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RESEARCH ARTICLE

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Three new additions to the genus *Talaromyces* isolated from Atlantis sandveld fynbos soils

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Key words

ITS and β-tubulin phylogeny
morphology
Penicillium subg. *Biverticillium*
South Africa
Western Cape

Abstract During a survey of *Penicillium* spp. in soils from the diverse fynbos region in the Western Cape, South Africa, a number of previously undescribed species were isolated. Three of these belong to subg. *Biverticillium* sensu Pitt, recently incorporated into its previously associated teleomorph genus, *Talaromyces* s.str. These species displayed symmetrical biverticillate penicilli, acerose phialides and poor growth at reduced water activity, typical of this group. Morphological characters of the new species were compared to known *Talaromyces* species. The ITS and β-tubulin gene regions were used for phylogenetic comparisons, which confirmed the distinct nature of the three fynbos soil species described here as *Talaromyces chloroloma* sp. nov., *T. ptychoconidium* sp. nov. and *T. solicola* sp. nov., respectively. *Talaromyces chloroloma* is typically recognised by its strongly funiculose colony texture and after prolonged incubation, synnemata can be observed on CYA. *Talaromyces ptychoconidium* is characterised by closely appressed conidiophores that produce spirally rough-walled conidia, while *T. solicola* typically struggle to grow on CYA and is distinguished from similar species by its prominently rough-walled, spheroid conidia.

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INTRODUCTION

The fynbos biome situated in the Western Cape, South Africa, is considered one of the most botanically diverse habitats on earth with approximately 9 030 vascular plant species. This accounts for 44 % of the total floral inventory of South Africa (Goldblatt & Manning 2002). Based on the association between plants and fungi, Hawksworth (1991, 2001) estimated the scope of fungal diversity on earth to be 1.5 million species, using a plant vs fungus ratio of 1 : 6 (Hawksworth 1991, 2001). Crous et al. (2006) suggested this ratio to be 1 : 7 for South Africa and estimated that 171 500 fungal species may be present in these unique habitats. Extrapolating this ratio to the vascular plants from the fynbos area, approximately 63 210 fungal species are expected to occur in this botanically diverse region of the Western Cape. However, only a fraction of this estimate has been documented from this habitat thus far.

Similar to other global soil surveys (Christensen et al. 2000), *Penicillium* was found to be one of the dominant fungal genera in fynbos soil, contributing to the majority of the species diversity in this niche. Previous surveys conducted in the fynbos, focused on genera other than *Penicillium* s.l., with Allsopp et al. (1987) representing the only study identifying strains to species level. Considering the inherent diverse and unique nature of the fynbos biome one would expect that the soils harbour a large number of fungi, particularly species that are unknown to science (Hawksworth 1991, 2001, Crous et al. 2006).

Penicillium subg. *Biverticillium* is one of the better-defined subgenera of *Penicillium* s.l., with 23 species accepted by Pitt (1979). These species are easily distinguished from other groups by their distinct symmetrical biverticillate conidiophores that bear typical acerose phialides, while some species produce ampulliform-like phialides that end in a long and fine neck. Species characteristically have a metula to phialide length ratio of ± 1–1.2 and poor growth at reduced water activity (Pitt 1979, Pitt & Hocking 1997, Samson et al. 2011). Penicillus dimensions and shapes within species of this group are very similar. Colony characters were, therefore, often the criterion used to distinguish between closely related species, since they often contain brightly pigmented features such as mycelia, soluble pigments, exudates and/or reverse colony colour (Pitt 1979, Pitt & Hocking 1997). *Penicillium* s.l. has been widely accepted as being polyphyletic, with two distinct phylogenetic clades that correspond to the teleomorphic genera *Eupenicillium* and *Talaromyces*, respectively (LoBuglio et al. 1993, Berbee et al. 1995, Peterson 2000, Heredia et al. 2001, Seifert et al. 2004, Visagie et al. 2009, Houbraken & Samson 2011, Samson et al. 2011). In accordance with changes made to the ICBN and movement towards single-name nomenclature for fungi (Hawksworth et al. 2011, Norvell 2011), Houbraken & Samson (2011) incorporated *Eupenicillium* as a synonym of *Penicillium* s.str. and transferred species in *Penicillium* subg. *Biverticillium* to *Talaromyces* s.str. as new combinations (Samson et al. 2011), accepting 74 species and excluding species, i.e. *P. rubrum* and *P. lignorum*, with questionable taxonomic positions (Samson et al. 2011).

During a survey of the microbial diversity of fynbos soils, eight distinct taxa belonging to *Talaromyces* were isolated from Atlantis sandveld fynbos soil. Three of the isolated species were found to be novel. The aim of this study was, therefore, to compare and describe the new species with previously described *Talaromyces* spp. using morphological and phylogenetic characters. In addition to this, an identification key to the new species and their close relatives is included.

MATERIALS AND METHODS

Isolations

Strains were collected from sandy fynbos soil, situated near Malmesbury in the Western Cape, at Camphill Village (S 33,59787°; E 18,56433°), Kalbaskraal (S 33,57061°; E 18,62861°), Pella

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(S 33,51022°; E 18,55236°) and Riverlands (S 33,49066°; E 18,58388°). At each sampling site, random soil samples were collected from different plots. Five grams of each soil sample were added to 100 mL dH_2O. A dilution series were prepared from this, with the 10^{-1} and 10^{-2} dilutions plated onto Potato-Dextrose Agar (PDA; Biolab, Johannesburg, South Africa), containing 50 ppm Streptomycin (Applichem, South Africa) and 100 ppm Chloramphenicol (Applichem, South Africa). Plates were incubated at 25 °C for 6–7 d, after which colonies resembling those of *Penicillium* were transferred to Oatmeal Agar (OA). Strains were incubated for a further 7 d at 25 °C, after which single spore cultures were prepared.

Morphology

Spore suspensions of strains were prepared in a semi-solid agar (0.2 % agar; 0.05 % Tween 80). Inoculations, from these spore suspensions, were done in three point style on Czapek Yeast Autolysate Agar (CYA), Malt Extract Agar (MEA: after Blakeslee 1915), Yeast Extract Sucrose Agar (YES) and 25 % Glycerol Nitrate Agar (G25N) (Pitt 1973, 1979, Samson & Pitt 1985, Frisvad & Samson 2004). Media were modified by adding copper-sulphate ($CuSO_4 \cdot 5H_2O$) and zink-sulphate ($ZnSO_4 \cdot 7H_2O$) (Frisvad & Samson 2004). The inoculated Petri dishes (90 mm), containing 20 mL of media, were incubated at 25 °C (CYA, MEA, YES, G25N), 5 °C (CYA) and 37 °C (CYA) (Pitt 1979, Samson & Pitt 1985), in the dark with plates left unwrapped to allow for sufficient aeration (Okuda et al. 2000). Additional inoculated CYA and MEA plates were incubated in incidental light at room temperature (± 23 °C) for 7–21 d. Strains were characterised and described following the methods of Pitt (1979), Samson & Pitt (1985), Okuda et al. (2000) and Frisvad & Samson (2004). Colour names and codes follow that of Kornerup & Wanscher (1967). Reference strains for *T. calidicanius* (CBS 112002), *T. cecidicola* (KAS 509), *T. dendriticus* (KAS 849 & KAS 1190), *T. diversus* (CBS 320.48), *T. duclauxii* (MUCL 28672), *T. erythromellis* (CBS 644.80), *P. lignorum* (CBS 709.68), *T. palmae* (CBS 442.88), *T. phialosporus* (CBS 233.60), *T. panamensis* (CBS 128.89), *T. pseudostromaticus* (CBS 470.70), *T. ramulosus* (CMV 113 = DAOM 241015, CV 2804, CV 2805), *T. rugulosus* (CBS 255.31) and *T. purpureus* (CBS 475.71), were examined and compared to the new species.

Phylogenetic analysis

DNA was extracted from strains grown on MEA for 7 d using the ZR Fungal/Bacterial DNA Kit (Zymo Research, California, USA). The subsequent PCR of the ITS1–5.8S–ITS2 rDNA region were prepared in 25 µL total volume reactions and consisted of 2.5 µL 10X Kapa Taq High Yield Buffer A, 2.5 U Kapa Taq (Kapa Biosystems, Woburn, USA), 250 µM dNTPs and 0.25 µM of primers ITS1 and ITS4, respectively (White et al. 1990). The thermal cycle profile had an initial denaturing step at 94 °C for 5 min, followed by 36 cycles at 94 °C for 45 s, 56 °C for 45 s, 72 °C for 60 s, followed by a final elongation step at 72 °C for 10 min. The β-tubulin gene was amplified using the same reagents as for the ITS PCR, except for primers Bt2a and Bt2b used (Glass & Donaldson 1995). The thermal cycle profile had an initial denaturing step at 94 °C for 3 min, followed by 36 cycles at 94 °C for 45 s, 52 °C for 45 s, 72 °C for 60 s, and was completed with a final elongation step at 72 °C for 10 min.

PCR products were purified using the MSB®Spin PCRapace (Invitek, Berlin) kit. Sequencing reactions of the PCR products were set up using a Big Dye terminator cycle sequencing premix kit (Applied Biosystems, CA). The thermal cycle profile had an initial denaturing step at 94 °C for 5 min, followed by 25 cycles at 94 °C for 10 s, 55 °C for 10 s and 60 °C for 4 min. Sequence reactions were analysed with an ABI PRISM 310 genetic analyser, with the subsequent sequence contigs assembled in CodonCode Aligner (v. 3.7.1.1, Codon Code Corporation). The ITS and β-tubulin sequences of the fynbos soil strains were included in datasets containing sequences from GenBank of known *Penicillium* subg. *Biverticillium* and *Talaromyces* species (LoBuglio et al. 1993, Peterson 2000, Heredia et al. 2001, Seifert et al. 2004, Visagie et al. 2009).

Datasets were aligned in MAFFT v. 6.850b (Katoh et al. 2009) with the L-INS-i option selected. All of the aligned datasets can be obtained from TreeBASE (Submission ID 11467). Sequence analysis was done in PAUP v. 4.0b10 (Swofford 2000), using the BioNJ option for calculating a single tree for each dataset (Gascuel 1997). Confidence in nodes was calculated using a bootstrap analysis of 1 000 replicates. *Talaromyces thermophilus* was chosen as outgroup for the ITS phylogeny (Heredia et al. 2001). For β-tubulin, clade specific phylogenies were done which gave a better alignment than a total phylogeny. *Penicillium lignorum* and *T. phialosporus* were chosen as outgroups for the *T. solicola* clade phylogeny. For the *T. chloroloma* / *T. ptychoconidium* clade, synnemata-producing species *T. calidicanius*, *T. duclauxii*, *T. palmae* and *T. panamensis* were chosen as outgroups.

RESULTS

Isolations made from soil dilutions yielded 434 *Penicillium* s.l. strains. The isolates were placed into their respective taxa, using colony characters on CYA, MEA and YES. Sixty-six strains represented seven distinct morphological taxa belonging to *Talaromyces*. All of them had biverticillate conidiophores with acerose phialides and showed poor growth at reduced water activity. Taxa were identified as *Penicillium rubrum* (van Reenen-Hoekstra et al. 1990), *T. rugulosus*, *T. verruculosus* (Pitt 1979) and *T. ramulosus* (Visagie et al. 2009).

PCR reactions yielded amplicons of ± 600 and ± 500 bp in length for ITS and β-tubulin, respectively. The aligned ITS dataset (Fig. 1) was 611 base pairs long. The aligned β-tubulin datasets was 382 and 374 bp long, for the *T. solicola* clade (Fig. 1, Clade 1) and *T. chloroloma* / *T. ptychoconidium* clade (Fig. 1, Clade 2), respectively. Morphological identifications were confirmed by neighbour-joining analysis that resolved the fynbos strains in the larger clade containing *Talaromyces* spp. (Fig. 1). Sequenced strains from the three, presumed to be new species, clustered in three separate clades according to their respective morphological characters. The three groups could also be separate from previously described species, based on morphological differences, supporting their novelty.

One of the fynbos strains resembled *P. minioluteum.* Pitt's 1979 description of this species was based on isolate FRR1714 (= IMI 89377, CBS 642.68) as ex-neotype. This isolate, however, more closely resembles *P. rubrum* sensu Raper & Thom and was renamed accordingly (van Reenen-Hoekstra et al. 1990). The fynbos isolates displayed characters typical of this species, growing faster than *P. minioluteum*, as well as being strongly funiculose and producing the greenish to red colony reverse. The phylogenetic placement of *P. rubrum* and *P. minioluteum* makes sense considering their morphological characters (Fig. 1). *Penicillium minioluteum* is resolved in a clade containing slow growing species on CYA, whereas *P. rubrum* is closely related to *P. funiculosum*, *P. pinophilum* and *P. verruculosum* which all grow more rapidly on CYA. Samson et al. (2011) did not transfer *P. rubrum* to *Talaromyces* since its taxonomy remains unresolved because no type material was located. A number of fynbos strains, however, did match sequences of strain FRR 1714, as well as the description provided by van Reenen-Hoekstra et al. (1990). These were thus identified as *P. rubrum*.

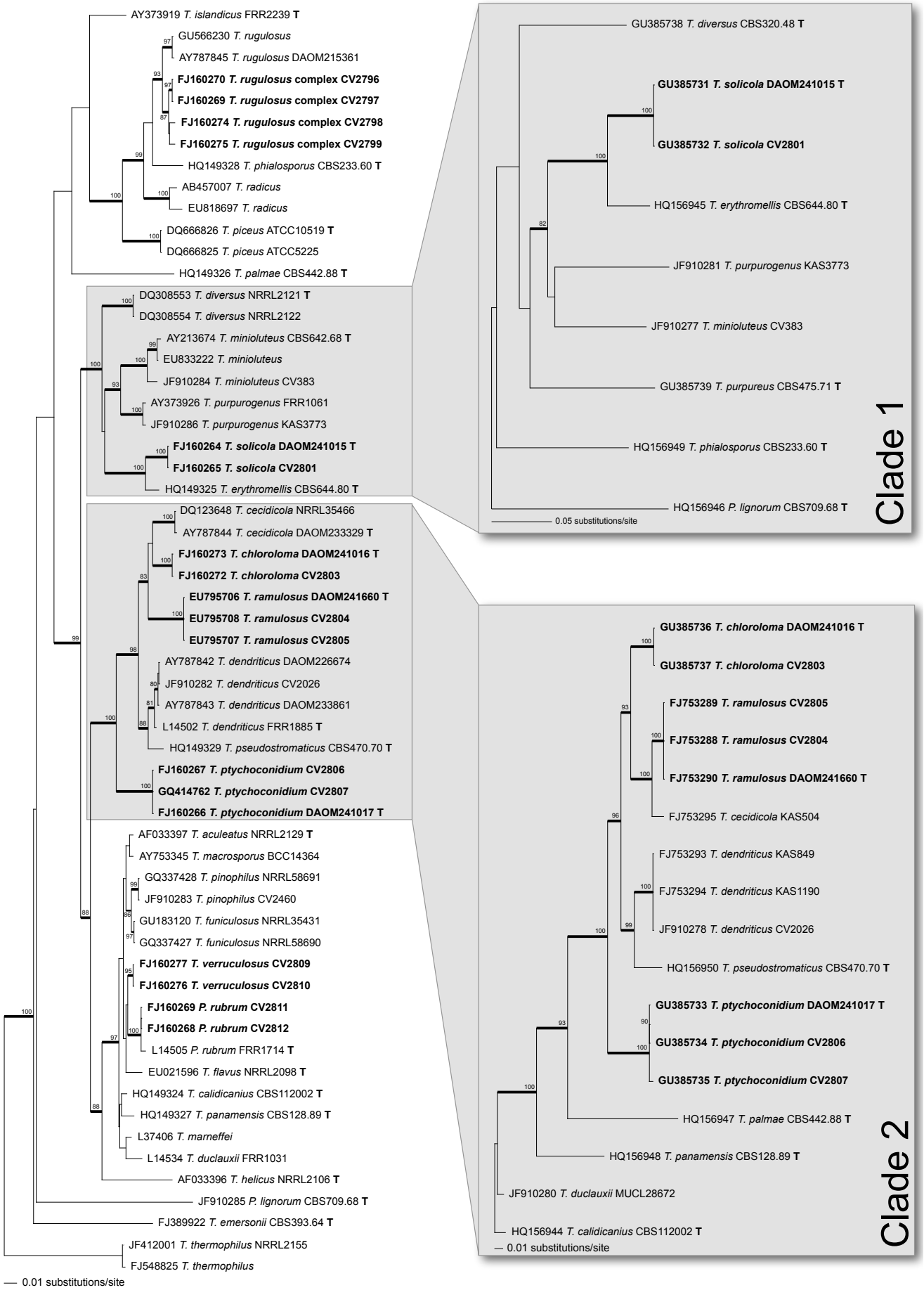

Fig. 1 Neighbour-joining trees based on the ITS1–5.8S–ITS2 rDNA and β-tubulin (in blocks) gene regions, showing relationships within *Talaromyces* spp. and strains isolated from fynbos soil. Numbers at branching nodes represent bootstrap values (1 000 replicates), with **bold** branches indicating bootstrap values higher than 80 %. *Talaromyces thermophilus* was selected as outgroup for the ITS phylogeny. *Penicillium lignorum* and *T. phialosporus* was chosen as outgroup for the *T. solicola* clade (top block = Clade 1) phylogeny, with *T. calidicanius*, *T. duclauxii*, *T. palmae* and *T. panamensis* the outgroup for the *T. chloroloma / T. ptychoconidium* clade (bottom block = Clade 2) phylogeny.

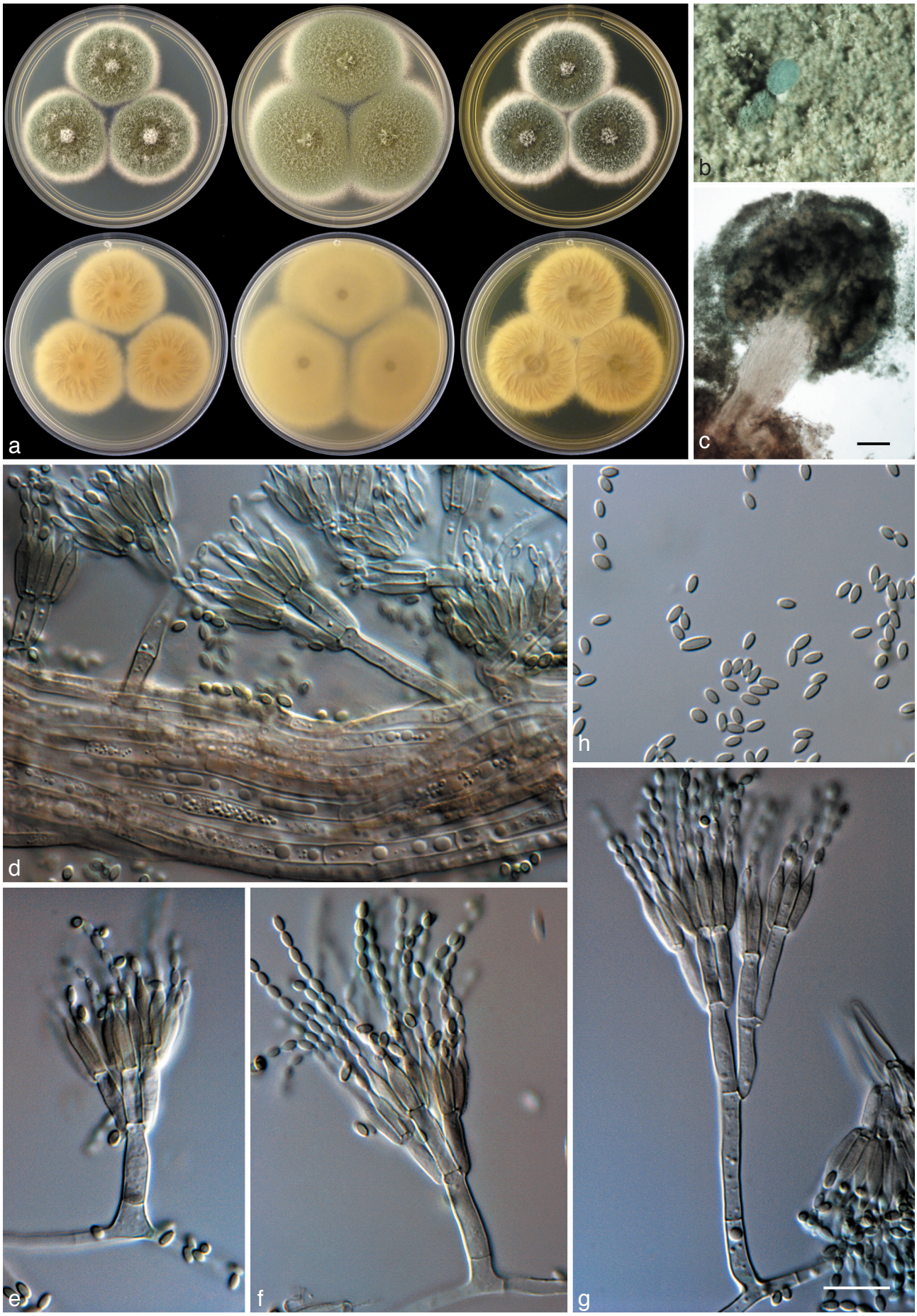

Fig. 2 Most important taxonomic characters of *T. chloroloma*, holotype (PREM 60033), distinguishing it from closely related species. a. Colonies of *T. chloroloma* incubated on CYA, MEA and YES from left to right (top row = obverse, bottom row = reverse); b, c. synnemata produced on CYA after prolonged incubation; d–g. conidiophores produced in culture; h. ellipsoidal, smooth-walled conidia. — Scale bars: b, c = 50 µm; in g = 10 µm, applies to d–h.

Fynbos strains identified as *T. verruculosus*, produced bright yellow mycelia on CYA and MEA, as well as spheroid, verrucose conidia, which are definitive characters for the species (Pitt 1979). *Talaromyces rugulosus* strains grew slowly on both CYA and MEA, and produce conidiophores that often are multi-ramulate, ending in acerose to almost ampulliform-like phialides. The recently described *T. ramulosus* (Visagie et al. 2009), characterised by strongly funiculose colonies, producing clear glutinous exudates and short synnema after prolonged incubation, was also isolated during the survey. Three of the fynbos taxa exhibited unique morphological characters that did not conform to descriptions of any known species. Based on these analyses, these are presumed to be new to science and are described below.

TAXONOMY

Talaromyces chloroloma Visagie & K. Jacobs, *sp. nov.* — MycoBank MB564326; Fig. 2, 3

Etymology. Latin, *chloroloma*: *chloros-* = green + *-loma* = fringe. Indicating the green conidia en masse near fringes.

Colony morphology, CYA, 25 °C, 7 d: Colonies 34–37 mm diam, plane, moderately dense; texture floccose with funicles present, determinate synnemata produced in incidental sunlight after prolonged incubation; margins subsurface, irregular, mycelia white, 4–5 mm wide, characteristic spiral growth at edge of colonies; conidiogenesis medium to heavy, olive-brown (4e5–4e7) at centre, pastel green (27a4) at edge; exudate and soluble pigment absent, reverse greyish ruby (12c3–12c4) at centre, greenish white (26a2) elsewhere. At 5 °C, 7 d: Germination; 37 °C, 7 d: Colonies 9–12 mm diam, plane; moderately dense; texture floccose to loosely funiculose; white mycelia; conidiogenesis sparse to medium, dark green (25f7); exudate and soluble pigment absent, reverse olive (1e6). MEA, 25 °C, 7 d: Colonies 43–48 mm diam, plane, moderately dense; texture strongly funiculose to floccose; margins subsurface, 4–5 mm, irregular, mycelia white; conidiogenesis medium to dense, olive-brown (27a4) at centre, greyish green (25c6–25d6) elsewhere; exudate and soluble pigment absent, reverse greyish green (29d5). YES, 25 °C, 7 d: Colonies 39–42 mm diam, plane, moderately dense; texture strongly funiculose; margins wide, 3–5 mm, regular, mycelia white; conidiogenesis medium to heavy, dull green to greyish green (26E4–26E6); exudate and soluble pigment absent, reverse greyish yellow (2A3–2A4). G25N, 25 °C, 7 d: Micro-colonies.

Conidiophores borne from well-defined funicles, having an olive-like pigmentation, *stipes* smooth-walled, 12–45 × 3–4 µm when borne on funicles, and 700–1200 µm when borne in synnema, bearing terminal biverticillate penicilli, with terverticillate penicilli also present; *branches*, when present in appressed to almost parallel whorls of 2–4, but mostly 3 branches, 11–12(–15) × 3–3.5 µm; *metulae* cylindrical in whorls of 3–5, appressed, 7.5–9(–10.5) × 2–3 µm; *phialides* 4–5 per metula, closely appressed, acerose, 7–8(–8.5) × 1.5–2.5 µm, tapering to fine apical pore, 0.5–1 µm; *conidia* ellipsoidal, 2.5–3.5 × 1.5–2 µm, smooth-walled, borne in disordered chains, sparse larger conidia present 4.5–5(–6) × 2–2.5 µm; *synnemata* on CYA produced after 14–21 d of growth in incidental sunlight determinate, unbranched white stalk 700–1200 × 80–150 µm, 400–580 µm across apex, conidiophores bearing a powdery, dark green conidial mass at the apex.

Specimens examined. South Africa, Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, holotype PREM 60033, culture ex-type CV2802 (KAS 4250, DAOM 241016); Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, paratype PREM 60034, culture ex-type CV 2803 (KAS 4251).

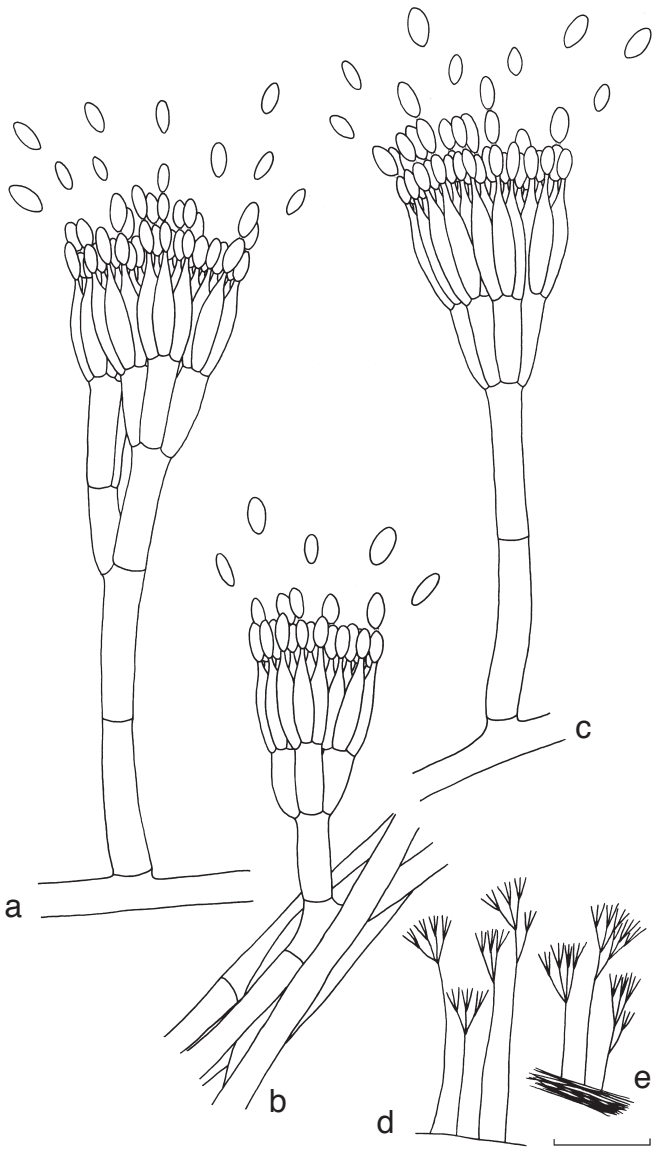

Fig. 3 *Talaromyces chloroloma* line drawings from holotype (PREM 60033) material. — Scale bar: 10 µm for a–c; 50 µm for d, e.

Talaromyces ptychoconidium Visagie & K. Jacobs, *sp. nov.* — MycoBank MB564327; Fig. 4, 5

Etymology. Latin, *ptychoconidium*: *ptycho* = ridge. Refer to the ridges on the conidia that are distinctive in this species.

Colony morphology, CYA, 25 °C, 7 d: Colonies 8–12 mm diam, plane, loose to moderately dense; texture floccose; margins subsurface, 3–4 mm wide, regular, mycelia white; conidiogenesis sometimes absent, mostly sparse, conidia en masse greyish green (1d4) when present; clear sticky exudate produced, soluble pigment absent, reverse pale to greyish yellow (1d4). At 5 °C, 7 d: No germination; 37 °C, 7 d: Colonies 8–9 mm diam, plane; texture velutinous; white mycelia; conidiogenesis sparse, brownish orange (6c3–6c5); exudate and soluble pigment absent, reverse pale (6b2). MEA, 25 °C, 7 d: Colonies 15–21 mm diam, plane, loose, sometimes having a somewhat pinkish colour; texture loosely funiculose; margins subsurface, narrow, irregular, mycelia yellow; conidiogenesis sparse to moderate, dark green (28f4); clear to pale slimy exudate produced, soluble pigment absent, reverse raw umber (5f8). YES, 25 °C, 7 d: Colonies 20–25 mm diam, moderately sulcate to umbonate; margins low, mycelia white, margins wide; conidiogenesis sparse to moderate, conidia en masse spinach green to dull

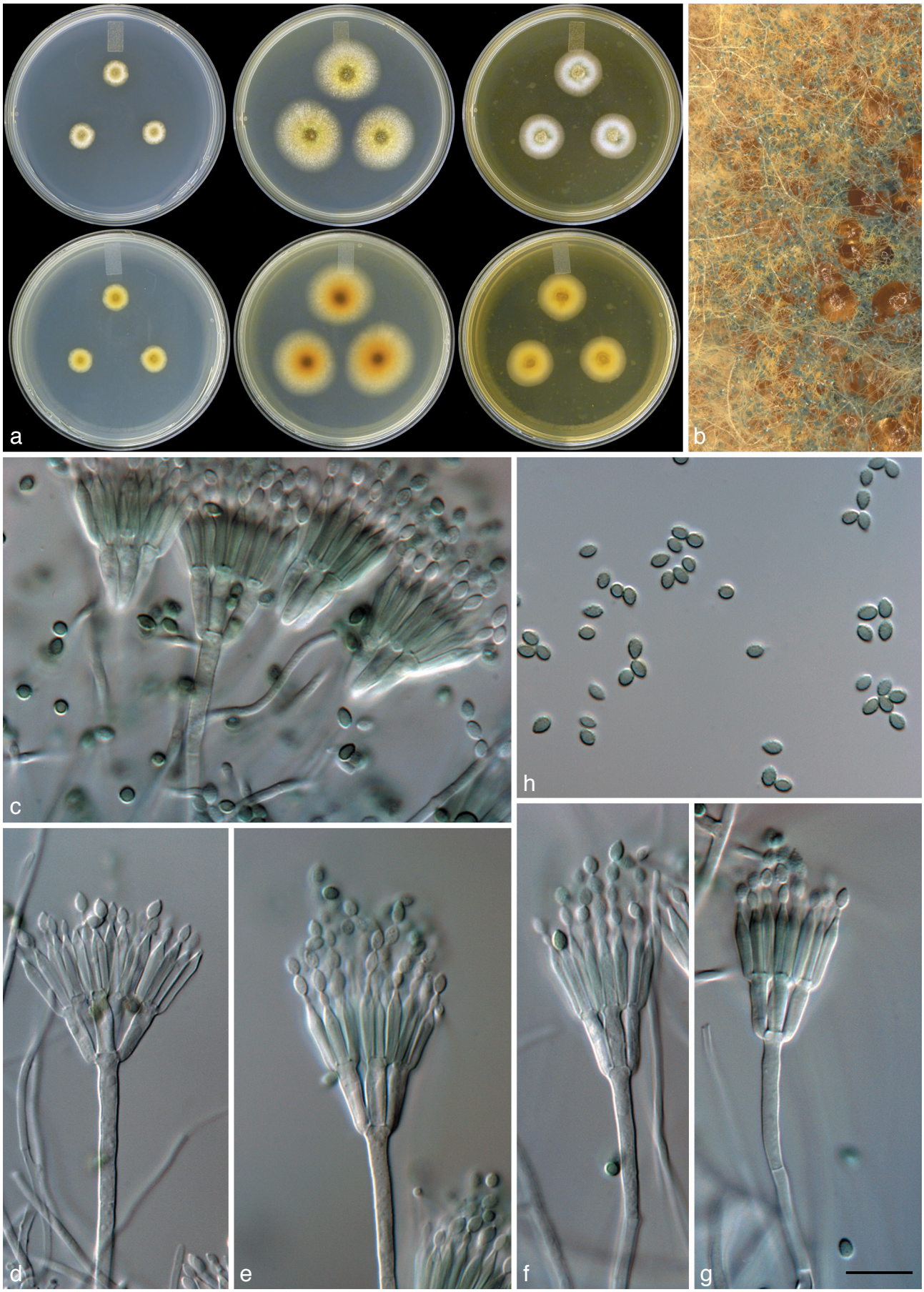

Fig. 4 The most important taxonomic characters distinguishing *T. ptychoconidium*, holotype (PREM 60041), from closely related species. a. Colonies of *T. ptychoconidium* grown on CYA, MEA and YES from left to right (top row = obverse, bottom row = reverse); b. typical loosely funiculose texture seen on MEA, together with abundant exudate and yellow mycelia; c–g. conidiophores produced in culture; h. ellipsoidal, fusiform, spirally roughened conidia. — Scale bar: in g = 10 µm, applies to c–g.

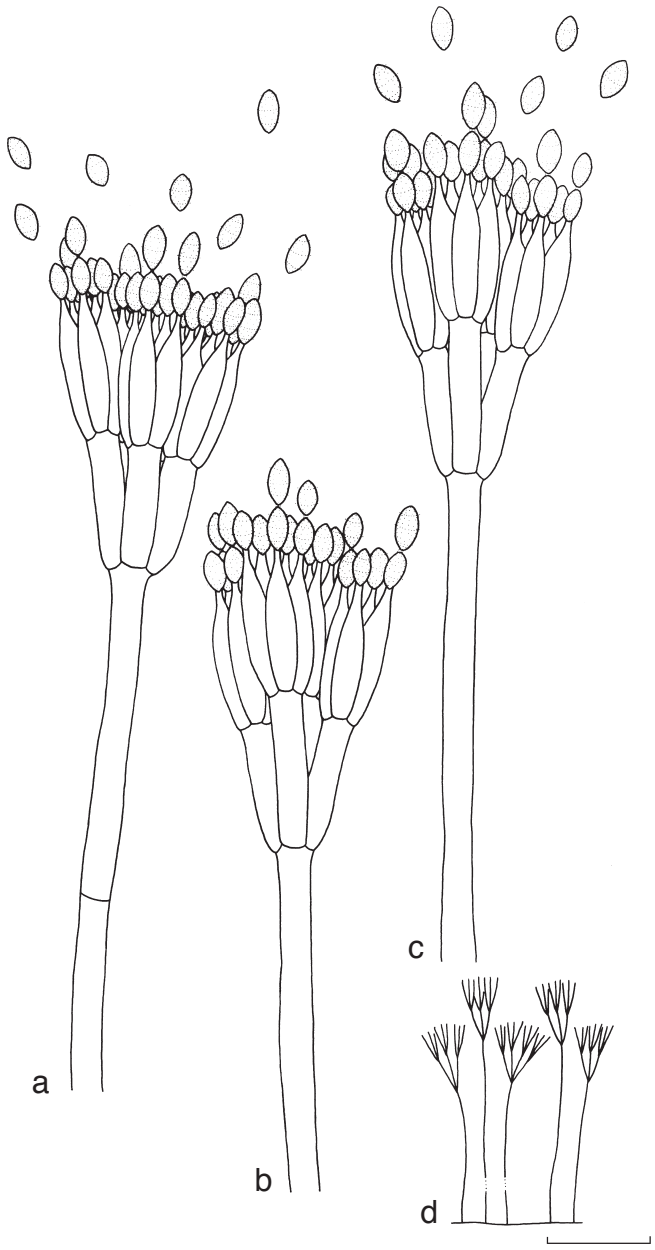

Fig. 5 *Talaromyces ptychoconidium* line drawings from holotype (PREM 60041) material. — Scale bar: 10 µm for a–c; 50 µm for d.

green (29e3–29e6); yellow soluble pigment yellow (2a5–2a6), abundant yellow to golden exudate, reverse persian red (8e8) at centre, becoming light brown (6d7–6d8) nearer to edge. G25N, 25 °C, 7 d: Colonies 4–5 mm diam, plane; margins low, white mycelia; conidiogenesis absent; clear exudate produced, soluble pigment absent, reverse white.

Conidiophores borne from aerial hyphae and loose funicles, having a greenish pigment, *stipes* smooth-walled, (38–)60–93 × 2.5–3.5 µm when borne on aerial hyphae and funicles, bearing strictly terminal biverticillate penicilli; *metulae* cylindrical in whorls of (3–)5–7, appressed, 10–11.5(–12.5) × 2–3 µm; *phialides* 3–4 per metula, closely appressed, acerose, 10–12 × 2–2.5 µm, tapering to fine apical pore, 0.5–1 µm; *conidia* ellipsoidal, apiculate, 3–4.5(–5) × 2–3 µm, spirally rough-walled, borne in disordered chains.

Specimens examined. South Africa, Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, holotype PREM 60041, culture ex-type CV 2808 (KAS 4245, DAOM 241017); Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, paratype PREM 60043, culture ex-type CV 2806 (KAS 4246), paratype PREM 60042, culture ex-type CV 2807 (KAS 4247).

Talaromyces solicola Visagie & K. Jacobs, *sp. nov.* — MycoBank MB564328; Fig. 6, 7

Etymology. Latin, *solicola*: *solum* = soil; *-cola* = an inhabitant, an inhabitant of soil.

Colony morphology, CYA, 25 °C, 7 d: Colonies 3 mm diam, sometimes only germination, low, plane, loose; texture floccose; margins low, irregular, mycelia white; conidiogenesis absent to sparse, conidia en masse white when present; clear to orange exudate produced, soluble pigment absent, reverse pale white. At 5 °C, 7 d: No germination. 37 °C, 7 d: No germination. MEA, 25 °C, 7 d: Colonies 20–21 mm diam, sometimes up to 25 mm, low, sulcate and sometimes slightly sunken at centre, loose, sometimes having a brownish orange colour; texture floccose to velutinous; margins low, 3–4 mm wide, regular, mycelia white; conidiogenesis medium, dark green (28f4); clear exudate produced, soluble pigment absent, reverse colouration brownish grey (6d2) at centre and greyish green (1c3) elsewhere. YES, 25 °C, 7 d: Colonies 8–10 mm diam, dense; texture floccose; margins low, irregular, mycelia white; conidiogenesis sparse to moderate, conidia en masse greyish green, but colour rather distorted because of reddish pink exudates produced; soluble pigment absent, reverse dark brown (8F6–8F8). G25N, 25 °C, 7 d: No germination or growth.

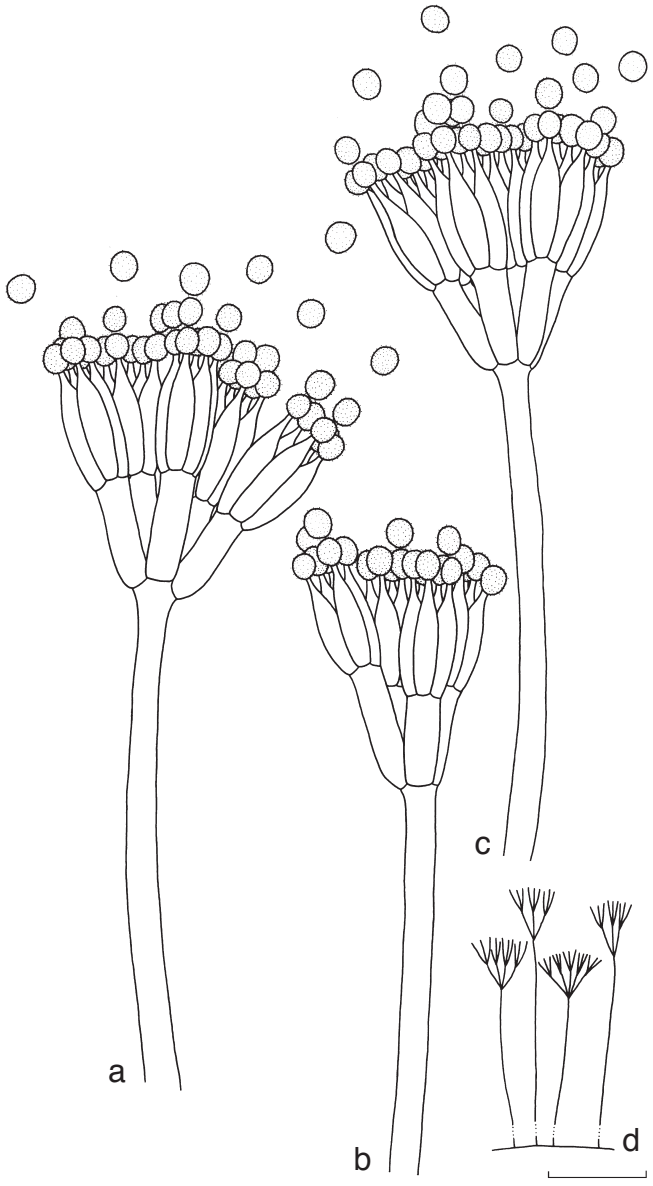

Fig. 6 *Talaromyces solicola* line drawings from holotype (PREM 60037) material. — Scale bar: 10 µm for a–c; 50 µm for d.

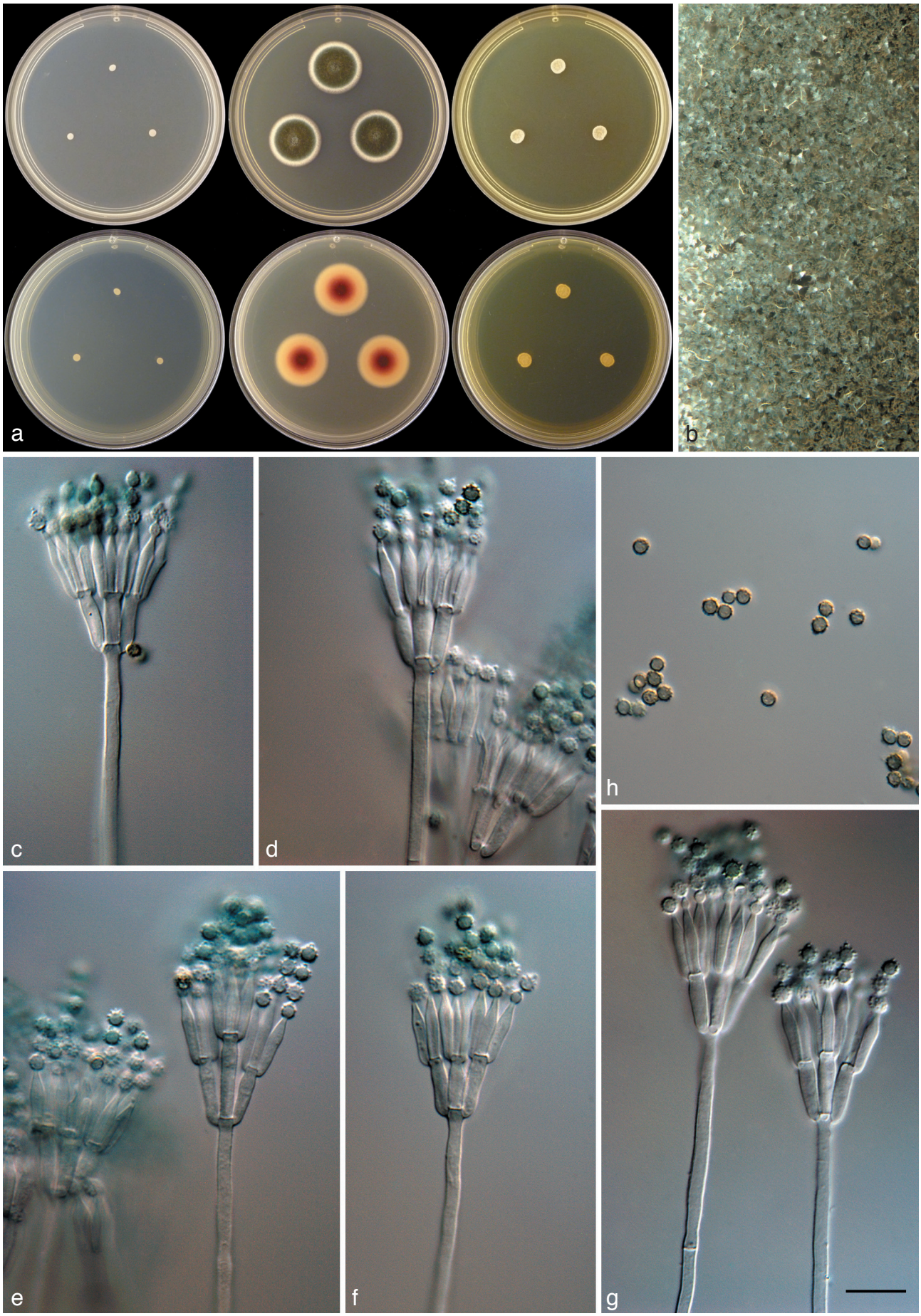

Fig. 7 The most important taxonomic characters distinguishing *T. solicola*, holotype (PREM 60037), from closely related species. a. Colonies of *T. solicola* grown on CYA, MEA and YES from left to right (top row = obverse, bottom row = reverse); b. floccose to velutinous colony texture on MEA; c–g. conidiophores produced in culture; h. subspheroidal, rough-walled verrucose conidia. — Scale bar: in g = 10 µm, applies to c–g.

Conidiophores borne from aerial hyphae, *stipes* smooth-walled, (90–)160–230 × 2.5–3.5 µm, bearing strictly terminal biverticillate penicilli; *metulae* cylindrical in whorls of 5–8 sometimes up to 10, appressed, 8.5–10(–11) × 2.5–3.5 µm; *phialides* 3–4 per metula, closely appressed, acerose 9–11 × 2–2.5 µm, tapering to fine apical pore, 0.5–1 µm; *conidia* subspheroidal, verrucose, (2–)2.5–3.5 × 2–2.5 µm, rough-walled, borne in disordered chains.

Specimens examined. South Africa, Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, holotype PREM 60037, culture ex-type CV 2800 (KAS 4235, DAOM 241015); Western Cape Province, Malmesbury, Riverlands S 33,49066°; E 18,58388°, isolated from soil, 21 Feb. 2007, collected by *C.M. Visagie*, paratype PREM 60038, culture ex-type CV 2801 (KAS 4236).

KEY TO THE NEWLY DESCRIBED *TALAROMYCES* SPECIES AND THE CLOSELY RELATED SISTER TAXA

1. Colonies on CYA < 5 mm 2
1. Colonies on CYA > 5 mm 4
2. Conidia distinctly rough-walled . . . . . *T. solicola**
2. Conidia smooth-walled 3
3. Growth only on acidified (pH 5/less) CYA; stipes < 100 µm . . . . . *P. lignorum*
3. Colonies on CYA 2–4 mm, sometimes only microcolony; stipes > 100 µm . . . . . *T. diversus*
4. Conidia ornamented with transverse/spiral striations . . . 5
4. Conidia without striations 6
5. Mycelia red on CYA and MEA; stipes vesiculate . . . . . *T. purpureus*
5. Mycelia white on CYA and MEA; stipes non-vesiculate . . . . . *T. ptychoconidium**
6. Colonies on CYA < 12 mm and MEA < 20 mm 7
6. Colonies on CYA > 12 mm or MEA > 20 mm 8
7. Red colony pigments commonly produced on CYA and MEA; Metulae borne on vesiculated stipes . . . . . *T. erythromellis*
7. No red colony pigmentation; stipes non-vesiculated . . . . . *T. rugulosus*
8. Synnemata never produced 9
8. Synnemata produce, mostly after prolonged incubation 10
9. Colonies on CYA > 20 mm, conidia rough-walled . . . . . *T. purpurogenus*
9. Colonies on CYA < 20 mm, conidia smooth-walled . . . . . *T. minioluteus*
10. Synnemata stalks and mycelia on CYA and MEA pigmented 11
10. Synnemata stalks and mycelia white 12
11. Determinate synnemata > 2000 µm . . . . . *T. dendriticus*
11. Indeterminate synnemata < 2000 µm . . . . . *T. pseudostromaticus*
12. Conidia en masse on CYA pink to greyish green; synnemata 100–250 µm tall; abundant sticky exudates produced . . . . . *T. ramulosus*
12. Synnemata > 250 µm 13
13. Colonies on CYA 34–37 mm and MEA 43–48 mm; conidia en masse olive brown, with light green edge . . . . . *T. chloroloma**
13. Colonies on CYA 19–31 mm and MEA 32–40 mm; conidia en masse greyish green to greyish turquoise *T. cecidicola*

* Species described in this paper

DISCUSSION

Talaromyces is distinguished from closely related genera by its cleistothecial ascomata that have a soft hyphal exterior giving it a yellow, cream, pink or reddish colour (Samson et al. 2011). In its anamorph state, typical symmetric biverticillate, sometimes terverticillate, conidiophores are produced that bear thin acerose phialides. A few species do produce an ampulliform-like phialide with a thin, long neck (Pitt 1979, Samson et al. 2011). Species typically have a metula to phialide length ratio of ± 1–1.2, as well as poor growth (Pitt 1979). Seven taxa isolated from Atlantis sand fynbos soil conform to these characters, although no ascomata was observed, and was subsequently placed in *Talaromyces*. Species isolated included *Penicillium rubrum*, *T. ramulosus* *T. rugulosus*-like strains and *T. verruculosus*, together with the three species described here (Fig. 1).

In a similar study, Allsopp et al. (1987) recorded *P. verruculosum* (= *T. verruculosus*), *P. purpurogenum* (= *T. purpurogenus*) and *P. funiculosum* (= *T. funiculosus*) from soil collected at Riverlands Nature Reserve. Thus, they only isolated three species from the *Talaromyces* clade, compared to the eight isolated from this study. This may, however, be due to the fact that we mostly found this group to occur in low numbers and often in only one soil sample. In their study, Allsopp et al. (1987) included only species that occurred in 10 or more samples, which may explain the low species diversity reported for this habitat. This seems to hold true for new isolations made from additional fynbos biotypes. In a current study, a large number of strains representing *Talaromyces* were isolated. Once again these also seem to be habitat specific and occur in low numbers. Reasons for this species distribution remain unclear, although the heterogeneous nature of the fynbos, with its high beta-diversity (Goldblatt & Manning 2002), may play a role. In addition, we believe that some of these species might have alternative dispersal strategies, opposed to air dispersal, and have associations with specific plant species occurring in the fynbos (Visagie et al. 2009).

This idea was also voiced by Pitt (1979), who mentioned species with possible narrow spectrum habitats and alternative dispersal strategies. Also, new species from this group are often isolated from very specific habitats. For example, *Talaromyces cecidicola*, was isolated and described from wasp galls on *Quercus pacifica* trees (Seifert et al. 2004), *T. dendriticus* always seems to be associated with *Eucalyptus* (Pitt 1979, Seifert et al. 2004), *T. pseudostromaticus* was isolated from various bird species in Minnesota (Hodges et al. 1970), and *P. aureocephalum* is currently only known to occur on dried-out leaf litter from Barcelona (Muntañola-Cvetković et al. 2001, Llimona et al. 2006). Distribution patterns and dispersal methods in *Penicillium* and *Talaromyces* will be investigated in future studies.

One of the species isolated from fynbos soils, conformed to Pitt's (1979) description for *T. rugulosus*. The description of this slow-growing species is, however, considered too broad. For instance, conidia were described as smooth to verrucose. This allows for a broad spectrum of morphological variation within the taxon. Comparisons of the ITS gene region showed that the fynbos strains form a distinct clade next to previously sequenced *P. rugulosum* strains. In addition to this, a morphologically distinct species, also isolated during this study, forms a clade within the larger *P. rugulosum* group. β-tubulin sequences did, however, not resolve these into well-defined clades. Without a more detailed sequence dataset of *T. rugulosus*, its previously designated synonyms and close relatives, this issue cannot be resolved. We, therefore, consider *T. rugulosus* to possibly represent a species complex and this needs to be addressed in future studies.

Talaromyces chloroloma is characterised by strongly funiculose colonies producing olive coloured conidia en masse. Determinate synnemata are produced on CYA after 2 wk of incubation. The species is further characterised by appressed olive-coloured conidiophores, borne on short stipes. *Talaromyces chloroloma* is morphologically similar to other synnemata producers, *T. cecidicola*, *T. dendriticus* and *T. ramulosus*. The new species is easily distinguished from *T. cecidicola* by faster growth on CYA (34–37 vs 19–31 mm) and MEA (43–48 vs 32–40 mm) at 25 °C and its ability to grow at 37 °C on CYA. In addition, *T. chloroloma* conidiophores have shorter stipes (12–45 vs 20–80 µm), but generally, longer synnemata (700–1200 vs 250–1250 µm) compared to *T. cecidicola*. The longer synnemata produced by *T. chloroloma* also distinguishes it from *T. ramulosus* which has very short synnemata (110–150 µm) produced on MEA. Conidial structures in *T. chloroloma* are also much denser and conidia have an olive-brown colour compared to the pink to greyish green conidia of *T. ramulosus*. *Talaromyces dendriticus* is easily distinguished by its production of long (2–4 mm) synnemata with yellow stalks, compared to *T. chloroloma* that has shorter synnemata and white stalks. The morphological relationship of these species are reflected in their phylogeny and are sister taxa with well-supported branches in a neighbour-joining tree (Fig. 1).

Talaromyces ptychoconidium characteristically displays restricted growth on CYA and MEA. Its most striking feature is the closely appressed conidiophores producing spirally rough-walled ellipsoid conidia (Fig. 4, 5). The phylogenetic analysis suggests an affinity to *T. purpureus* as its sister taxon. Interestingly, both of these species produce spirally ornamented conidia. These two species are readily distinguished from each other, based on the red mycelia produced by *T. purpureus* compared to the white mycelia of *T. ptychoconidium.* Microscopically, *T. purpureus* has vesiculate stipes with both metulae (7–10 µm) and phialides (6–10 µm) shorter than that of *T. ptychoconidium.*

The third species, *T. solicola*, is distinguished by its characteristic poor growth on CYA. Its phylogenetic placement resolves *T. solicola* in a clade where species all display similar restricted growth. *Talaromyces erythromellis*, *T. diversus*, *T. purpurogenus* and *P. lignorum* all display similarly poor growth on CYA and MEA, the latter species only growing on acidified CYA (Pitt 1979). *Talaromyces solicola* is easily distinguished from all close relatives based on its heavy rough-walled, spheroidal to subspheroidal conidia, compared to the smooth-walled conidia of its sister taxa. In addition to the texture of its conidial walls, *P. lignorum* produces shorter stipes (15–50(–100) µm). *Talaromyces diversus* often produces yellow coloured mycelia at colony peripheral zones, which are absent in *T. solicola*. On MEA, *T. erythromellis* grows much faster than the new species. Phylogenetic analysis of both the ITS and β-tubulin genes confirmed the novelty of *T. solicola*, forming well-defined clades separate from any close relatives.

Penicillium and *Talaromyces* in general are regarded to have soil as its principle habitat, with a large proportion of species only known from soil (Pitt 1979). Fynbos soil is considered an especially harsh environment because of its acidity and poor nutrient levels (Kruger et al. 1983), which is worsened by the low rainfall and high temperatures during summer and high rainfall and low temperatures during winter (Richards et al. 1997). Other external factors influencing organisms that live in the fynbos is the heterogeneity of the soil and plants, as well as the constant fires associated with the habitat (Goldblatt & Manning 2002). These are just some factors that affect and place constant evolutionary pressure on organisms that actively live in this habitat. It is, however, of interest to note that the species isolated in this study does not seem to be confined to the soil environment. *Talaromyces ramulosus* has been isolated from *Protea* infructescences (Visagie et al. 2008) and together with a number of other *Penicillium* and *Talaromyces* spp. were also isolated from mites in these infructescences. *Talaromyces ramulosum* and *T. chloroloma* were also isolated from apple orchards in the Western Cape (van der Walt et al. 2010). The occurrence and distribution pattern of species occurring in fynbos from the Western Cape are still unknown. With more studies like these, uncovering and describing species from this unique area, which has diverse fungal communities, might lead to surprising discoveries related to their distribution and ecology.

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