the inner conidiomatal cavity, hyaline, 1–3-celled, 3–15 × 2.5–6 µm. *Conidiogenous cells* variable in shape, conical to subulate or subcylindrical, doliiform, broadly or elongated ampulliform, sometimes with a long neck, phialidic with periclinical wall thickening or with one or more percurrent proliferations near the apex, 2.5–5 × 3–7 µm. *Conidia* initially hyaline, mature conidia pale brown, subcylindrical to ellipsoidal, both ends obtuse, 1-celled, smooth-walled to fine verruculose and thin-walled, sometimes with two small polar droplets, (2.5–)3–4(–5) × 1–2(–2.5) µm, mean ± SD = 3.3 ± 0.6 × 1.5 ± 0.3 µm, average L/W ratio = 2.2. *Vegetative hyphae* 1.5–5 µm wide, hyaline to pale brown, septate, smooth, chlamydospores absent. On OA parts of the hyaline vegetative hyphae are transformed to very dark-walled hyphal pieces (delimited by septa), which can become locally swollen or accumulate amorphous brown material on the outer wall surface (Fig. 3d).

Cultural characteristics — Colonies on OA reaching 32 mm after 7 d, 46–53 mm after 14 d (15 °C, nuv, 12 h light : 12 h dark; 43 mm in 7 d at 20 °C, in diffuse daylight), flat, with an even to slightly ruffled colourless and glabrous margin, immersed mycelium slowly developing, at first honey, then isabelline to somewhat greenish olivaceous pigmentation, sometimes also with conspicuous brick to cinnamon sectors, aerial mycelium absent or consisting of sparse, scattered white to greyish tufts; reverse concolourous, entire edge. Colonies on CMA reaching

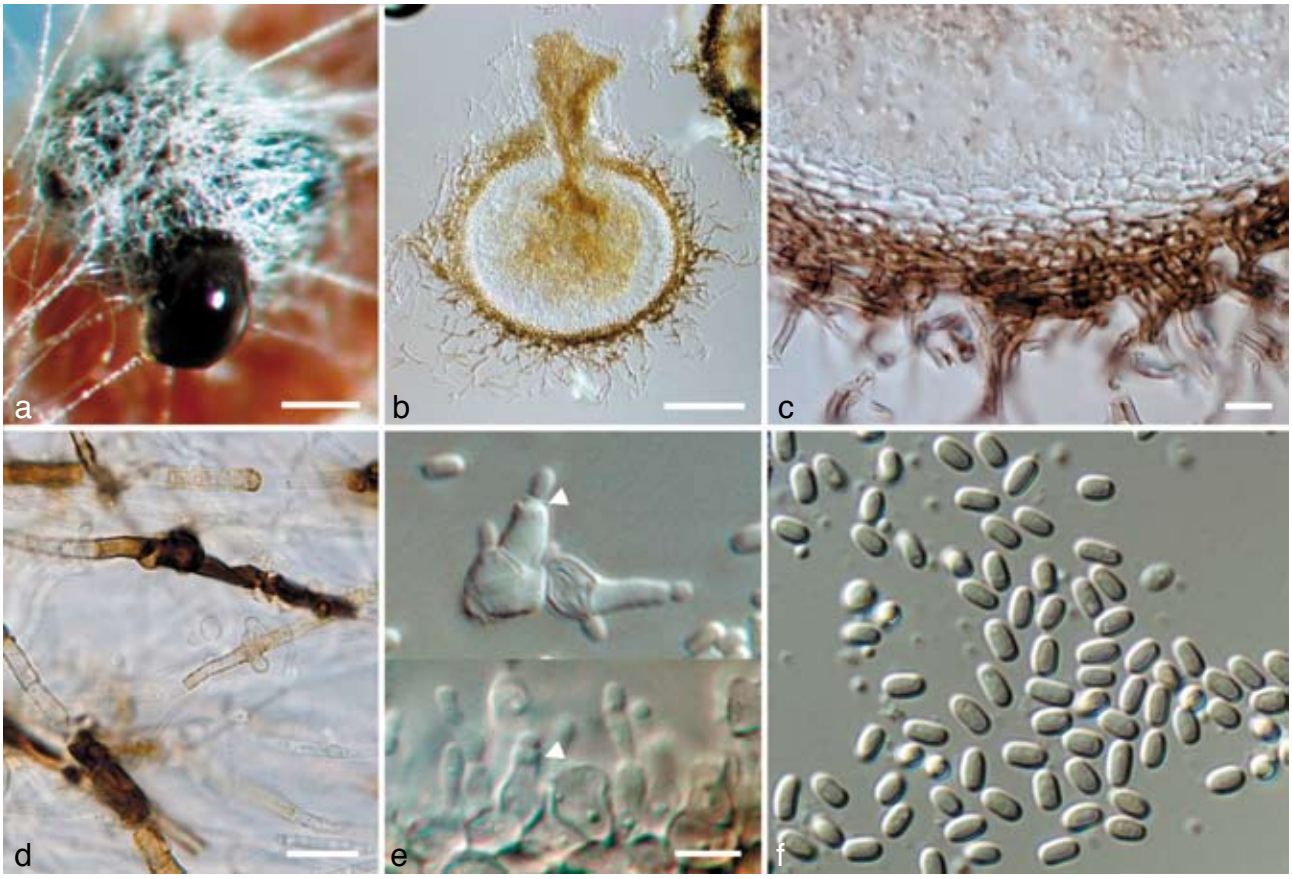

Fig. 3 *Paraconiothyrium variabile.* a. Conidia oozing from pycnidia; b. longitudinal section through a pycnidium; c. pycnidial wall with conidiophores; d. dark-walled hyphae on OA; e. conidiophores (arrow heads indicate percurrent proliferations and periclinical wall thickening); f. conidia; all from CBS H-20004 (holotype); a: DM, b–f: DIC. — Scale bars: a, b = 100 µm; c, d = 10 µm; e = 5 µm; e applies to e & f.

45–50 mm diam in 14 d (15 °C, nuv; 44 mm in 7 d at 20 °C in diffuse daylight), as on OA, but without or very sparse greenish olivaceous aerial mycelium. Colonies on MEA reaching 25–35 mm diam in 7 d, 43–45 mm in 14 d (15 °C, nuv; 42 mm in 7 d at 20 °C in diffuse daylight), low convex, with an even to slightly ruffled, glabrous and colourless margin, most of the colony surface covered by dense woolly-floccose, first whitish or pale primrose, then smoke-grey to grey-olivaceous to olivaceous aerial mycelium; reverse ochreous to fulvous, with some darker cinnamon to isabelline patches or entire centre cinnamon to isabelline or olivaceous, entire edge. Conditions for growth: max 35 °C, opt 20–25 °C.

Hosts — *Actinidia chinensis*, *Actinidia deliciosa*, *Laurus nobilis, Prunus persica, Prunus salicina.*

Distribution — South Africa, Italy, Turkey.

Specimens examined. South Africa, Western Cape Province, Paarl, from discoloured wood of *Prunus persica* close to a pruning wound, 10 June 2004, *U. Damm*, H-20004 holotype, culture ex-type CBS 121163 = STE-U 6311; from small reddish brown V-shaped necrosis under cracked bark of *Prunus salicina*, 10 June 2004, *U. Damm*, CBS 121754 = STE-U 6313. – Italy, Latina, from discoloured wood of decaying vine of *Actinidia chinensis*, 16 June 2005, *L. Riccioni*, CBS 119486 = ER1380; from discoloured wood in trunk, of *Actinidia chinensis*, 5 May 2006, *L. Riccioni*, CBS120014 = ER1386. – Turkey, Western Anatolia, Günlüce / Marmaris, from leaves of *Laurus nobilis, M.E. Göre & C. Bucak*, CBS 119633 = Det 303-2005 nr 41.

Notes — Conidia of *P. variabile* are similar to those of *P. estuarinum*, but smaller than those of most other *Paraconiothyrium* species, and longer and narrower than conidia of *P. cyclothyrioides* (Camara et al. 2001, Verkley et al. 2004, Domsch et al. 2007). However, *P. variabile* grows more slowly than *P. estuarinum* and the shape of the conidiogenous cells is more variable. On OA, *P. variabile* has paler colours and forms no or only restricted aerial mycelium as well as dark-walled hyphal pieces (Verkley et al. 2004).

Paraconiothyrium africanum Damm, Verkley & Crous, *sp. nov.* — MycoBank MB511291; Fig. 4

Conidiomata pycnidialia 100–600 µm diam, conidiophora cellulis conidiogenis reducta. Cellulae conidiogenae ampulliformes ad doliiformes, phialidicae vel semel ad repetite percurrentes, 3–8 × 2–6 µm. Conidia primum hyalina demum brunnea, cylindrica, ellipsoidea vel ovoidea, utrinque obtusa vel basi truncata, plerumque continua vel 1-septata, raro 2–3-septata, septum atrobrunneum crassum, pariete verruculoso, (4–)6.5–9.5(–12) × (2.5–)3–4(–5) µm, 2.3 × longiora quam lata.

Etymology. Named after the continent of origin, Africa.

Conidiomata pycnidial, produced on pine needles on SNA after 2–4 wk, solitary, subglobose, ampulliform or flattened, brown, 100–600 µm diam, semi-immersed, immersed or superficial,

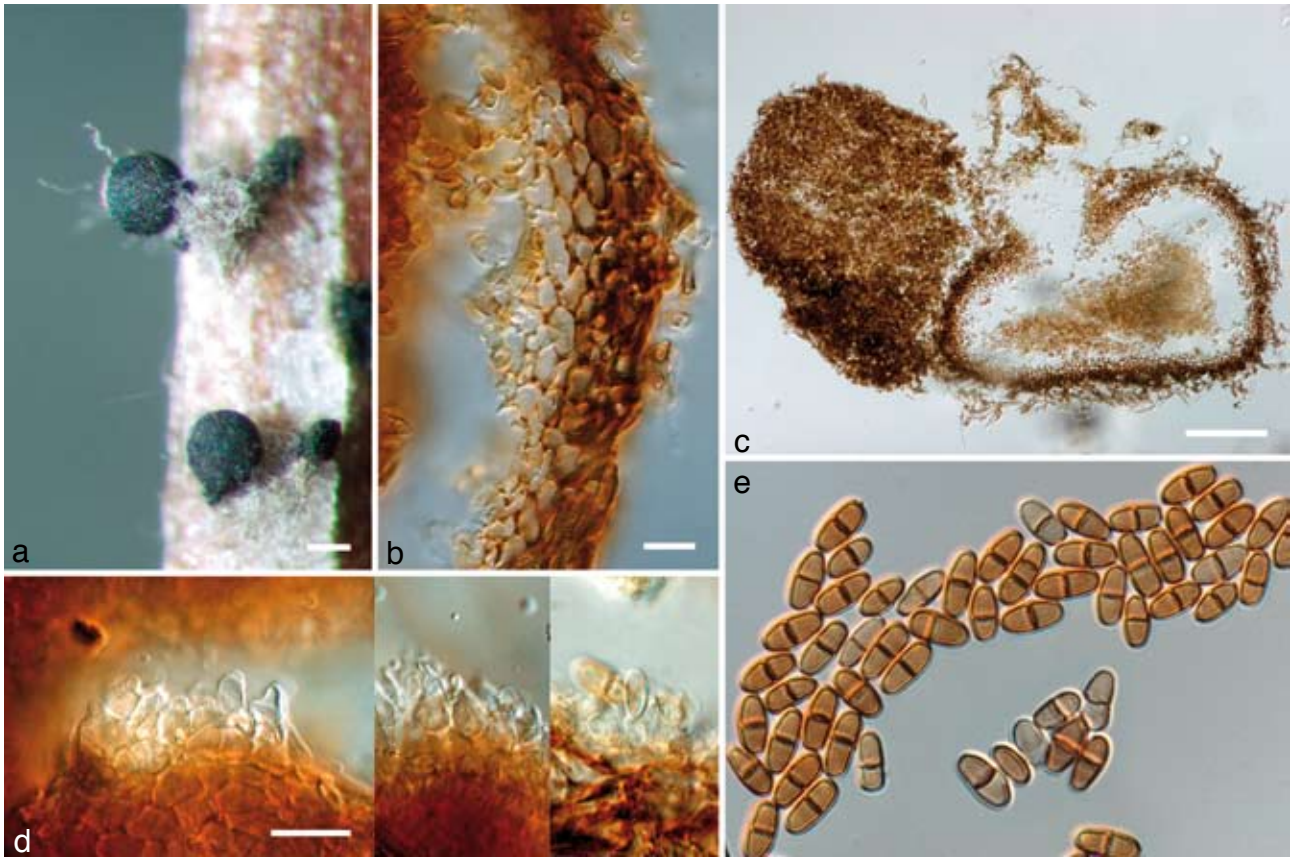

Fig. 4 *Paraconiothyrium africanum*. a. Conidia oozing from pycnidia; b. pycnidial wall; c. longitudinal section through a pycnidium; d. conidiophores; e. conidia; all from CBS H-19847 (holotype); a: DM, b–e: DIC. — Scale bars: a = 100 µm; b = 10 µm; c = 100 µm; d = 10 µm; d applies to d & e.

1–2-locular, central ostiole, wall consisting of 4–10 layers (15–30 µm) of dark brown *textura angularis*, becoming pale brown to hyaline towards the inside of the pycnidium, that is surrounded by brown, verruculose hyphal appendages. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* lining the inner conidiomatal cavity, inconspicuous, hyaline to pale brown, ampulliform to doliiform, phialidic, but also proliferating percurrently, 3–8 × 2–6 µm. *Conidia* initially hyaline, mature conidia medium brown, cylindrical, ellipsoidal or ovoid, generally both ends obtuse, sometimes one end truncate, mainly (1–)2-celled, rarely 3- or 4-celled, septum dark and thick-walled, verruculose, (4–)6.5–9.5(–12) × (2.5–)3–4(–5), mean ± SD = 8.1±1.5 × 3.5±0.6 µm, average L/W ratio = 2.3. *Vegetative hyphae*: 2–6 µm, hyaline to brown, verrucose.

Cultural characteristics — Colonies on OA reaching 24 mm after 7 d, 60–64 mm after 14 d (15 °C, nuv, 12 h light : 12 h dark; 44 mm in 7 d at 20 °C, in diffuse daylight), flat, with an even, colourless and glabrous margin, immersed mycelium honey to olivaceous buff, aerial mycelium absent or consisting of sparse, white to greyish tufts in the centre; reverse concolourous, entire edge. Colonies on CMA reaching 23 mm after 7 d, 56–62 mm diam after 14 d (15 °C, nuv; 38 mm after 7 d at 20 °C in diffuse daylight), as on OA, flat, with an even, colourless and glabrous margin, immersed mycelium honey, olivaceous buff to greenish olivaceous, aerial mycelium absent or consisting of sparse, white to greyish tufts in the centre; reverse concolourous, entire edge. Colonies on MEA reaching 22 mm diam after 7 d, 52 mm after 14 d (15 °C, nuv; 40 mm after 7 d, 70 mm after 14 d at 20 °C in diffuse daylight), flat, with an even, glabrous and colourless margin, most of the colony surface covered by felty floccose, white to pale smoke-grey aerial mycelium, immersed mycelium buff or honey to greenish olivaceous, reverse buff or honey to greenish olivaceous or olivaceous, entire edge. Conditions for growth: min < 5 °C, max 30 °C, opt 20 °C.

Host — *Prunus persica*.

Distribution — Paarl (South Africa, Western Cape Province).

Specimen examined. South Africa, Western Cape Province, Paarl, from pycnidia on the bark of *Prunus persica*, 10 June 2004, *U. Damm*, H-19847 holotype, culture ex-type CBS 121166 = STE-U 6316.

Notes — The conidia of *P. africanum* are (1- or) 2-celled, resembling those of *Paraconiothyrium hawaiiense* (*Microdiplodia hawaiiensis*) (Crous & Groenewald 2006). However, conidia of *P. hawaiiensis* are generally much larger, (10–)12–13 × (4–)5(–5.5) µm. *Paraconiothyrium fungicola* produces only occasionally 2-celled conidia with similar size (6–8 × 4.5–5.2 µm) (Verkley et al. 2004). Other known *Paraconiothyrium* species only produce aseptate, smaller conidia (Verkley et al. 2004). Conidia of *Coniothyrium palmarum* are the same size, but are 0- or 1-euseptate, and the conidiogenous cells are exlusively annellidic (Sutton 1980). According to SSU phylogeny, *C. palmarum* is not closely related to *P. africanum* (Fig. 1).

Paraconiothyrium hawaiiense (Crous) Damm, Crous & Verkley, *comb. nov.* — MycoBank MB511292

Basionym. *Microdiplodia hawaiiensis* Crous, Fungal Planet 7 (2006).
Holotypus. CBS H-19778, ex-type culture: CBS 120025 = CPC 12265.

DISCUSSION

After a first attempt to reveal the phylogenetic relationship of coniothyrium-like fungi and establishing the genus *Paraconiothyrium* to accommodate new as well as well-known species by Verkley et al. (2004), we found this to be a commonly occurring fungal genus. Species were frequently isolated from wood and leaves of *Prunus*, *Actinidia* and *Laurus*, and two additional species, *P. africanum* and *P. variabile* could be distinguished based on their DNA sequence data and unique morphological characteristics.

Paraconiothyrium brasiliense was recently described from a fruit of *Coffea arabica* in Brazil (Verkley et al. 2004). According to DNA sequence data deposited in GenBank, *P. brasiliense* also occurs endophytically in *Ginkgo biloba* (DQ094168, unidentified fungus) and *Pinus tabulaeformis* (AY546076, unidentified fungus), in leaves of *Picea glauca* in Canada (AY561200, AY566890, unidentified fungi), *Alliaria petiolata* in the USA (EF432267), in a marine fish (*Pennahia argentata*) in China (AJ619957, identified as *Myrothecium*) and in surface water in wetland in Japan (AB303550). The fungus has also been isolated from discoloured wood of a living tree of *Platanus* × *acerifolia* in Rome, Italy (M. Pilotti, CRA-PAV, Rome, Italy, pers. comm.). In our study, however, *P. brasiliense* was isolated from peach, nectarine and plum trees in two different areas in South Africa. This is the first report of this fungus on *Prunus*, as well as from South Africa. This fungus was isolated both from wood with necrotic symptoms found on living trees of *Prunus salicina* and *Prunus persica* var. *nucipersica,* as well as from pycnidia on the bark of pruning debris of *Prunus persica* collected on the orchard floor. The species seems to be widespread (different countries and continents) and common on a wide range of host plants and other habitats.

The novel species, *P. variabile,* could be distinguished based on DNA sequence data and morphology, and fits well in the concept of the genus *Paraconiothyrium* by producing smooth-walled to verruculose, pale brown, 1-celled conidia from inconspicuous phialides with periclinal thickening or percurrent proliferations (Camara et al. 2003, Verkley et al. 2004). The fungus was frequently isolated from wood necroses and pycnidia on the bark of *Prunus persica* in South Africa, and from wood of *Prunus salicina,* in association with wood necrosis symptoms. Additionally, *P. variabile* was frequently isolated from wood of *Actinidia chinensis* and *A. deliciosa* in Italy in association with trunk or vine disorders and from necrotic wood under pruning cut surfaces (Riccioni et al. 2007), and from leaves of *Laurus nobilis* in Turkey (Göre & Bucak 2007). This indicates that it may also have a broad host range, including several distantly related host plants and a wide geographical distribution. However, this species has so far only been found in and on wood and leaves of woody hosts.

Paraconiothyrium africanum was isolated from the bark of *Prunus persica* in South Africa. Conidia of *P. africanum* resemble those of *Microdiplodia hawaiiensis*, a species recently described on stems of *Sophora chrysophylla* in Hawaii (Crous & Groenewald 2006), and shown to be phylogenetically distinct from similar anamorphs in the Botryosphaeriaceae (Crous et al. 2006). In both species, the conidia, formed on phialidic as well as percurrently proliferating conidiogenous cells, are mainly 2-celled, but conidia of *P. africanum* are much smaller than those of *M. hawaiiensis*. *Microdiplodia hawaiiensis* was shown to belong to the genus *Paraconiothyrium* by means of ITS and SSU sequence data and is therefore renamed as *Paraconiothyrium hawaiiense*. This shows, that the genus *Paraconiothyrium* comprises not only species with pigmented 1-celled conidia, but also species with pigmented 2-celled conidia that would be considered as *Microdiplodia* species. There are 349 records listed in Index Fungorum (http://www.speciesfungorum.org) under *Microdiplodia*, most of them are not connected to any teleomorph genus and of uncertain position within the Ascomycetes. However, there is no type species of *Microdiplodia* designated (Sutton 1977) nor any authentic cultures, and thus its taxonomic position remains uncertain.

Within the genus *Paraconiothyrium*, there are closely related species with mainly 1-celled conidia, that formerly belonged to or would have been addressed as *Coniothyrium* or *Microsphaeropsis*, as well as 2-celled conidia, that formerly would have been regarded as *Microdiplodia*. One species, *P. fungicola,* mainly forms 1-celled, but occasionally also 2-celled, conidia. According to this study, the concept of the genus *Paraconiothyrium* should be amended to also accommodate species with predominantly 2-celled conidia. While comparing *Coniothyrium* and *Microsphaeropsis*, Morgan-Jones (1974) emphasised that conidia of *C. palmarum* are septate at maturity, while those of *M. olivacea* are always unicellular. However, since conidia of *C. eucalypticola* are unicellular also, Morgan-Jones (1974) considered the presence of a septum as a less significant feature within the genus *Coniothyrium*, as can now also be concluded with regards to *Paraconiothyrium*.

Confirming observations of Buck et al. (1998), we also found coniothyrium-like fungi to be common on peach bark. Furthermore, we found *Paraconiothyrium* species in necrotic wood of peach, plum and nectarine wood with necrotic symptoms. It is, however, not known whether the *Paraconiothyrium* species found here have antagonistic activities against other fungi as shown for *P. minitans* (Whipps & Gerlagh 1992), and for *Coniothyrium olivaceum* isolated from peach bark (Buck & Traquair 1998). It has also not yet been determined whether the *Paraconiothyrium* species isolated from *Prunus* wood could cause disease on these hosts.

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