The polyphasic taxonomy of *Penicillium* and *Talaromyces* spp. isolated from the diverse Fynbos biome

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Summary

The genus *Penicillium* is well known and occurs in a diverse range of terrestrial environments. Its main function is the decomposition of organic materials and the impact on our everyday lives is far reaching. They cause various rots of food crops as pre- and postharvest pathogens, produce an immense range of enzymes and secondary metabolites important for biotechnology and are common indoor air irritants. These characters contribute to their economic importance. In the movement towards single name nomenclature, recent taxonomic revision of the family *Trichocomaceae* resulted in the incorporation of *Penicillium* subgenus *Biverticillium* into its previously associated teleomorph genus *Talaromyces*. Approximately 300 species are currently accepted for *Penicillium* and *Talaromyces*. However, despite the importance of this genus, very little is known about the distribution and ecology of this group in South Africa. Studies traditionally focused on environments of economic importance and some of the unique natural habitats were neglected. The importance of the conservation of biodiversity has, however, resulted in a number of studies that explore biodiversity in habitats such as the unique Fynbos biome.

The current study focused on the exploration of *Penicillium* and *Talaromyces* diversity in the Fynbos biome of the Western Cape. Three sampling sites were chosen, representing three Fynbos types. Sites include Stellenbosch Mountain (Boland granite Fynbos), Malmesbury (Atlantis sand Fynbos) and Struisbaai (Agulhas sand Fynbos). Soil and air samples, as well as *Protea repens* infructescences were collected and fungi isolated from these. A total of 2500 isolates were obtained, of which *ca*. 1700 represented *Penicillium* and *ca.* 200 *Talaromyces*. Other genera isolated, include *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* and *Trichoderma*.

One of the most important objectives of this study was to make identification of Fynbos species as easy and logical as possible. The bulk of this study is presented in the second chapter. For the identification of these strains, a polyphasic species concept, which included morphological and genetic data, was adopted. Isolates were initially placed into tentative morphological taxa based on colony morphology on CYA and MEA. The ITS gene region of representative strains from each group were also sequenced. The subsequent ITS phylogeny was used for dividing taxa into their respective sections of the Houbraken & Samson (2011) reclassification of *Penicillium*. Fynbos species were included in multigene phylogenies and compared to their close relatives based on the respective sections. Descriptions and full color photoplates are provided for each of the 61 *Penicillium* and 15 *Talaromyces* species isolated. 25 *Penicillium* and 5 *Talaromyces* spp. could not be identified and are considered to be novel. Identification keys for all 76 species were compiled as part of the study. The descriptions, full color photoplates and gene sequences that serve as DNA barcode-like sequences should provide the much-needed stable identification system for South African species.

In the final chapter, the ecology of *Penicillium* and *Talaromyces* from the three Fynbos sites was explored. A presence-absence data matrix was created for species that occurred in each sample. This was used to compare species richness between the different sampling sites and different habitats at each site. The effect of site locality and infructescence age on *Penicillium* and *Talaromyces* communities was determined using multi-dimensional scaling combined with permANOVA analysis. Possible dispersal methods were investigated by calculating area-proportional Venn diagrams, in which communities were compared between the mite, *Protea repens* infructescence and combined soil and air communities. A clear trend was observed. Site locality had a significant effect on communities. However, individual plants did not have a significant effect on communities. Venn diagrams showed that at each plant, communities between mites and *Protea repens* infructescences were similar and was different from the soil and air communities. This adds to the idea that mites could possibly act as transport vectors of *Penicillium* and *Talaromyces* species. For future studies, it was proposed that a specific site is extensively sampled in order to investigate vectored dispersal of these species.

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Opsomming

Penicillium is 'n algemeen bekende swam en kom voor in feitlik alle terrestriële omgewings. Hierdie fungus se hoof funksie is die afbraak van organiese materiale. As gevolg hiervan, veroorsaak dit skadelike verrotting van voedsel produkte, as voor- en na-oes patogene. Daarmee saam produseer hierdie swamme 'n wye verskeidenheid van ensieme en sekondêre metaboliete wat belangrik vir biotegnologie is en wat ook kan optree as algemene irritante in binnenshuise omgewings. *Penicillium* vorm dus 'n belangrike komponent van die mens se alledaagse lewe. In 'n onlangse hersiening van die familie *Trichocomaceae*, gekombineerd met die veskuiwing na enkelnaam nomenklatuur, is *Penicillium* subgenus *Biverticillium* verwyder uit *Penicillium* s.l. en ingesluit in sy voorheen geassosieerde teleomorf genus, *Talaromyces*. Tans word ongeveer 300 spesies van *Penicillium* en *Talaromyces* aanvaar in literatuur. Ongelukkig is kennis van hierdie twee genera in die Suid Afrikaanse konteks beperk. Vorige studies het grootliks gefokus op areas van ekonomiese belang en die unieke natuurlike biome van Suid Afrika is afgeskeep. Die bewaring van biodiversiteit is tans wêreldwyd van groot belang en dit het gelei tot 'n aantal studies wat begin het om unieke omgewings soos die Fynbos te verken.

Hierdie studie het daarop gefokus om kennis oor *Penicillium* en *Talaromyces* in die Fynbos te verbreed. Monsters is versamel by drie plekke in die Wes-Kaap, elkeen met 'n unieke fynbos tipe. Dit sluit Stellenbosch berg (Boland graniet Fynbos), Malmesbury (Atlantis sandveld Fynbos) en Struisbaai (Agulhas sandveld Fynbos) in. Grondmonsters, lug en *Protea repens* vrughofie monsters is by elke plek versamel. Ongeveer 2500 fungusstamme is geïsoleer uit hierdie monsters. In totaal behoort ongeveer 1700 aan *Penicillium* en 200 aan *Talaromyces*. 'n Polifasiese spesies konsep wat morfologie en genetiese data kombineer, is gebruik in hierdie studie. Isolate is aanvanklik in tentatiewe taksa geplaas op grond van hul kolonie-eienskappe op CYA en MEA. Die ITS-geen se basispaar-opeenvolgings is bepaal vir verteenwoordigers van elke takson, en gebruik in 'n groot ITS filogenie. Hierdie filogenie is dan as basis gebruik om taksa in hulle onderskeie seksies te plaas, soos beskryf in Houbraken & Samson (2011). Fynbos spesies is verder in meer diepte geklassifiseer binne hierdie seksies. In totaal is 61 *Penicillium* en 15 *Talaromyces* spesies gekarakteriseer deur multi-geen filogenië asook morfologiese beskrywings en volkleur fotoplate. Vanuit hierdie groep, word 25 *Penicillium* en 5 *Talaromyces* spesies as nuut beskou en sal as sodaande beskryf word. Identifikasie sleutels is ook ingesluit wat spesifiek fokus op die identifikasie van Fynbos spesies. Die doel is om die identifikasie van hierdie spesies so maklik as moontlik te maak vir die gebruiker. Die beskrywings, volkleur fotoplate en DNS-volgordes, wat soos DNS-strepieskodes kan werk, sal dus die broodnodige stabiele identifikasie sisteem vir Suid Afrikaanse spesies skep.

Die ekologie van *Penicillium* en *Talaromyces* is ondersoek in hoofstuk 4. 'n Aanwesig-afwesig data matriks is opgestel vir elke spesie wat in die onderskeie monsters voorgekom het. Gebaseer op hierdie spesie-matriks, was spesierykheid vergelyk tussen die verskillende versamelplekke en verskillende habitatte by elke versamelplek. Die effek van versamelplek en vrughofie ouderdom was bepaal met multi-dimensionele skale, asook permANOVA analises. Moontlike verspreidings-strategieë is ondersoek met behulp van area-proportionele Venn diagramme, waarmee populasies vanaf myte, *Protea repens* vrughofies en gekombineerde grond en lug monsters vergelyk is. Die permANOVA analises het gewys dat lokaliteit 'n beduidende effek het op die populasies en dat individuele plante geen effek het op die samestelling van die populasies nie. Venn diagramme het verder gewys dat populasies tussen myte en vrughofies baie eenders is, met die grond- en lugpopulasies uniek. Dit voeg by tot die vermoede dat myte as verspreidingsvektore vir *Penicillium* and *Talaromyces* kan optree in hierdie habitat. Aangesien lokaliteit 'n beduidende effek het op populasiesamestelling, is dit voorgestel dat nuwe studies monsters by 'n spesifieke area intensief versamel, om sodoende verspreidings-strategieë beter te kan bestudeer.

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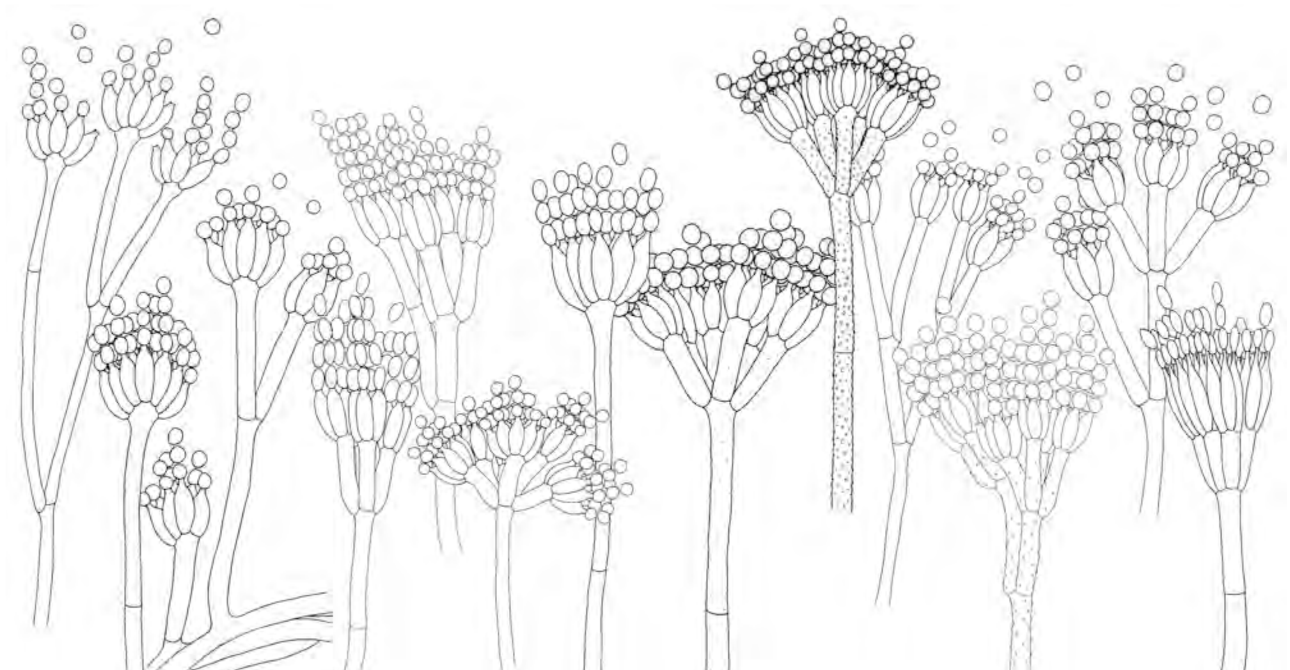
CHAPTER 1
Literature study: The 200 years of *Penicillium* taxonomy



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Literature study: The 200 years of *Penicillium* taxonomy

1. Introduction

More than 200 years has passed since Link (1809) first described *Penicillium*. The first reference of *Penicillium*, however, is most probably the Micheli (1729) sketch (FIGURE 1) of a fungus that closely resembles a penicillus. Members of *Penicillium* greatly influenced human history. It was made famous by the antibiotic activity of penicillin, discovered by Alexander Fleming in 1929, and subsequent purification as pharmaceutical in the 1940's by Howard Florey and his team of researchers at the Sir William Dunn School of Pathology, University of Oxford. Pitt (1979) mentions that it must have been fate that led to *P. chrysogenum*, today correctly identified as *P. rubens* (Houbraken *et al*. 2011c), to be Fleming's contaminant and not one of the many other mycotoxin producers. It would be prudent to bear in mind that "Penicillin is a mycotoxin which affects only bacteria, rather than saying that antibiotics from *Penicillium* have generally proved too toxic for use in human therapy" (Pitt 1979).

In nature, however, the primary function of *Penicillium* is as decomposers of dead organic matter. Pitt (1979) paraphrased Voltaire, "If Penicillia did not exist, they would have to be invented". This character makes it also a nuisance, as it is responsible for, damaging rots of foodstuffs as pre- and/or postharvest pathogens. The spores of this fungus are typically carried by air currents, which aid in its dispersal. *Penicillium* is, therefore, commonly isolated in indoor environments where it can cause asthma or allergies. These fungi are in fact so ubiquitous that "Rare indeed must be the human individual who has not encountered fungi of the genus *Penicillium* or been affected by one of the many metabolites produced by them" (Pitt 1979).

Its taxonomy has also been of great importance. *Penicillium* taxonomists through history have often been at the front of new taxonomic developments (Raper & Thom 1949, Pitt 1979, Seifert *et al*. 2007, Frisvad & Samson 2004, Houbraken & Samson 2011, Samson *et al*. 2011). This is because *Penicillium* is so diverse and is taxonomically a very difficult group to work with. Its diverse nature has resulted in identifications that are near impossible using only a morphological species concept. *Penicillium* taxonomists thus applied the polyphasic species concept to the genus, which incorporates morphology, physiology and phylogeny. Arguably, phylogenetic data carries more weight, but the other two components still are very important (Frisvad & Samson 2004, Houbraken & Samson 2011).

Accurate and consistent identification of the close to 300 accepted species (Pitt *et al*. 2000, Houbraken & Samson 2011) (FIGURE 2) remain problematic. Its ubiquitous and diverse nature, as well as its important function in nature and role they or their metabolites play in our everyday lives, validates the need for a system where one could produce fast and accurate identifications. This goal to make identification easier has been the main focus of many workers of *Penicillium* in its more than 200 year history.

1.1. Link and the typification of *Penicillium*

The generic name *Penicillium* (Latin: penicillus, painter's brush) was first introduced by Link (1809). The generic description[1] can roughly be translated as "fungi that have a wooly covering that grow in tufts, conidiophores erect, and simple or branched, conidia collecting at conidiophore apex". He also described three species, *Penicillium glaucum, P. candidum* and *P. expansum*, each distinguished by the appearance of its penicilli. Link (1824) then wrongfully applied *P. glaucum* to all green *Penicillium* spp., including *P. expansum*. The name *P. glaucum* thus had no meaning and no type material was available. It was eventually considered a *nomen dubium* and the name rejected as invalid (Pitt 1979).

The International Code of Botanical Nomenclature (ICBN) governs formal naming of plants and other organisms traditionally considered to be plants. The ICBN and changes made to the code had a big impact on the nomenclature of *Penicillium*. Until recently, the starting point for fungal names had been Fries (1821) and Persoon (1801). This had the implication that Link (1809) did not validly publish the name *Penicillium*. Gray (1821) was the first publication that accepted *Penicillium* as a valid generic name, although he did not designate a type. The first typification of *Penicillium*, according to Hawksworth *et al*. (1976), was by Fries (1829, 1832) who based the name on *Mucor crustaceus* Linnaeus. Linneaus' (1753) concept of *M. crustaceus*, however, was based on the sketch of Micheli (1729) Tab. 91 Fig. 3 *Botrytis*, not the *Penicillium*-like Fig. 3 under *Aspergillus* on the same Tablet (FIGURE 1). Because Fries (1829, 1832) wrongfully typified the genus with a *Botrytis*-like fungus, the concept of *Penicillium* was threatened. Hawksworth *et al*. (1976) proposed the conservation of the name *Penicillium*, under article 13f of the ICBN. *Penicillium expansum* was widely used, against nomenclatural rules, as type for the genus following Thom (1910, 1930) and Raper & Thom (1949). For conservation, *Penicillium* Link ex Gray was thus proposed over *Penicillium* Fr., with *P. expansum* Link ex Gray (IMI39761, lectotype) as

[1] "*Thallus e floccis cæspitosis, septatis, simplicibus aut ramosis, fertilibus erectis apie penicillatis. Sporodia in apicibus penicillatis collecta*"

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generic type (Hawksworth *et al*. 1976). Jørgenson and Gunnerbeck (1977) questioned the type concept of Fries (1932) and thus did not consider conservation necessary. The Special Committee for Fungi and Lichens later decided conservation was not necessary (Peterson 1980). Changes made to the code in Sydney at the 13th International Botanical Congress (1981) resolved this issue. The starting point for fungal names was changed to Linneaus (1753), meaning that Link (1809) validly published the name *Penicillium* and the correct citation for the genus is *Penicillium* Link. A type strain could be selected from one of his original species, with *P. expansum* [CBS H-7485 (herbarium), IMI39761, CBS325.48, NRRL976, ATCC7861] selected as generic type. Fries (1932) citation of *M. crustaceus* L. does not comply with the generic description of *Penicillium* Link, and thus looses its nomenclatural relevance (Hawksworth 1985).

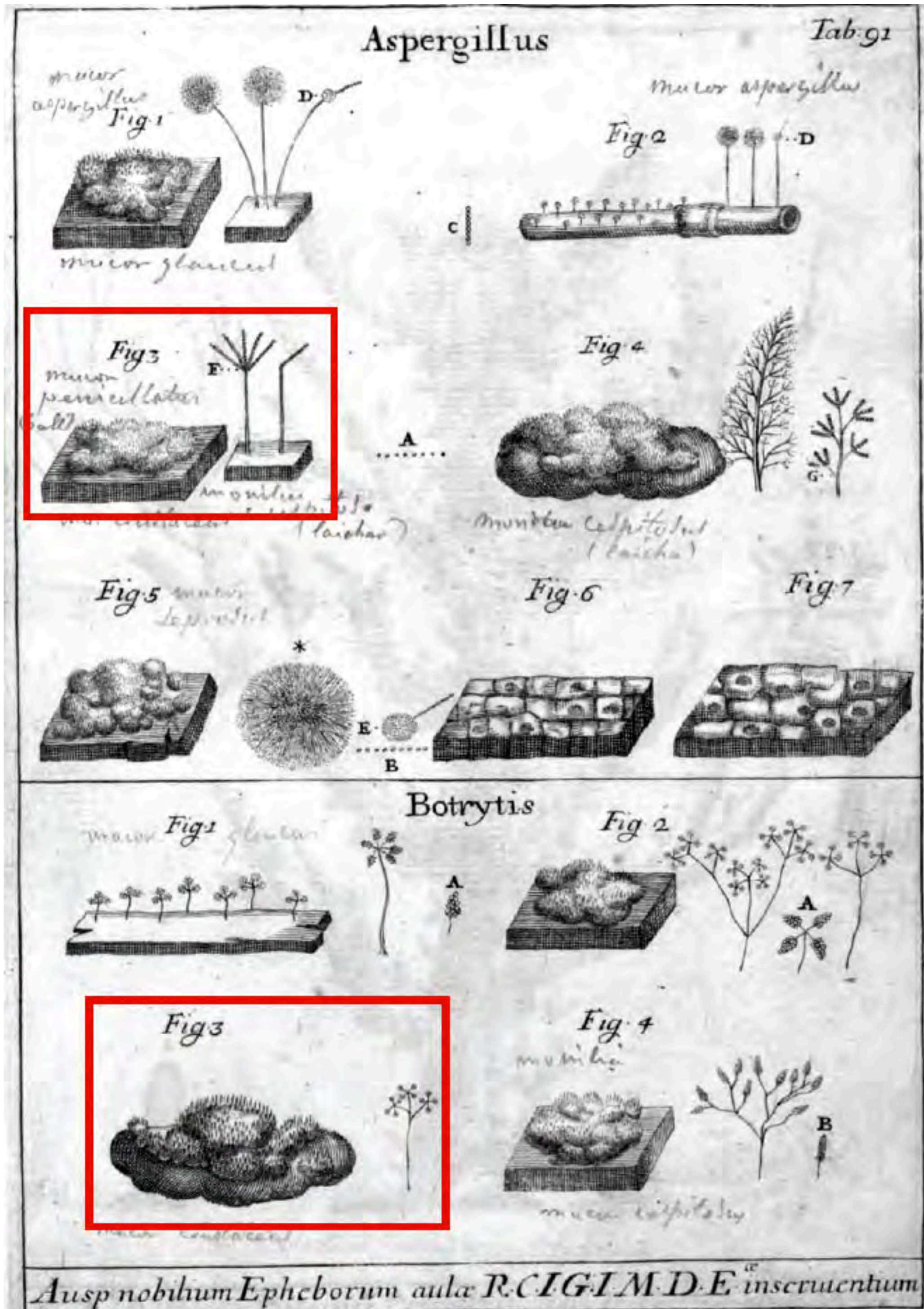

FIGURE 1: Micheli's (1729) Tablet 91 with the first illustration of a *Penicillium*-like conidiophore under *Aspergillus* (Fig. 3), not under *Botrytis* (Fig. 3) which Fries (1832) used for typifcation of *M. crustaceus*.

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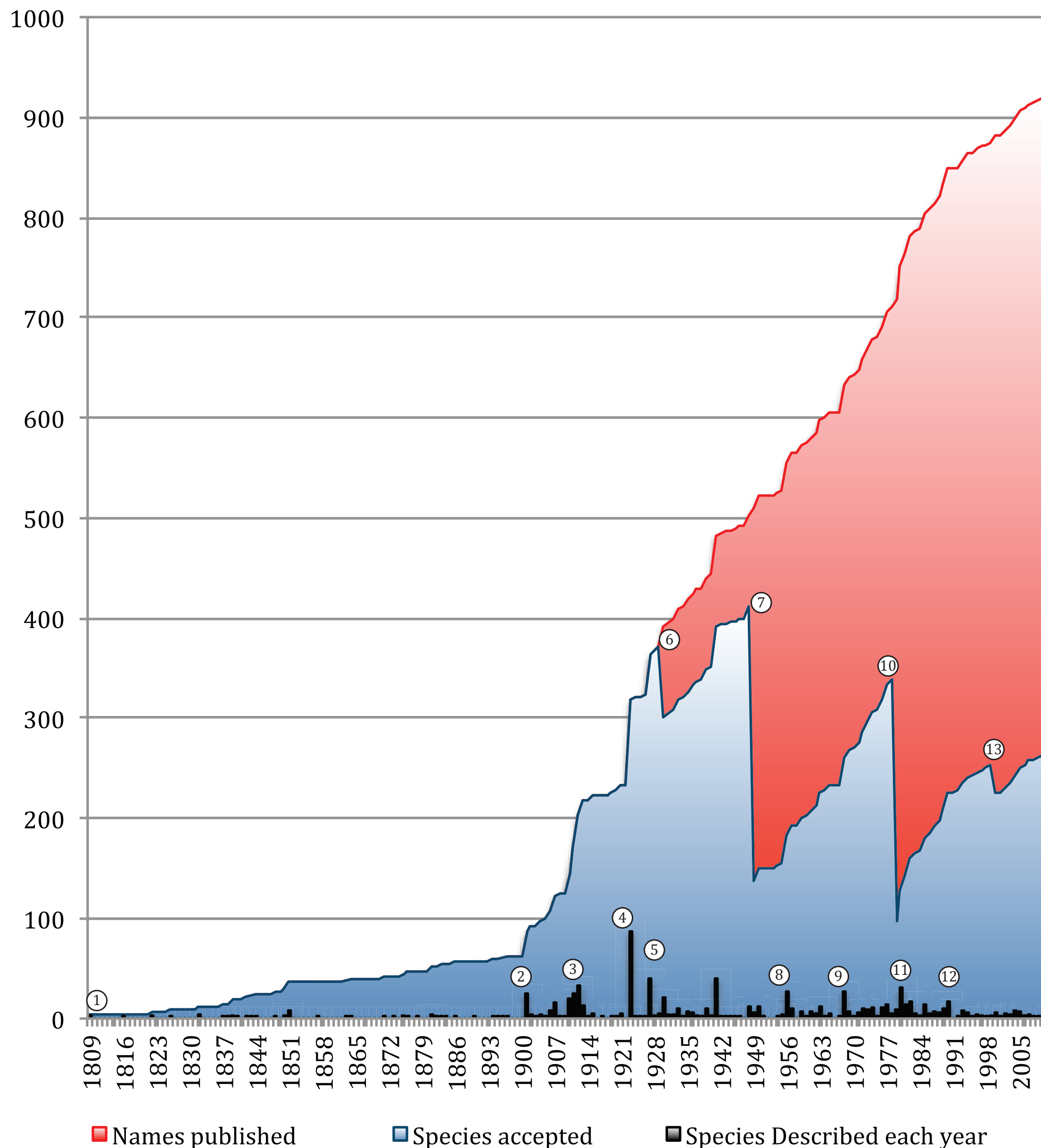

FIGURE 2: *Penicillium* sensu lato species described until 2010, since Link (1) introduced the name *Penicillium* in 1809. The red graph indicates the number of names introduced to *Penicillium*. The blue graph is represents the number of species accepted after major revisions of the genus and includes the rejection of a large proportion of names, as well as reduction down to synonymies. The black graph shows the number of species described each year. (2) Dierckx 1901. (3) Thom 1910, Westling 1911, Sopp 1912. (4) Biourge 1923. (5) Zalesky 1927. (6) Thom 1930. (7) Raper & Thom 1949. (8) Abe 1956. (9) Scott 1968, Baghdadi 1968. (10) Pitt 1979. (11) Pitt 1979, Ramirez 1982. (12) Frisvad 1989, Frisvad, Samson & Stolk 1989, Samson, Frisvad & Stolk 1989. (13) Pitt & Samson 1993.

1.2. Pre-Thom (1930)

A large number of *Penicillium* spp. were described from 1830 up to the 1900's. Thom (1906) considered that identification of a species described during this period was done by pure accident, as there was no concept of type material, pure culturing techniques or standard descriptions. As such, most species described during this period remain unrecognizable today. Pasteur, Koch and Petri only established pure culturing techniques in the 1880's, meaning that species were typically described from their natural substrata in mixed culture. Morphology is, however, a direct response to the environment, which made species comparisons from different substrates unreliable (Pitt 1979, Okuda *et al.* 2000). Brefeld (1874) first emphasized the use of standardized culturing techniques, when he illustrated the stages of what he considered to be *P. glaucum's* different life cycles, including the sexual state today recognizable as *Eupenicillium* Ludwig (Thom 1930, Raper & Thom 1949, Pitt 1979). Delacroix (1891) was the first to realize the importance of distributing "type" material when he described *P. duclauxii* in pure culture and distributed it to other workers. The practice of distributing cultures made species comparisons much easier.

Dierckx (1901) and Biourge (1923) both made great contributions to *Penicillium* taxonomy.

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Biourge studied the bacterial diseases of beer. During the course of his studies he isolated numerous *Penicillium* strains from germinating barley, malt infusions, hops, brewery water, cheese and fruit (Biourge 1923, Hennebert 1985). Importantly he had realized that he isolated more than one species and could not apply the name *P. glaucum* L., which was the accepted name for green *Penicillium* at the time. Work on the first *Penicillium* monograph thus began (Hennebert 1985). Progress on the monograph, however, was slow until Dierckx decided to prepare his doctorate thesis under the supervision of Biourge. He took over all the strains and in 1901 published his thesis that contained descriptions and illustrations for 25 species of which 23 were described as new (Dierckx 1901). He also provided the first subgeneric classification, when he divided the genus into subgenera *Aspergilloides* and *Eupenicillium* based on conidiophore branching patterns. Unfortunately, Dierckx lost all the *Penicillium* strains during his subsequent travels. Although he attempted to re-isolate all of his species after he returned to Biourge's laboratory (Pitt 1979, Hennebert 1985), the project was later abandoned. Biourge then took over all of Dierckx' sketches, descriptions and new strains in an attempt to salvage the project. His first task was to catalogue all of the material into a collection, also adding new strains he had isolated (Hennebert 1985). Eventually he finished the monograph, which was published in 1920 and 1923. It contained descriptions for 125 *Penicillium* species, of which 60 were new to science. Biourge (1923) also managed to describe 21 of Dierckx' original species. He accepted the subgeneric classification of Dierckx (1901), although he changed the subgeneric name *Aspergilloides* to *Monoverticillium* (Biourge 1923). The contributions of Dierckx (1901) and Biourge (1923) to *Penicillium* taxonomy were very important. In addition to the large number of species described, they emphasized the use of pure culturing techniques, standardized culture conditions, defined growth conditions and the distribution of live cultures as type material (Thom 1905).

During the Dierckx (1901) and Biourge (1923) studies, a number of taxonomists published important works and new species descriptions. These include Bainier (1905, 1906, 1907), Bainier & Sartory (1912a, 1912b, 1913) who introduced 35 new *Penicillium* and *Citromyces* (an accepted synonym of *Penicillium*) species (Biourge 1923, Thom 1930, Raper & Thom 1949, Houbraken & Samson 2011, Seifert *et al.* 2011). Westling (1911) published a monograph and described 18 new species from Scandinavia. Sopp (1912) published a *Penicillium* monograph focused on species from Norway and described 41 new species. Zaleski (1927) monographed *Penicillium* isolated from soil in Poland and described 35 new species. In all these studies, the researchers went through great trouble in order to meticulously describe their species. Most of them distributed ex-type cultures to collections, which resulted in a large number of these species that remain recognizable today. Culture conditions were in most cases well defined, however, these conditions were not consistent across the different studies. Comparisons between the large numbers of species described from these studies were very difficult, which resulted in *Penicillium* taxonomy to some degree be "chaotic" (Thom 1930) and identification at best problematic. It needed stability and that is what Charles Thom (1872–1956) brought to *Penicillium* taxonomy.

1.3. Charles Thom

Thom devoted a life's work on the genera *Aspergillus* and *Penicillium* at the United States Department of Agriculture (USDA). His aim was to sort out, as he described it, the mess in *Penicillium*. In the process, concepts on speciation and subgeneric classification were established and formed the basis on which all understanding of *Penicillium* was built. "Fungi in cheese ripening (1906)" was his first major publication on *Penicillium*; with *P. camemberti* and *P. roqueforti* described as new species. More importantly it laid the foundations for the use of colony morphology as a taxonomic character, still very important today. Within the morphological concept of a species, comparative data must not only include microscopic details of the conidiophores, but also the appearance of the colony (Thom 1905, 1906, 1910). Thom (1905) was preoccupied to make species descriptions logical and sensible. As such, he took great care to explain each character that he considered to be taxonomically informative. He also made very important observations that today form part of the foundation of *Penicillium* taxonomy. Media composition affects the size and appearance of colonies and conidiophores. A species grown on different media will thus display different sets of morphological characters that are taxonomically informative. Strains from a particular species will also share morphological features on a particular medium, even after repetitive culturing (Thom 1905, 1906, 1910). Thom (1910) also noticed that two species might display similar morphological and physiological characters on one medium, but appear totally different on another, which make it possible to distinguish between two species. It was proposed that a species description be done from multiple media, and that descriptions include media formulations and incubation conditions, as well as illustrations of species that include habit details (Thom 1905, 1906, 1910). In addition to the fundamental principals instilled with this work, Thom (1910) studied thousands of strains in an attempt to bring together all knowledge of the genus at the time. He proposed *P. expansum* as the

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type for the genus, provided the first identification key to *Penicillium* spp., described and sketched 39 species and varieties of which 13 were new, as well as documenting the effect and taxonomic importance temperature have on the growth of *Penicillium* (Thom 1910). This publication eventually formed the foundation for the more comprehensive review of the genus, "The Penicillia" (Thom 1930). In this work, he provided descriptions for the 300 accepted species, grown under standardized conditions. The genus was divided into four divisions, 12 sections and 18 subsections, which formed the basis for the identification key to all described species. Species were divided into their respective divisions based on conidiophore branching patterns, similar to the concept of Dierckx (1901).

Division *Monoverticillata* represented species where one branching stage (whorl of phialides born on stipe) was typical (Thom 1930). He included species that sometimes have biverticillate conidiophores, with two branching stages between stipe and phialides, where the terminal penicillus of each branch remain separate. It contained species previously designated to the genus *Citromyces* and subgenus *Aspergilloides* (Dierckx 1901)/*Monoverticillium* Biourge (1923). Division *Assymetrica* was characterized by biverticillate and terverticillate species that have assymetrical conidiophores. Thom (1930) transferred Biourge's (1923) section *Bulliardum*/*Assymetrica* and some species of Biourge (1923) and Zaleski (1927) in his section *Biverticillium* and *Monoverticillium* into division *Assymetrica*. Species with symmetrical biverticillate conidiophores was placed into division *Biverticillata-symmetrica*. Division *Polyverticillata-symmetrica* housed species that produce symmetrical conidiophores with three or more branching stages and conidiophores borne on funicles or fascicles. It was mainly included to accommodate the *Synpenicillium* Constantine form species (excluding the type *S. album*), described by Biourge. Thom (1930) did, however, question whether this group should in fact be included in *Penicillium*. *Synpenicillium*, typified by *S. album*, is today considered a synonym of *Cephalotrichum*. None of the species Thom (1930) and Raper & Thom (1949) placed in division *Polyverticillata-symmetrica* were accepted as *Penicillium* in subsequent studies (Pitt 1979, Pitt *et al*. 2000). Colony textures were emphasized for further segregation into sections and sub-sections (Thom 1930).

The importance of the contributions Thom made to *Penicillium* taxonomy cannot be overemphasized. He laid the foundations and created a framework for future taxonomists to take on the challenges *Penicillium* present. He also made great contributions to general mycology and readers are referred to Raper (1957) for further reading on Thom's contributions to mycology.

1.4. Kenneth B. Raper

In his first job working for Thom as junior mycologist, Raper cultured isolations from a diverse range of natural habitats. His most important job, however, was the proper preservation of strains (Cavender *et al*. 1988). This eventually led him to pioneer the use of lyophilisation for long-term storage of fungal strains (Raper & Alexander 1945). Although his first love was the study of slime-moulds, especially the genus *Dictyostelium*, he made great contributions to *Penicillium* and *Aspergillus* taxonomy, while working as microbiologist at the National Regional Research Laboratory (NRRL), USDA (Cavender 1988). The industrial importance of these groups resulted in a large number of strains isolated from various habitats. Raper himself had isolated numerous strains in the search for a strain producing large quantities of penicillin, after Howard Florey and Norman Heatley visited in the search of help for large scale production of the antibiotic (Cavender 1988). It was in fact one of his strains, *P. chrysogenum* (NRRL1951), isolated from cantaloupe and later a mutant of this strain, Wisconsin Q176 that was used for the largest proportion of penicillin produced worldwide. The economic importance of *Penicillium*, helped by additional strains isolated at the NRRL, necessitated a taxonomic review of the genus. With technical assistance from Dorothy Fennell and input from Thom, Raper published the "Manual of the Penicillia" in 1949. Raper & Thom (1949) accepted 137 species, divided them into 4 sections and 41 series, with concepts and working methods similar to that of Thom (1930). The effect of standard growth media and incubation temperatures was further explored. From his point of view, he aimed to make the manual as practical as possible and made major advances in the protocol for *Penicillium* identifications (Pitt 1979). Their understanding of the taxonomy of *Penicillium*, logic approach, careful and thorough species descriptions and work methods resulted in the manual to still be useful for *Penicillium* species identification today.

1.5. John I. Pitt

Pitt (1979) with "The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*" was the next step in the evolution of *Penicillium* taxonomy. The monograph became the standard for, not only species identification, but also classification and description of new species. Even with large DNA barcoding initiatives that provide a quick means of comparing species to ex-type material, Pitt's (1979) monograph remain useful as a first step identification tool. The DNA data have, however, showed that many species reduced down to synonymy under the morphological species

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concept by Pitt (1979), are in fact distinct species. The species concepts, standardization of work methods and species descriptions, as well as Pitt's logic approach to working with the genus are major contributions to *Penicillium* and its taxonomy.

Pitt (1973) stressed the importance for the further standardization of working methods from those instilled by Thom (1930) and Raper & Thom (1949). Based on his study on the effect of water potential on fungi (Pitt and Christian 1968), Pitt (1973) proposed the use of colony diameters of strains, under standardized inoculation and growth conditions, at different temperatures and water potential as taxonomic informative characters. Growth rates of species was emphasized in Pitt (1979), with cultures incubated for 7 days on Czapek Yeast Autolysate Agar (CYA) at 5, 25 and 37 °C, as well as on additional media such as Malt Extract Agar (MEA) and G25N at 25 °C. Less emphasis was, however, placed on characters such as colony texture and conidial colors, in comparison to Raper & Thom (1949). However, Pitt (1979) applied the same concepts as proposed by Raper & Thom (1949) to micromorphological characters.

Ninety-seven species was accepted and divided into 4 subgenera, 10 sections and 21 series. Subgeneric classification was based on penicillus branching patterns. Subgenus *Aspergilloides* was characterized by strictly monoverticillate species, thereby removing species of section *Monoverticillate* series *Ramigena* (Raper & Thom 1949) and placing it in subgenus *Furcatum*. Subgenus *Furcatum,* effectively, became the "dumping ground" of species that did not belong in either subgenera *Penicillium* or *Biverticillium*. It contained species that Raper & Thom (1949) classified in section *Assymetrica*, subsections *Divaricata* and *Velutina*, section *Monoverticillata* series *Ramigena* and section *Biverticillata-Symmetrica* series *Penicillium herquei*. Subgenus *Furcatum* is characterized by species that are predominantly biverticillate, although monoverticillate and terverticillate conidiophore are not uncommon, and end in ampulliform phialides. Subgenus *Penicillium* is characterized by species that have predominantly terminal terverticillate or more complex conidiophores, with minor proportion biverticillate. It contained the rest of the species Raper & Thom (1949) classified in section *Assymetrica*. Most species from Raper & Thom's (1949) section *Biverticillata-Symmetrica* were transferred into subgenus *Biverticillium*. It was defined by species that are predominantly biverticillate, or less commonly terverticillate, more or less symmetrical and end in thin acerose phialides. Subgenus *Biverticillium* species also have conidiophores with a phialide to metulae length ratio of 1:1–1.2, compared to subgenus *Furcatum* species that have a length ratio bigger than 1.2 (Pitt and Hocking 1997, Pitt 2000).

Teleomorph-anamorph associations were ignored, against the ICBN regulations, in previous monographs (Thom 1930, Raper & Thom 1949), since they believed that it created unnecessary confusion and was illogical to its taxonomy. Pitt (1979), however, included species descriptions of the teleomorphs *Eupenicillium* and *Talaromyces*, accepting 37 and 16 species, respectively. He also noted that it was only subgenus *Biverticillium* species that were associated with *Talaromyces* while the other subgenera had a *Eupenicillium* teleomorph. Descriptions for asci and ascospores, as well as conidiophores were included and incorporated into identification keys.

John Pitt's understanding of the genus lead to his species concepts and methods to be recommended as standard for *Penicillium* at the First International *Penicillium* and *Aspergillus* Workshop (Samson and Pitt 1985). His industrial mycology background led him to emphasize the importance of rapid identification techniques. Although the definition of the genus, its subgeneric classification etc., are today rapidly changing mainly due to genetic work, the use of different growth conditions as informative characters still lie deep in the roots of *Penicillium* taxonomy. His monograph contains a wealth of information and is still a first step for species identifications and will probably remain that way for many years. He was also an integral part of the three workshops and subsequent manuscripts on the taxonomy of *Penicillium* and *Aspergillus* published in 1985, 1989 and 2000.

1.6. *Penicillium* taxonomy enters the modern era

After Pitt (1979) published his monograph on the genus, Ramirez (1982) published his "Manual and atlas of the Penicillia". His work was unfortunately never considered as important as that of Pitt (1979). He used the taxonomic scheme and concepts of Raper & Thom (1949) and took great care in standardizing his work methods, in order to emphasize colony characters as the most important taxonomic criterion. Many of his descriptions were based on single specimen isolates, which can be problematic. The biggest issue was, however, the 14-day incubation time compared to the 7 days of Pitt (1979). Pitt (1979) emphasized the need for making identifications as fast as possible and thus 14 days will always be too long. The 14-day incubation period also made subsequent species comparisons between the two studies problematic. Ramirez (1982) did, however, recognize the importance of full color photoplates and line drawings as an important aid to species identifications. Plates and drawings were considered as the first step in the identification stage for the novice *Penicillium* taxonomist. In the spirit of its predecessors (Thom 1930, Raper & Thom 1949), the aim was to publish a manual that provide a means of bringing all knowledge on

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Penicillium at a particular date together and to make species identification as easy as possible. Although not commonly used for identifications, it is still a very important part of the history of *Penicillium*. Ramirez (1982) managed to use the technology and knowledge available to him at the time and incorporate all the principles that Thom (1906, 1910, 1930) was passionate about, successfully illustrating and describing species.

The aforementioned publications set the trends and standards for the taxonomy of *Penicillium*. After the Thom (1930) and Raper & Thom (1949) monographs was published, a large number of new species and names was introduced for *Penicillium*, *Eupenicillium* and *Talaromyces* (Van Beyma 1929, Vuillemin 1931, Swift 1932, Van Beyma 1933, Smith 1933, Stapp & Bortels 1935, Van Beyma 1935, 1937, 1940, Von Szilvinyi 1941, Van Beyma 1942, 1944, Chalabuda 1950, Abe 1956, Smith 1957, 1961, 1962, 1963, 1965, Stolk 1965, Scott & Stolk 1967, Baghdadi 1968, Scott 1968a, 1968b, Stolk 1968, Udagawa 1968, Smith 1969, Stolk 1969, Pidoplichko 1972, Stolk & Samson 1972, Udagawa & Horie 1972, Udagawa & Horie 1973, Samson *et al*. 1976, Fassatiová 1977, Martinez & Ramirez 1978, Ramirez *et al*. 1978). Many are still recognizable and were accepted as valid and distinct species (Pitt 1979, Pitt *et al*. 2000). However, many of these described species have not been accepted. This is mainly due to unsatisfactory comparisons to type material of known strains and species, as well as a lack of type material of the new species distributed. Although Pitt (1979) and Ramirez (1982) attempted to add these species into their monographs, many of these species will probably remain unrecognizable in future.

From the 1980's, a large number of new species were described from a wide variety of habitats (Ramirez *et al*. 1980, Ramirez *et al*. 1981, Quintanilla 1981, Quintanilla 1982, Udagawa & Euda 1982, Quintanilla 1983, Stolk & Samson 1983, Takada & Udagawa 1983, Quintanilla 1984, Ramirez and Muntañola-Cvetkovic 1984, Pitt & Hocking 1985a, 1985b, Quintanilla 1985, Ramirez 1985, Seifert & Samson 1985, Gochenaur & Cochrane 1986, Ramirez 1986, Frisvad *et al*. 1987, Hsieh *et al*. 1987, Kong & Qi 1988, Manoch & Ramirez 1988, Takada & Udagawa 1988, Frisvad & Filtenborg 1989, Valla *et al*. 1989, Vincent & Pitt 1989, Frisvad *et al*. 1990, Quintanilla 1990, Ramirez 1990, Tzean *et al*. 1992, Udagawa 1993, Udagawa *et al*. 1993, Yaguchi *et al*. 1993, Seifert *et al*. 1993, Udagawa *et al*. 1994, Lund & Frisvad 1994, Frisvad *et al*. 1994, Ueda 1995, Boysen *et al*. 1996, Banke *et al*. 1997, Frisvad *et al*. 1997, Hocking *et al*. 1998, McRae *et al*. 1999, Peterson *et al*. 1999, Yaguchi *et al*. 1999, Frisvad *et al*. 2000, Wang & Kong 2000, Kong 2000, Heredia *et al*. 2001, Muntañola-Cvetkovic *et al*. 2001, Chen *et al*. 2002, Peterson & Sigler 2002, Tuthill and Frisvad 2002, Kong & Liang 2003, Overy & Frisvad 2003, Peterson *et al*. 2003, Frisvad & Samson 2004, Peterson *et al*. 2004, Seifert *et al*. 2004, Wang *et al*. 2004, Janso *et al*. 2005, Kwasna & Nirenberg 2005, Peterson *et al*. 2005, Wang & Zhuang 2005, Frisvad *et al*. 2006, Serra & Peterson 2007, Wang *et al*. 2007, Sonjak *et al*. 2007, Visagie *et al*. 2009, Peterson & Horn 2009, Houbraken *et al*. 2010, Hsieh *et al*. 2010, Houbraken *et al*. 2011a, 2011b, Rivera & Seifert 2011, Peterson *et al*. 2011, Barreto *et al*. 2011, Nonaka *et al*. 2011, Visagie & Jacobs 2012). Most of these described species are accepted as valid and distinct, with only a few species not recognized. Technological advances made since the 1980's, however, resulted in a number of important publications that changed the way we approach fungal taxonomy. The incorporation of additional taxonomic criteria such as chemical/physiological and genetic characters challenged what we considered a species to be.

It is particularly evident in *Penicillium* subgenus *Penicillium.* Species of this subgenus are of great economic importance as pre- and postharvest pathogens and producers of a diverse range of mycotoxins (Frisvad & Samson 2004). They form a group of closely related species that are very difficult to distinguish from each other using morphology. This group is, therefore, a good model for testing new ideas, concepts and techniques and in the end changed the way *Penicillium* taxonomy is approached. Raper & Thom (1949) acknowledged the difficulties within section *Assymetrica* subsections *Lanata*, *Funiculosa* and *Fasciculata* (subgenus *Penicillium* Pitt 1979). Raper & Thom (1949) distinguished species mainly by colony characters such as colors and textures, which may vary between different strains. Often species characters would be merged together without definite demarcations that make the placement of species within their respective series difficult (Raper & Thom 1949, Samson *et al*. 1976, Pitt 1979). Intraspecies variation could result in strains from different species sharing certain characteristic features, also known as intergrading strains. This is sometimes observed in strains of *P. crustosum* and *P. cyclopium* (Samson *et al*. 1976). This creates obvious problems in the identification process. In a revision of subsection *Fasciculata*, as well as some species from sections *Funiculosa* and *Lanata*, Samson *et al*. (1976) proposed a new classification scheme based on conidiophore morphology. A synoptic and dichotomous key that included only micromorphological characters was included for identification of the 19 species and varieties treated in the paper. It is, however, unfortunate that many of these species have similar conidiophores. To further complicate the issue, Pitt (1979) reclassified section *Assymetrica* into subgenus *Penicillium* using concepts different from Samson *et al*. (1976) (Pitt & Cruickshank 1990). Morphological identification was thus possible, albeit extremely difficult (Raper

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& Thom 1949, Ciegler & Pitt 1970, Samson *et al.* 1976, Pitt 1979, Frisvad 1981). In order to circumvent the problem of morphological identifications, taxonomists searched for alternative ways to characterize, separate and delineate species. With a common goal of a definitive taxonomy for *Penicillium* subgenus *Penicillium*, the taxonomic approach to the genus were changed and resulted in a polyphasic species concept adopted, which incorporates morphological, physiological and genetic data.

1.6.1. Physiological species concept and secondary metabolites

The first major step towards a definitive taxonomy was the characterization of species based on their physiological properties. Growth characters under different conditions, eg. temperature, media and water-potential, were already used for distinguishing between closely related species (Thom 1910, 1930, Raper & Thom 1949, Abe 1956, Pitt 1973, 1974, 1979, Ramirez 1982). Pitt (1979) mentioned that one of *Penicillium's* claims to notoriety was the production of a diverse range of mycotoxins. Ciegler & Pitt (1970) first reported the possible application of mycotoxin production as taxonomic informative data for *Penicillium*. This concept was also reported in a study to differentiate between *Aspergillus* species from the *Aspergillus flavus* group (Vincent & Kulik 1970). Scott *et al.* (1970) developed a Thin-Layer-Chromatography (TLC) method for detection of 18 mycotoxins commonly produced by *Penicillium, Aspergillus* and *Fusarium*. They also proposed Yeast Extract Sucrose Agar (YES) as a medium for detecting these mycotoxins. Kulik & Vincent (1973) used Pyrolysis-Gas-Liquid-Chromatography (PGLC) for comparing multiple strains from nine subgenus *Penicillium* species. Although they found some intergrading strains based on the chemical results, they did report that species identification was possible using pyrolysis of strains' conidia. Frisvad (1981) further explored the use of physiological data for the taxonomy of *Penicillium*. A number of physiological criteria were proposed. These include growth on the newly developed NO_2 agar and Creatine Sucrose Agar (CREA), which include acid production, as well as extracellular chemical detection using the simple agar plug method with (Filtenborg & Frisvad 1980). This TLC method was proposed as confirmation tool rather than sole criterion because of somewhat inconsistent results in some species. Filtenborg & Frisvad (1983) argued that colors produced by *Penicillium* colonies are as a result of secondary metabolites and melanins produced. Thus, these colors could be detected more accurately using TLC. Based on results, they hypothesized that species could be defined by specific profiles of consistently produced secondary metabolites. Subsequently, the total secondary metabolite profile was found to be species specific and in general consistent. As such it was proposed that future revisions in *Penicillium* always include secondary metabolite data together with the already used morphological and physiological data (Frisvad 1985a, 1985b). In a follow-up paper, Frisvad & Filtenborg (1989) chemically analyzed more than 4000 terverticillate *Penicillium* strains and divided this group mainly on chemical data, although they also included morphological and physiological data. They also revised a number of strain identities, species synonymies and introduced a number of varieties. Frisvad & Thrane (1987, 1993) optimized the use of High-Performance-Liquid-Chromatography (HPLC) for secondary metabolite detection. This technique was eventually used in the revision of subgenus *Penicillium*. The secondary metabolite profiles were a key component in the polyphasic species concept that resulted in 58 species accepted in the subgenus. Subsequent studies started to also include secondary metabolite data for revisions or descriptions of new species in *Penicillium* groups (Frisvad & Stolk 1989, Frisvad *et al.* 1990a, 1990b, Lund & Frisvad 1994, Boysen *et al.* 1996, Frisvad *et al.* 1997, Christensen *et al.* 1999, Seifert *et al.* 2004, Frisvad & Samson 2004, Frisvad *et al.* 2006, Baretto *et al.* 2011, Houbraken *et al.* 2011b). The characterization of *Penicillium* strains with this technique, combined with morphological and genetic data have become a powerful taxonomic tool.

1.6.2. DNA sequencing and the reclassification of *Penicillium*

Since the discovery of the polymerase chain reaction (PCR) by Kary Mullis (1983), a number of PCR typing methods, such as RAPD/AFLP/ RFLP, have been used for distinguishing between *Penicillium* species (Scott & Straus 2000). RFLP, for instance, has successfully been used for fingerprinting the human pathogen, *Penicillium marneffei* (Trewatcharegon *et al.* 2001, Fischer *et al.* 2004, Lasker & Ran 2004, Vanittanakom 2006). Reproducibility, standardization and the limited information these techniques carry, compared to DNA sequence data, resulted in them not really used for taxonomic purposes (Scott & Straus 2000).

During the 1990's, DNA sequencing quickly became one of the most powerful tools available to taxonomists. Comparisons between DNA sequences give taxonomists the opportunity to infer evolutionary relationships between groups of species. As such, this gave valuable information for the assessment and evaluation of classification schemes and relationships in *Penicillium* and the *Trichocomaceae* family (LoBuglio *et al.* 1993, Berbee *et al.* 1995, Boysen *et al.* 1996, Skouboe *et al.* 1999, Geiser *et al.* 2000, Ogawa & Sugiyama 2000, Peterson 2000, Heredia *et al.* 2001, Peterson *et al.*

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2004, Samson *et al*. 2004, Seifert *et al*. 2004, Peterson *et al*. 2005, Seifert *et al*. 2007, Wang & Zhuang 2007, Serra *et al*. 2008, Peterson & Horn 2008, Houbraken *et al*. 2010, Peterson *et al*. 2010, Houbraken *et al*. 2011a,b,c, Houbraken & Samson 2011, Barreto *et al*. 2011, Samson *et al*. 2011).

LoBuglio *et al*. (1993) published the first phylogenetic study on *Penicillium* species. The study focused on the relationships in the sexual *Talaromyces* and relationships with selected associated anamorph genera. The data showed that *Penicillium* subgenus *Biverticillium* forms a clade together with *Talaromyces* species distinct from the other *Penicillium* species. These species often have a very close relationship, as predicted by morphological (Pitt 1979) and chemical (Frisvad *et al*. 1990) data. Berbee *et al*. (1995) then showed that *Penicillium* was polyphyletic in the family *Trichocomaceae*, with *Eupenicillium* associated teleomorphs closer related to *Eurotium* associated *Aspergillus* species than *Talaromyces*. This was also confirmed by a number of additional studies with a suggestion that *Penicillium* subgenus *Biverticillium* should be included into its own monophyletic genus (Heredia *et al*. 2001, Ogawa & Sugiyama 2000, Peterson 2000, Seifert *et al*. 2004).

The recent movement towards single-name nomenclature (Hawksworth *et al*. 2011, Norvell 2011) provided the perfect platform for redefining genera. Houbraken & Samson (2011) studied the phylogenetic relationships in the family *Trichocomaceae* based on a four-gene phylogeny. As a result, subgenus *Biverticillium* was removed from *Penicillium sensu stricto* and incorporated into *Talaromyces* (Samson *et al*. 2011), reflecting both the distinct morphology and genetic characters of this group. In addition, a new sectional classification was proposed, similar to Peterson (2000) who divided the genus into six clades and mentioned the possibility of it representing a future subgeneric classification. This also confirmed results of Peterson (2000) that showed that subgeneric classification based on conidiophore branching patterns was superficial and not based on true evolutionary relationships. Although terverticillate species were resolved in a monophyletic clade, species from subgenera *Aspergilloides* and *Furcatum* were intermixed. In the recent reclassification of *Penicillium*, Houbraken & Samson (2011) accepted subgenera *Aspergilloides* and *Penicillium*, and divided the remainder of the genus into 25 sections. Houbraken & Samson (2011) also synonymized a number of genera to *Penicillium* and as such redefined its generic concept.

A species name contains vital scientific information about that particular species. As such, all biological research is based on correct species identifications (Seifert *et al*. 2007). The large DNA barcoding initiative aims to make species identification possible for anybody, by use of a short DNA sequence (Blaxter 2003, Hebert *et al*. 2003, Tautz *et al*. 2003, Blaxter *et al*. 2005, DeSalle *et al*. 2005, Min and Hickey 2007, Ratnasingham and Hebert 2007, Seifert *et al*. 2007, Schoch *et al*. 2012). The internal transcribed spacer (ITS) region was recently approved as the official DNA barcode for fungi (Schoch *et al*. 2012). However, ITS is not variable enough for separation of closely related species (Skouboe *et al*. 1999, Seifert *et al*. 2007, Schoch *et al*. 2012). It does, however, present the best chance of a correct identification in a broad sense, with secondary barcodes that can be developed for identifications in a narrow sense (Seifert *et al*. 2007, Schoch *et al*. 2012). There are a number of possibilities for a secondary DNA barcode for *Penicillium* species identifications. β-tubulin was successfully used for separation of the closely related subgenus *Penicillium* species (Samson *et al*. 2004). Possible alternative genes for species separation in *Penicillium* also include Calmodulin, the RNA polymerase II genes RPB1 and RPB2, and Elongation Factor 1-α (Peterson *et al*. 2002, Peterson *et al*. 2004, Samson *et al*. 2004, Seifert *et al*. 2004, Peterson *et al*. 2005, Seifert *et al*. 2007, Wang & Zhuang 2007, Serra *et al*. 2008, Peterson & Horn 2008, Visagie *et al*. 2009, Houbraken *et al*. 2010, Peterson *et al*. 2010, Houbraken *et al*. 2011a,b,c, Barreto *et al*. 2011, Samson *et al*. 2011, Visagie & Jacobs 2012). Because of introns, however, these genes are very difficult to align across a diverse genus such as *Penicillium* (Houbraken & Samson 2011). RPB1 and RPB2, however, lack these introns, which makes their alignments very easy. Also, these genes were shown to have enough variation for close species separation (Houbraken *et al*. 2011a, b, c, Samson *et al*. 2011). As such, RPB2 at the moment seems to be the preferred region as secondary DNA barcode, although a more in depth study on this is required (Seifert, Houbraken & Samson, personal comm.).

2. The *Trichocomaceae* family and *Penicillium* sensu lato teleomorphs

Penicillium belongs to the diverse *Trichocomaceae* family. Fischer (1987) introduced the family for genus *Trichocoma*, which are associated with a biverticillate *Penicillium* anamorph (Geisert & LoBuglio 2001) and are characterized by the production of cleistothecial ascocarps (Malloch and Cain 1972, Fennel 1973, Benny and Kimbrough 1980, Malloch 1981, Malloch 1985a, Malloch 1985b, Berbee *et al*. 1995, Ogawa and Sugiyama 2000, Pitt *et al*. 2000, Tamura *et al*. 2000, Stchigel and Guarro 2007). Until the recent reclassification of *Trichocomaceae,* it had 29 accepted genera associated with it. Nine was anamorphic, most notably *Penicillium* and *Aspergillus*, and 20 holomorphic (Ogawa & Sugiyama 2000, Pitt *et al*. 2000, Tamura *et al*. 2000).

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A number of studies have proposed different classifications for the family (Benny & Kimbrough 1980, von Arx 1987, Malloch 1985a), but these were never supported by phylogenetic data (Berbee *et al*. 1995, Ogawa *et al*. 1997, Sugiyama 1998, Ogawa and Sugiyama 2000, Tamura *et al*. 2000, Stchigel and Guarro 2007, Houbraken & Samson 2011). Houbraken & Samson (2011) studied the relationships between genera that historically were classified in the *Trichocomaceae* based on a four-gene phylogeny. This resulted in the identification of three distinct lineages or families. *Penicillium* s. str. was resolved in the lineage *Aspergillaceae* together with genera such as *Aspergillus*, and its many associated teleomorphs, *Hamigera*, *Leiothecium*, *Monascus*, *Penicilliopsis*, *Phialomyces*, *Phialosimplex*, *Polypaecilum*, *Sclerocleista*, *Warcupiella* and *Xeromyces*. Also, the genera *Chromocleista*, *Eladia*, *Eupenicillium*, *Hemicarpenteles*, *Thysanophora* and *Torulomyces* were placed in synonymy with *Penicillium.* The second lineage identified was *Thermoascaceae* for *Paecilomyces*, its teleomorph *Byssochlamys*, and *Thermoascus*. The *Trichocomaceae* lineage was reduced down to *Dendrospheara*, *Rasamsonia*, *Sagenomella*, *Thermomyces*, *Trichocoma* and *Talaromyces* that include *Sagenoma*, *Erythrogymnothecia* and *Penicillium* subgenus *Biverticillium* as synonyms.

2.1. *Eupenicillium* (*Penicillium* s. str.)

Brefeld (1874) made the first report of a perfect state in *Penicillium* when he provided a description for the full life-cycle of "*Penicillium crustaceum* (Link) Fries (*P. glaucum*)". Brefeld (1874) also included illustrations for the maturation of cleistothecia and the production of ascospores in the species. Winter (1887) considered Brefeld's fungus as *P. crustaceum* (Link) Fries, which Ludwig (1892) used as type for the teleomorph genus *Eupenicillium*. Langeron (1922) was not aware of Ludwig's (1892) paper and introduced *Carpenteles* for the sexual state of *P. glaucum* (Link) Brefeld, even though he did not observe any cultures (Shear 1934). His peers therefore, never accepted *Carpenteles* as valid. Shear (1934) then re-isolated what he considered to be *P. glaucum* as described by Brefeld (1874) and re-described it as *Carpenteles asperum* (Shear 1934, Raper & Thom 1949, Stolk & Scott 1967, Pitt 1979, Stolk & Samson 1983).

Benjamin (1955) reviewed the ascocarpic forms associated with *Aspergillus* and *Penicillium*. As a result, *Carpenteles* was reintroduced as a *Penicillium* perfect state with *C. asperum* the accepted type. *Eupenicillium* Ludwig (1892) was, however, the older name and eventually correctly reinstated as one of the teleomorphs associated with *Penicillium* and *E. crustaceum* Ludwig accepted as generic type (Stolk & Scott 1967). Scott (1968a, b) subsequently published a monograph on 26 *Eupenicillium* species, as well as providing an identification key. Species was distinguished based on characters of cleistothecia, which may take up to a month or longer to mature. As a result, Pitt (1974) published a synoptic key to 36 *Eupenicillium* species and 22 sclerotigenic *Penicillium* species. Pitt (1974) added characters such as colony diameters on different media and incubation conditions, as well as conidiophore morphology. As a result, identifications were made possible even in non-mature colonies (Pitt 1979). Stolk & Samson (1983) emphasized the use of micromorphological characters, which include conidiophores, cleistothecia and ascospores, for their reclassification of *Eupenicillium*. Houbraken & Samson (2011) showed in their four-gene phylogeny that *Eupenicillium* species are resolved in many of the redefined sections of *Penicillium.* With article 59 removed from ICBN, (Norvell *et al*. 2011), *Eupenicillium* was incorporated into the much more widely known and important *Penicillium*. A number of studies have also started to report on the production of sexual states in species or clades never expected to have sexual reproduction (Houbraken *et al*. 2011a, Houbraken & Samson personal communication). The fact that *Eupenicillium* is dispersed throughout the sections of *Penicillium*, and a number of sexual states have been induced for *Penicillium*, might also point to the possibility that sexual reproduction might be more common in nature than originally thought.

2.2. *Talaromyces* (*Penicillium* subgenus *Biverticillium*)

Van Tiegham (1874) discovered a soft cotton-like ascocarp without a wall for a *Penicillium* strain. Much later, Benjamin (1955) introduced the teleomorph name *Talaromyces* and designated *T. vermiculatus* (Dangeard) Benjamin as generic type. Stolk & Samson (1971) introduced *Hamigera* for *Talaromyces* spp. that have asci that occur solitary. Although Pitt (1979) considered *Hamigera* synonymous with *Talaromyces*, Houbraken & Samson (2011) confirmed it as a distinct genus. Pitt & Hocking (1979) and Pitt (1979b) also introduced two anamorph genera, *Merimbla* and *Geosmithia* respectively, for *Talaromyces* species that do not conform to the concept of *Penicillium*. In the list of species in use for the Trichocomaceae, Pitt *et al.* (2000) listed *Penicillium*, *Paecilomyces* and *Geosmithia* as anamorph genera to *Talaromyces*, with *Merimbla* associated with *Hamigera*. Houbraken & Samson (2011) confirmed many of these associations and considered *Penicillium* subgenus *Biverticillium* synonymous with *Talaromyces*. *Geosmithia* was excluded with data that show that it does not belong in the *Eurotiales* clade, even if some members are synonymous with *Penicillium* species. The Samson *et al.* (2011) phylogenetic review of *Talaromyces* included

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Penicillium subgenus *Biverticillium*, *Sagenoma*, *Erythrogymnothecia* and *Paratalaromyces* as synonyms.

Montagne (1845) introduced the monotypic genus *Lasioderma* for *L. flavovirens* Durieu & Montagne. Recently, Visagie *et al*. (accepted for publication in Mycotaxon) reported that *L. flavovirens* is conspecific with *P. aureocephalum* Munt.-Cvetk., Hoyo & Gómez-Bolea. Recent changes to the ICBN were not made at the time of the study, which means that *Lasioderma* is an older teleomorph name than *Talaromyces*. As such, for nomenclatural stability Seifert *et al*. (2012) proposed the conservation of the name *Talaromyces* against *Lasioderma*.

3. Review on the genus *Penicillium* and its teleomorphs, *Eupenicillium* and *Talaromyces*, in the South African context

Penicillium history in South Africa dates back to Pole-Evans (1911) who first recorded *P. digitatum* as the causative agent of citrus rot in the old Natal and Transvaal regions. Since then, *Penicillium* has been mentioned in a number of studies from diverse habitats. Schutte (1992) reviewed the state of *Penicillium* in South Africa, which included a list of species reported in literature. Unfortunately, a large proportion of studies did not attempt to identify *Penicillium* strains, often deeming it to be too difficult (Schutte 1992). Funding bodies traditionally do not provide funds for environmental fungal studies (Schutte 1992, Crous *et al*. 2006). As a result, mycologists focused on economically important habitats, such as bananas, beer industry, cork, citrus, dairy products, dried fruit, grapes, litchi, maize, mangoes, mushroom, nuts, pome fruits, wheat and wine industry (Pole Evans 1911, 1920, Doidge & Van der Plank 1936, Doidge 1950, Martin 1960, Roth 1963, Roth & Loest 1965, Roth 1967, Matthee 1968, Wehner & Rabie 1970, Le Roux *et al*. 1973, Marais & Kruger 1975, Lück *et al*. 1976, 1978, Lück & Wehner 1979, Wehner *et al*. 1981, Rabie & Lubben 1984, Smit 1984, Combrink *et al*. 1985, McLean & Berjak 1987, Wittaker *et al*. 1989, Rheeder *et al*. 1990, Johnston 2008). A limited number of environmental studies were, however, done in South Africa. Cohen (1950) completed the first fungal survey from soil in South Africa from the Transvaal region, when he identified seven *Penicillium* species, including two that he could not identify. Subsequent surveys from Transvaal and Natal were done from soil, *Acacia karroo*, *Pinus* and *Eucalyptus* leaf litter (Eicker 1969, 1970, 1973, 1976, Papendorf 1976, Eicker *et al*. 1982, Lundquist & Baxter 1985, Lundquist 1986, 1987). These studies often found *Penicillium* as one of the dominant genera. Species identification was, however, only attempted in a couple of these studies, with most only making mention that *Penicillium* was found. In papers where identifications were made, many of the species were reported as *Penicillium* sp. and possibly were new species. These were, however, rarely described subsequently. Scott (1968b) completed a taxonomic revision of *Eupenicillium* and in the process described eight new species from South African soils. Most of these species are still recognized today. Ramirez (1990) isolated a new species, *P. krugerii* in subgenus *Biverticillium* from soil collected in the Kruger National Park. The species has a questionable taxonomy and was not accepted in the recent revision by Samson *et al*. (2011), with type material not available. Allsopp *et al*. (1987) completed a soil survey from the Riverlands Nature Reserve in the Fynbos, one of the sites in this study. In total 66 fungal species were isolated, 16 from the genus *Penicillium*. Two of the *Penicillium* species remained unidentified and possibly represents new species. A fungal survey from *Protea* species, identified ten *Penicillium* species (Marais & Wingfield 1994, Lee *et al.* 2005). In a similar study, Roets (2006) reported that *Penicillium* was commonly found in infructescences. One species was found to be new and was included in the description of *P. ramulosum* (= *T. ramulosus*) (Visagie *et al*. 2009). A recent survey on *Penicillium* spp. that occur in apple orchards in the western Cape identified seven species and implicated two of them as possible causative agents of core rot in these apples (Van der Walt *et al*. 2010).

Over the last couple of years, biodiversity has become an important subject for South Africa, especially in relation to climate change (Midgley *et al*. 2002, Malcolm *et al*. 2006). As such, there has never been a better time for exploring fungal diversity in South Africa. Crous *et al*. (2006) estimated 171 500 fungal species to occur in South Africa. This was calculated in similar fashion to the Hawksworth (1991, 2001) worldwide estimate of 1.5 million species. This number was calculated based on the plant-fungi ratio (1:6) of the well-studied British Islands and extrapolated to the rest of the world. This ratio is, however, thought to be closer to 1:7 for South African habitats, when the diverse nature of fungi associated with Fynbos in the Western Cape is considered (Crous *et al.* 2006). Both these numbers are considered to be gross underestimates, since it does not take into account fungi associated with small animals such as insects. Of the South African estimate, the largest proportion of fungi is expected to occur in the Cape Floristic Region (CFR).

The Fynbos, which forms part of the CFR, is one of the world's 25 biodiversity hotspots (Myers *et al*. 2000), with 9030 vascular plant species that represents 44% of the total floral inventory of South Africa (Goldblatt & Manning 2002). With regards to β-diversity, a measure of diversity along environmental distances (Wilson & Shmida 1984, Kolef *et al*. 2003), the Fynbos is the most diverse

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habitat on earth (Goldblatt & Manning 2002). A number of reasons for the Fynbos diversity have been hypothesized. The Fynbos is highly heterogenous, contains rugged landscapes, soils that are acidic and nutrient poor (Kruger *et al*. 1983). Temperature fluctuates between seasons with hot and dry summers compared to the cold and wet winters (Richards *et al*. 1997). Also, its fire ecology is essential for healthy Fynbos (Goldblatt & Manning 2002). These factors all result in harsh living conditions. These are not unique in the Fynbos biome, but also play a role in the high diversity of southwest Australia and parts of California (Goldblatt & Manning 2002). The one factor that makes the western Cape unique is the climate that stayed consistent during the Pleistocene period, which for instance was characterized by the ice period (Meadows & Sugden 1991, Villagra'n 2004). Goldblatt & Manning (2002) thus suggested that organisms simply had more time to evolve and speciate in the western Cape area. Using the Crous *et al*. (2006) 1:7 ration, 63000 fungal species are expected to occur in the Fynbos. For plants, 70% of species are endemic (Goldblatt & Manning 2002). It is thus expected that a large proportion of the 63000 species estimate will be endemic too. With close to 780 fungi described from South Africa (Crous *et al*. 2006), many more waits to be discovered.

A recent survey from coastal Fynbos soil (Visagie 2008, Visagie *et al*. 2009, Visagie & jacobs 2012), reported *Penicillium* to be one of the dominant fungal groups in this habitat. Soil surveys conducted worldwide, also often reports *Penicillium* as one of the dominant genera (Christensen *et al*. 2000). Schutte (1992) mentioned that many South African strains show morphological differences from species descriptions provided in Pitt (1979). Based on observations by Visagie (2008), who reported 15 new species out of the 40 isolated, these strains also most probably represent new species. Unfortunately, strains from previous South African studies are extremely difficult, if not impossible, to obtain today. This is mainly due to strains that were commonly kept in private collections, which is most often inaccessible or has been lost. Previous studies only used morphology for identification, which may have resulted in a number of misidentifications. Frisvad (1989) reported on the misidentification of strains, which included some South African strains. New studies that incorporate gene sequences will, however, not only help for correct identifications for their studies, but also add to sequence databases for future studies. South Africa with its unique and diverse fungal communities, thus provide South African mycologists the opportunity to be major role players in the international understanding of Kingdom Fungi.

4. Conclusions and study objectives

It is now more than 200 years since Link (1809) introduced the genus *Penicillium*. Since then it has become one of the most commonly isolated and well-known genera worldwide (Thom 1930, Raper & Thom 1949, Pitt 1979, Frisvad & Samson 2004, Houbraken & Samson 2011). Difficulties in reliable identifications, has resulted in the genus often serving as model organism for modern concepts. As such, *Penicillium* taxonomists often change the taxonomic concepts applied for other genera. In nature, its success has been the degradation of dead organic matter. Members of the genus have also had a great impact on human life. Members produce a diverse range of chemical compounds, which has been used as antibiotics and has great potential as other medicinal purposes. As decomposers, they also produce enzymes that have been used for a wide range of biotechnological applications. On the negative side, they cause various rots and produce mycotoxins in the food industry, are common irritants in indoor air environments and it can cause penicilliosis. With the impact and common occurrence in mind, it is remarkable how little is known about this genus in South Africa. Schutte (1992) mentioned that this can mainly be attributed to the difficulties faced with *Penicillium* identifications from South Africa. This statement is true when taking into account that morphology was the only practical tool available for identification purposes. The modern era of *Penicillium* taxonomy, that incorporates morphology, secondary metabolite profiles and sequence data, have given South Africans the perfect basis from which to start exploring the genus in South Africa and contribute to international understanding of the genus.

Based on literature we, therefore, hypothesize that the *Penicillium* communities from Fynbos will reflect the diverse nature of plants in this biome. Also, similar to plant communities high endemic-levels are expected. As an additional interest, the ecology of *Penicillium* species and their distribution were also studied. The aims of the project were:

1. To isolate *Penicillium* and *Talaromyces* strains associated with *Protea repens* infructescences, mites living inside the infructescences, as well as surrounding soil and air, from three distinct Fynbos types.
2. To characterize Fynbos strains based on morphology and multi-gene phylogenies.
3. To provide descriptions of *Penicillium* and *Talaromyces* species that occurs in Fynbos, including full color photoplates and line drawings where considered informative.
4. To provide identification keys to species isolated and described during this study.
5. To investigate *Penicillium* communities' distribution and ecology from three Fynbos types.

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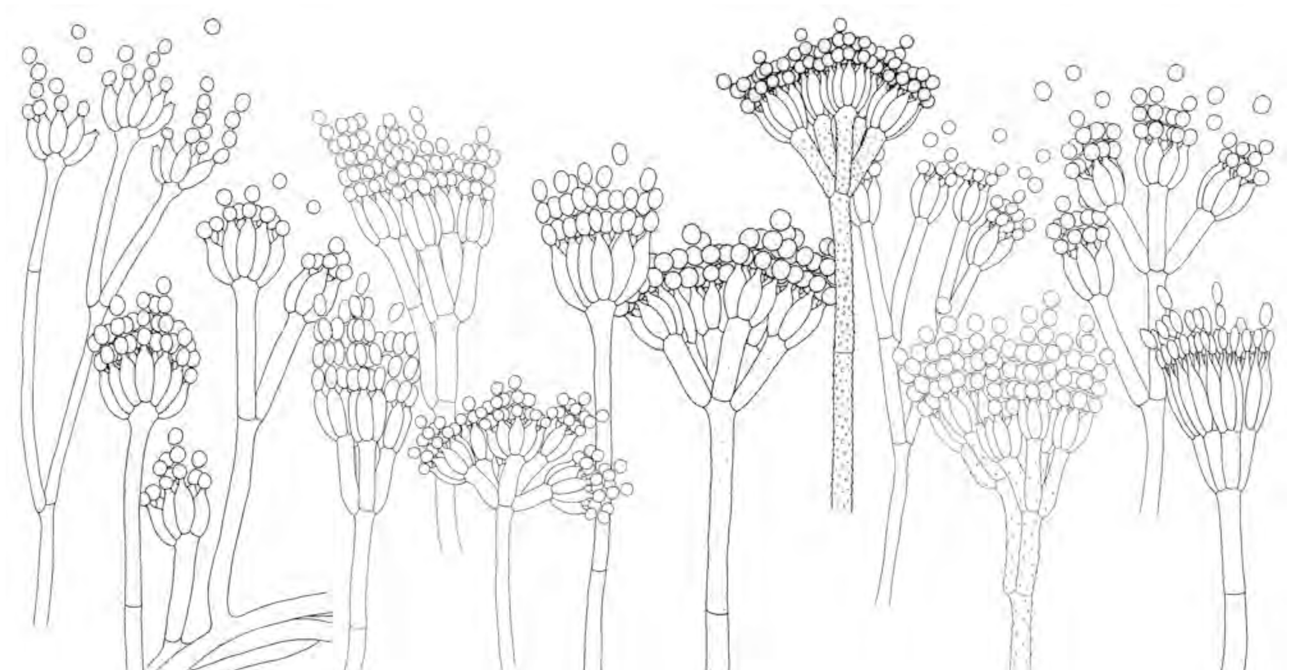
CHAPTER 2
The polyphasic taxonomy of *Penicillium* spp. isolated from the diverse Fynbos biome in the Western Cape, South Africa



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The polyphasic taxonomy of *Penicillium* spp. isolated from the diverse Fynbos biome in the Western Cape, South Africa

ABSTRACT — Currently there are more than 250 *Penicillium* species accepted in literature. This number increased rapidly over the last couple of years mainly due to improved sequencing techniques, as well as the exploration of new habitats. The Fynbos biome is one of the world's most unique biomes and is listed as one of UNESCO's world heritage sites. It is also listed as one of the 25 global biodiversity hotspots. This study aimed to provide a baseline inventory to *Penicillium* species that occur in the diverse Fynbos biome. Isolations from soil, air and *Protea repens* infructescences resulted in ±1700 *Penicillium* strains, which represented 61 species. Species were grouped into their respective sections based on ITS barcodes. A more detailed taxonomic evaluation was done based on the sections to which they belong. Within each section, multiple gene phylogenies are provided, followed by the descriptions, full color photoplates and line drawings for each species. In total, this study describes 25 new *Penicillium* species.

KEYWORDS — Morphology, Internal transcribed spacer region, β-tubulin, Calmodulin, RNA polymerase II second largest unit, Elongation Factor 1-α.

Introduction

The generic name *Penicillium*, meaning little brush, was introduced by Link (1809) in his *Observations in Ordines plantarum naturales*. In his original paper, he described three species, with *P. expansum* Link (CBS325.48^{NT}) as the generic type. According to online nomenclature databases such as MycoBank (www.mycobank.org) and Index Fungorum (www.indexfungorum.org), more or less a thousand names have since been introduced to *Penicillium.* Only *c*[1]*a*. 250 of these names are accepted as valid species in literature. The number of described species is, however, increasing at a rapid rate. This increase can be attributed to a solid foundation laid by works such as Thom (1930), Raper & Thom (1949), Pitt (1979) and Ramirez (1982), (see CHAPTER 1) as well as new taxonomic concepts introduced in the three *Aspergillus* and *Penicillium* workshop proceedings published in 1985, 1989 and 2000, respectively, as well as the Frisvad & Samson (2004), Samson *et al*. (2004) and Frisvad *et al*. (2004) treatments on the subgenus *Penicillium*. The combination of phylogeny, morphology and chemical data revolutionized the taxonomic approach. More bias are, however, placed on phylogenetic data as can be seen by the recently reclassification of the family *Trichocomaceae* mainly based on a four-gene phylogeny (Houbraken & Samson 2011). The use of DNA sequences for comparing unknown strains to the ex-type material of previously described species is critical. This is because reference strains, such as ex-type cultures, are often badly deteriorated and often do not display the characters seen in wild-type strains (Okuda *et al*. 1990, Barreto *et al*. 2011). This makes morphological comparisons very difficult. The use of DNA sequences, however, provides a means for easier and more consistent species comparisons. The move towards a bias on phylogenetic data, combined with the exploration of new and unique habitats, have greatly contributed to the sudden increase in the number of species described world-wide.

One of these unique habitats is situated at the Southwestern tip of Africa (PLATE 4a). The Fynbos biome with its ±9030 vascular plant species is one of the most unique and diverse habitats on earth (Myers *et al*. 2000, Goldblatt & Manning 2002, Midgeley *et al*. 2002, Crous *et al*. 2006, Mucina & Rutherford 2006). What is more remarkable is that close to 70% of plants in the area are endemic (Goldblatt & Manning 2002). The high diversity and endemic nature is also manifested in the fungal communities, especially for *Penicillium* and *Talaromyces* (Visagie *et al*. 2009, Visagie & Jacobs 2012). Surveys done for this project over the last six years have resulted in the isolation of close to 100 *Penicillium* sensu lato species. From indoor surveys, it is also known that these Fynbos species do occur in households in the Western Cape. As such, the aim of this project was to provide a monographic treatment for *Penicillium* and *Talaromyces* (CHAPTER 3) species that occur in the Fynbos, using a polyphasic taxonomic approach. Although *Talaromyces* are treated in CHAPTER 3, the species concepts used are the same and are discussed in this chapter. Characterization was done based on morphology and multigene phylogenies.

In this manuscript, emphasis was placed on simplifying and making identification of the treated taxa as logic and easy as possible. As a result, full color photoplates and line drawings are included with species descriptions. Identification keys to *Penicillium* and *Talaromyces* species occurring in Fynbos are also provided. For molecular identifications, ITS, β-tubulin, Calmodulin and in some cases RPB2 and Elongation Factor 1- α, have been sequenced for all species. The species concept adopted for this study is discussed in the following section. Also, all morphological characters examined for species descriptions are clearly described and illustrated, in order to make

[1] Repetition presented here are due to the preparation of individual manuscripts for publication

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morphological terms and descriptions easier to understand for non-experienced users.

Species concept

The specific concept used to delineate species is an important consideration in any taxonomic treatment. Defining this right from the start is not only imperative for that specific treatment, but also for future studies. This, unfortunately is no easy task for *Penicillium*. The first paper addressing the issue of species concepts in *Penicillium* was Thom (1954). In his paper, Thom (1954) emphasized the use of a logic approach and listed all characters he felt necessary for describing species in *Penicillium*. This list was mainly based on previous works (Thom 1906, Thom 1910, Thom 1930, Raper & Thom 1949), which described cultures from standardized incubation conditions. Thom (1954) was also aware of the fact that intra-species variation exists and took this into account for species delineation.

The biggest problem taxonomists faced was that they had only morphology for comparing strains and species. Added to this are obvious problems such as standardized working methods (Thom 1954, Samson & Pitt 1985), the degeneration of morphological characters in old reference cultures, as well as comparing dried herbarium material to freshly isolated strains. The shear number of *Penicillium* species estimated to occur worldwide complicates this even more. Both Thom (1954) and Pitt (1979) had the philosophy of "a species are only of value if others can recognize it". Pitt (1979) also considered two species to be the same if he could not satisfactorily separate them in an identification key, which lead to the synonymizing of many species. The revolution of fungal species concepts followed Pitt's (1979) work. New techniques incorporated into taxonomic studies included the physiological species concept (Frisvad 1981, Frisvad & Filtenborg 1983, 1989, 1990a, 1990b, Frisvad *et al*. 2004), phylogenetic species concepts and genealogical concordance species concept (Geiser *et al*. 1998, O'Donnell *et al*. 1998, Taylor *et al*. 2000). Christensen *et al*. (2000) once again mentioned the necessity of using morphological, chemical and genetic data combined into one species concept. This proposed polyphasic species concept was finally adopted in the Frisvad and Samson (2004) monograph for subgenus *Penicillium* and has since become the standard for working with *Penicillium* and *Talaromyces*.

For this study, a philosophy of practicality is adopted which will hopefully make species identification possible, also for non-taxonomists. Morphological descriptions were done under strict standardized conditions. Species are characterized using a wide range of morphological characters with these illustrated in full color photoplates and line drawings. This study did place a bias towards phylogenetic results for species identification and delineation. For descriptions of new species, sister species were identified based on molecular phylogenetic results, which made morphological comparisons between these possible. This combination of morphological and phylogenetic data was thus used for creating a coherent taxonomy for species from Fynbos.

For multigene phylogenies, ITS sequences were used to place Fynbos *Penicillium* spp. in their respective sections, similar to the Peterson (2000) and Houbraken & Samson (2011) studies. Based on the Houbraken & Samson (2011) sectional divisions, a more detailed analysis for each clade was done using β-tubulin, Calmodulin and for some clades, RPB2 and Elongation Factor 1-α. Genealogical concordance within a species was then confirmed with morphological characters. In cases where genealogical concordance was observed, but strains within the specific clade displayed morphological variation, the particular species was considered to have intra-species variations. In instances of concordance between two clades without obvious morphological differences the taxa are treated as a species complex. These complexes will need additional characterization, eg. chemical analysis or additional morphological characters such as growth optima in order to clarify boundaries between these species.

Although it is preferable to base species descriptions on a large number of isolates, studies in diverse regions such as the Fynbos will always have issues with single specimen isolates. Although morphologically they are often difficult to distinguish from their close relatives, gene sequences are considered reliable for making a decision on whether these isolates are in fact different or not. Raper & Thom (1949) made mention of the existence of mutant strains. We acknowledge the existence of these mutants. Having said that, in cases where morphology and gene sequences are considered unique enough, the strains are described as new species.

In cases where matches with ex-type species sequences were found, the fynbos strains was identified accordingly and species description provided. Notes on differences between our strains and previously published descriptions are provided at the bottom of each description. It is important to note that although a few descriptions are based on a single strain, the description will eventually contain a number of isolates from various studies from South Africa and this will only form a base from where a more comprehensive work on all *Penicillium* spp. that occur in South Africa can be done.

Morphological characterization

Morphology is an integral part in the taxonomy of *Penicillium* and together with the multigene

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phylogeny forms the species concept adopted in this study. Morphological features are however often difficult to interpret for non-experienced workers. As such, the characters typically used for *Penicillium* descriptions are explained in this section. Characters are divided into macromorphological (PLATE 2) and micromorphological characters (PLATE 1, 3).

Macromorphology

Growth rate — Thom (1930), Raper & Thom (1949) and Pitt (1973, 1979) considered the growth rate on various media as an important taxonomic feature. Colony diameters are measured across the widest part of the colonies, with the use of a ruler. When no growth is observed, points of inoculation are inspected for microcolonies or conidial germination (PLATE 2c–e), using a stereomicroscope.

Acid production on creatine sucrose agar (CREA) — Acid production is tested on CREA, with a yellow discoloration, indicative of acid production, while purple indicate the production of a base (PLATE 2b). Some species, such as *P. expansum* (PLATE 2b, center) initially produces acid, followed by base production.

Colony textures — Depending on the way a species produce or carry its conidiophores, it will create different colony textures. When conidiophores are borne directly in the media, it creates a velvety look or velutinous texture (PLATE 2f). Colonies are floccose (PLATE 2g) when conidiophores are borne on aerial hyphae. Species such as *P. expansum* produce fasciculate colonies (PLATE 2h), where large masses of conidiophores are borne together in tufts directly from media. *Penicillium crustosum* and *P. oxalicum* are typically velutinous, but conidiophores produce large masses of conidia that break off in crusts (PLATE 2i). Funicles are often seen in *Talaromyces* spp. colonies (PLATE 2j), as well as the production of synnemata (PLATE 2k–l). Funicles are characterized by a large number of conidiophores that are borne next to each other on the same aerial mycelia or "rope". Synnemata are produced when large masses of conidiophores are bundled together extending into the air. When conidiophores are produced only at the apex of synnema it is termed as determinate (PLATE 2l), compared to indeterminate (PLATE 2m), where conidiophores are produced along the entire length of the structure.

Medium buckling — Occasionally, medium buckling is typical of a species, although most often it is not taxonomically informative. It is also one of the first things noticed when observing *Penicillium* colonies. It is most often observed on CYA and YES and can be radiate, concentric, both radiate and concentric (PLATE 2a) or random. Some species may also produce crater-like colonies.

Colors produced in colonies — Colors can be useful for identifying species, although this is not always a stable character. Conidia *en masse* often dominate the color of the colony and typically range between blue and green. Mycelia can also be pigmented (PLATE 2g). For this study, mycelia color was recorded by observing colonies through a stereomicroscope. Sometimes a color could be observed when viewing the colony, even though this could not be observed for individual mycelia. This was then recorded as the colony having a color and not the mycelia. Other colors typically recorded are exudates (PLATE 2o) and soluble pigments (PLATE 2q). Colony reverse pigmentation also seems to be stable, especially in fresh isolates, and can range through a whole spectrum of colors.

Sclerotia — Some species typically produce sclerotia (PLATE 2n). As well as different sizes, they are also produced in different colors. However, sclerotia are, not always produced on top of media, but can also be produced inside media as is seen for *P. novae-zeelandiae* (PLATE 2p).

Micromorphology

Conidiophore branching (PLATE 1) — Traditionally conidiophore branching pattern has been a very important character used for *Penicillium* taxonomy. Subgeneric classification was based on the conidiophore branching pattern and although these subgenera have been shown to be superficial through phylogenetic studies (Peterson 2000, Houbraken & Samson 2011), a typical conidiophore branching pattern can still be observed in many of the sections. Branching patterns are divided into monoverticillate (simple) (PLATE 3n), biverticillate (one-stage branched) (PLATE 3o, p, r), terverticillate (two-stage branched) (PLATE 3q) and quaterverticillate (more complex), where branching stages are counted between the stipes and the phialides. Species that produce monophialidic conidiophores (PLATE 3m), previously belonging to the genus *Torulomyces* are considered a synonym of *Penicillium*. *Talaromyces* (= *Penicillium* subgenus *Biverticillium*) conidiophores (PLATE 3r) are easily recognizable based on the shape of its phialides, as well as the metulae to phialide length ration that is close to 1–1.2:1. Within *Penicillium*, two types of biverticillate conidiophore patterns are observed, with the terminal biverticillate conidiophores produced (PLATE 3o) compared to the more difficult to define irregular biverticillate conidiophores (PLATE 3p).

Stipes — Typically stipe walls can be classified as smooth (PLATE 3g), rough (PLATE 3h) or warted (PLATE 3i). It is important to note though that there are reports that this character is not only media dependent, but are also affected by factors such as oxygen availability (Frisvad *et al*. 2000, Frisvad & Samson 2004). However, during the course of the current study, this phenomenon was not observed.

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In especially monoverticillate species, the stipes can typically be swollen at the apex (PLATE 3n). A stipe is vesiculate when the vesicle width is, as a rule of thumb, more than twice the stipe width.

Metulae — The size, divergence and number of metulae are recorded for descriptions. In this study, the angle of metulae was also measured and was found to be somewhat taxonomically informative, with some species metulae forming big angles compared to smaller angles of others. Fresh isolates of specific species will typically produce swellings at the metula apex (commonly seen in *P. brevicompactum*).

Phialides — Most *Penicillium* spp. produce flask shaped (ampulliform) phialides (PLATE 3k), while some species produce cylindrical phialides (PLATE 3l). *Talaromyces* are easily distinguished from *Penicillium* by their acerose phialides (PLATE 3j) that are typically long and thin. Occasionally species produce phialides that have intermediate shapes, but those are mentioned in the species descriptions.

Conidia — Considerable variation in conidia size, shape and ornamentation are observed for *Penicillium*. Shapes include spheroid (PLATE 3a, e), subspheroid (PLATE 3b), broadly ellipsoid (PLATE 3c), ellipsoid (PLATE 3d) and cylindrical (PLATE 3f). Conidial shapes can, be difficult to determine (Frisvad *et al*. 2000) and thus width to length ratios are included in descriptions. Conidia can be smooth (PLATE 3c), slightly rough (PLATE 3b), rough (PLATE 3a), echinulate/spiny (PLATE 3e) or rough walled in patterns (PLATE 3d).

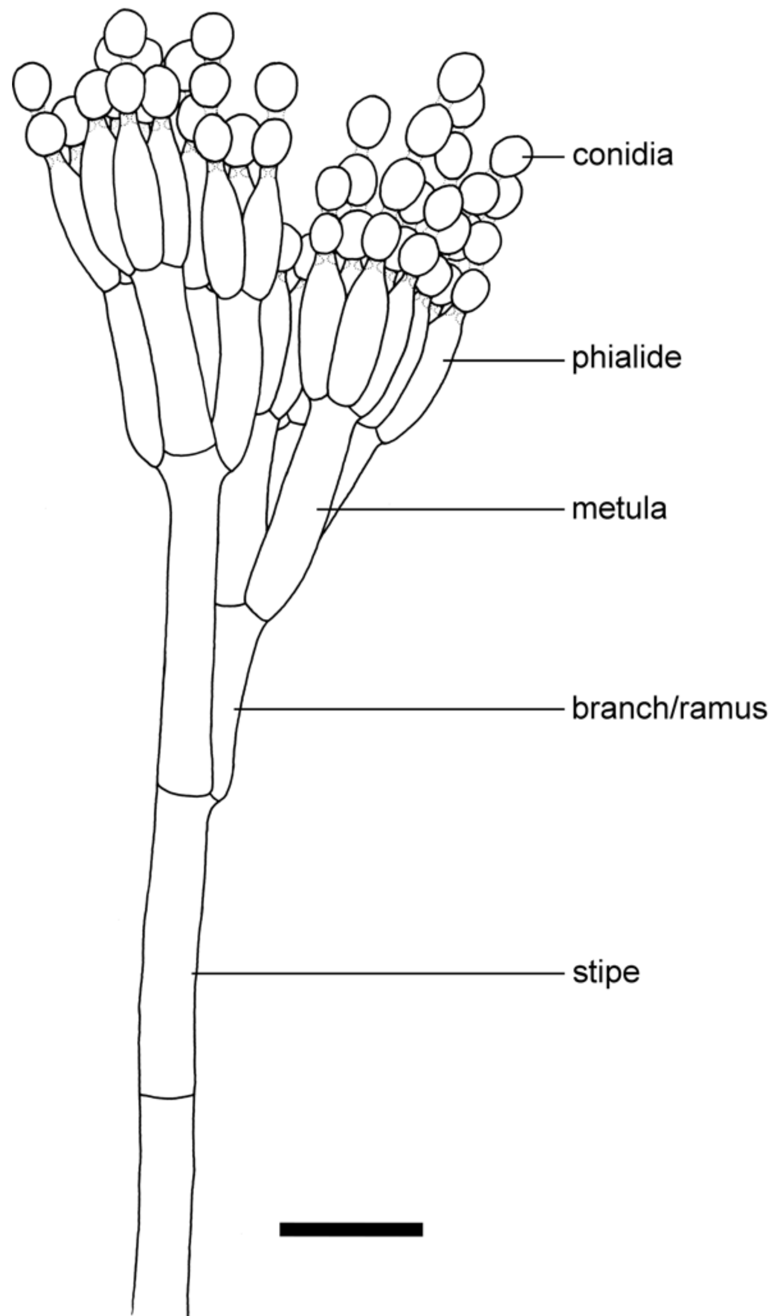

PLATE 1. Terminology used for describing the conidiophore of *Penicillium* (*P. expansum*, Scale bar = 10 µm).

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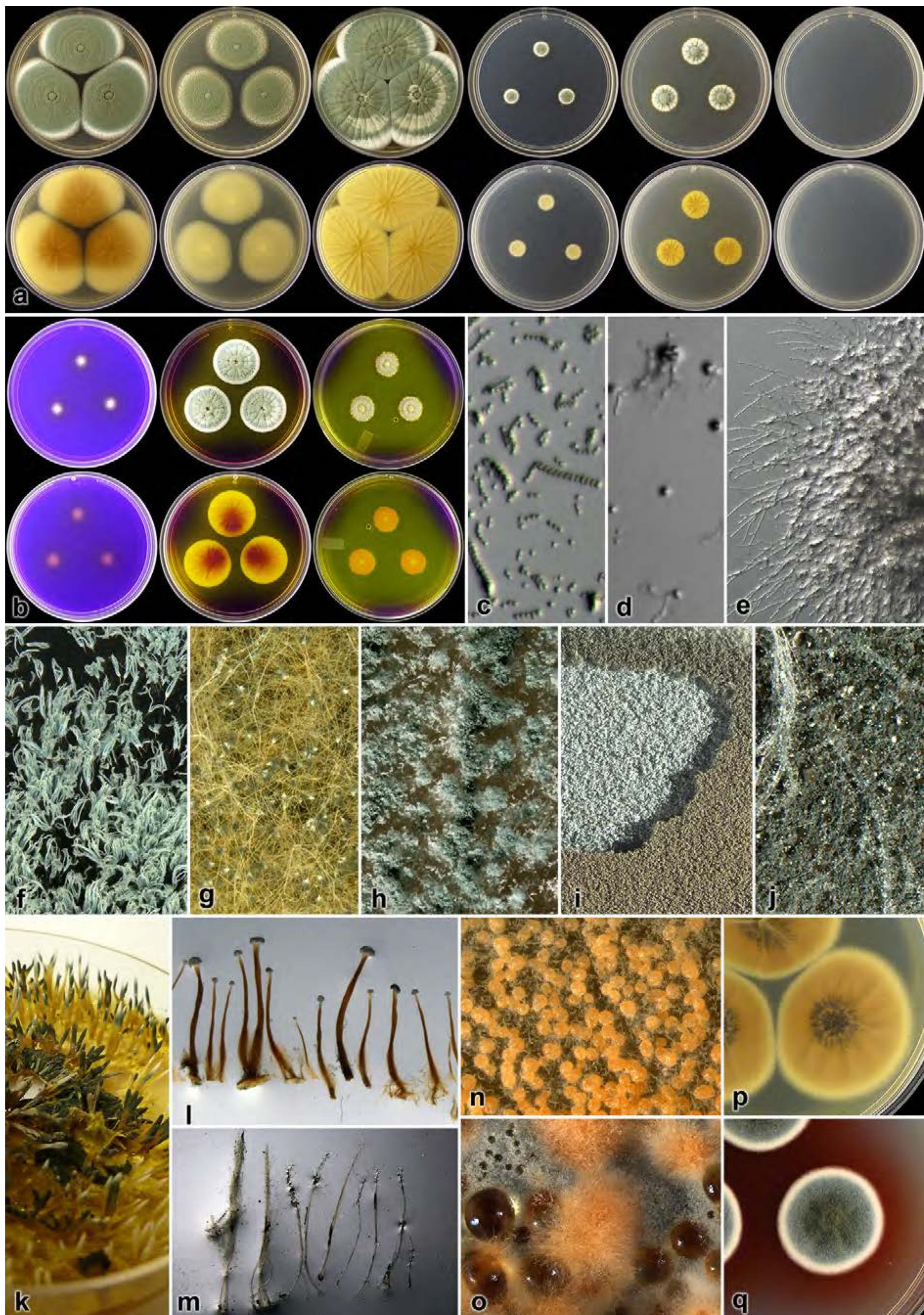

PLATE 2. Typical macromorphological characters. a. *Penicillium expansum* obverse and reverse colonies after 7 days incubation on, from left to right, CYA, MEA, YES, G25N, CYA (30 °C) & CYA (37 °C). b. Test for acid production on CREA, from left to right, no acid, moderate acid followed by base production and good acid production. c–e. CYA (5 °C): c. no germination, d. germination, e. microcolonies. f–m. Colony textures: f. velutinous (*P. rubens*), g. floccose (T. *pinophilus*), h. fasciculate (*P. expansum*), i. crusts (*P. oxalicum*), j. funiculose (*T. ramulosus*), k. synnema on CYA (*T. panamensis*), l. determinate synnema (*T. dendriticus*), m. indeterminate synnema (*T. duclauxii*). n. Sclerotia (*P. cumulacinatum* prov. nom.). o. Sclerotia and clear exudate (*P. sclerotiorum*). p. Sclerotia (*P. novae-zeelandiae*). q. Red soluble pigments (*T. atroroseus* prov. nom.).

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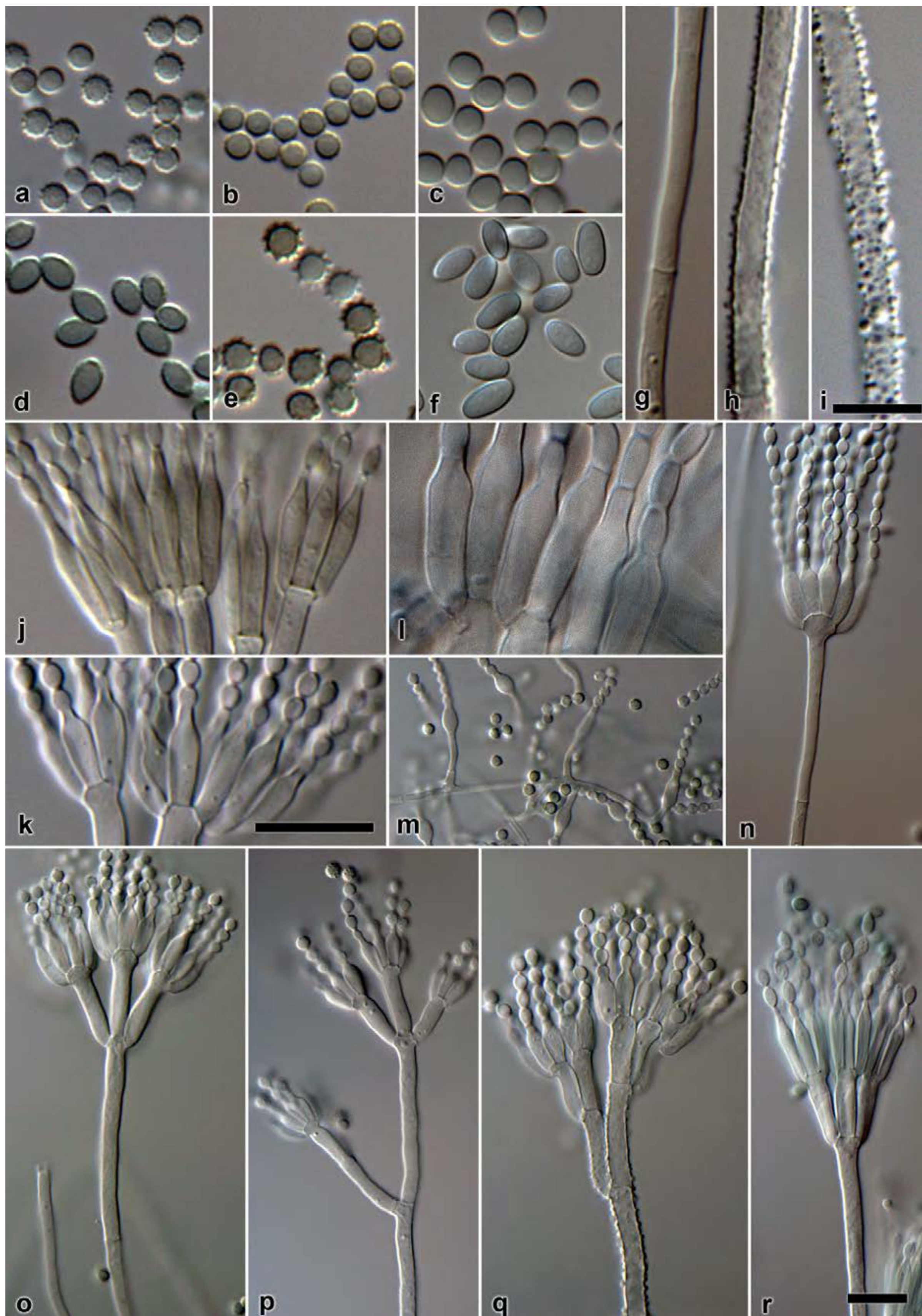

PLATE 3. Typical micromorphological characters. a–e. Variations on conidia: a. spheroid, rough (*P. fynbosense*), b. subspheroid, lightly rough (*P. sizovae*), c. broadly ellipsoid, smooth (*P. expansum*), d. ellipsoid, rough forming pattern (*T. ptychoconidium*), e. spheroid, echinulate (*T. solicola*), f. cylindrical, smooth (*P. digitatum*). g–i. Stipes: g. smooth (*P. sclerotiorum*), h. rough (*P. aurantiogriseum*), i. warted (*P. melinii*). j–l. Phialides: j. acerose (*T. chloroloma*), k. ampulliform (*P. sumatrense*), l. cylindrical (*P. digitatum*). m–r. Conidiophore branching patterns: m. monophialidic (*Torulomyces*), n. monoverticillate (*P. sclerotiorum*), o. biverticillate (subg. *Furcatum*) (*P. citrinum*), p. irregularly biverticillate (*P. canescens*), q. terverticillate (*P. aurantiogriseum*), r. biverticillate (*Talaromyces* = subg. *Biverticillium*) (*T. ptychoconidium*).

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Materials and Methods

Sampling and isolations

Three sites (PLATE 4a) representing different fynbos vegetation types were selected for this study. Samples were collected from Stellenbosch mountain (33°56’47”S 18°52’49”) representing Boland granite fynbos (PLATE 4b), Riverlands Nature Reserve near Malmesbury (33°29’46”S 18°35’16”) representing Atlantis sandveld fynbos (PLATE 4c) and Struisbaai (33°45’06”S 18°58’59”) representing Agulhas sand fynbos (PLATE 4d). At each site, three *Protea repens* plants were randomly selected. Infructescences (PLATE 4e), soil and air samples were collected at each plant. In total nine infructescences were collected from each plant, three each of the different age classes (1, 2 and 3 -year old). Soil samples were collected at the base of each plant and homogenized into one sample. Air samples were collected in triplicate around each plant.

For all isolations, potato dextrose agar (PDA) containing 100 ppm Streptomycin, 50 ppm Chloramphenicol and Dichloran (1 mL from 0.2% stock solution) were used. Plates were incubated for roughly 7 days after which fungal colonies were transferred onto oatmeal agar (OA). Single spore cultures were prepared on Czapek yeast autolysate agar (CYA) and cultures stored as water-plugs.

Isolations from Protea repens *infructescences and mites* — Infructescences were cut open and mites shaked out onto 1% agar plates (PLATE 4h). Mites were “sedated” by placing a tiny piece of cotton wool, dipped in chloroform, inside the petri dish. Mites were picked up with a needle and transferred to PDA. A subset of mites was crushed in eppendorf tubes and the solution plated out onto the same PDA plates. Plates were incubated on the lab bench (±21 °C) standing on water mite-traps.

Isolations from soil samples — A dilution series was prepared by adding 5 g of soil in 100 ml dH_2O and diluting that 10×, 100× and 1000× times. This was plated onto PDA and incubated at 25 °C.

Isolations from air samples — The MAS-Eco® air sampler was used for collecting 50 L samples at each *Protea repens* bush onto PDA. Plates were incubated at 25 °C.

Media and Incubation

Great care was taken when incubating cultures in order to standardize conditions (Okuda *et al*. 2000). Spore suspensions of strains were prepared in a semi-solid agar solution that contain 0.2% agar and 0.05% Tween80 (Pitt 1979). From here, strains were plated onto media (listed TABLE 1) in three-point inoculation style using a micropipette and each inoculum 0.5–1 µl. Media were prepared in 90 mm Petri dishes with a volume of 20 ml. Cultures were incubated for 7 days, in the dark with plates left unwrapped and not placed in boxes (Okuda *et al*. 2000). Each species were characterized using colony characters on CYA, MEA, YES, G25N and CREA grown at 25 °C, with additional CYA plates incubated at 5, 30 and 37 °C. CYA plates incubated at 30 and 37 °C were, however, wrapped with Parafilm to prevent plates from drying out. After incubation, strains were characterized using the methods of Pitt (1979) and Frisvad & Samson (2004). All color names and codes refer to the Methuen Handbook of Color (Kornerup & Wanscher 1967). For microscopy, slides were prepared from cultures grown on MEA, with 60% lactic acid used as mounting fluid. In species producing abundant conidia, 70% ethanol was used to wash away the excess spores. Microscopic examination was done using an Olympus BX50 light microscope and Olympus SZX12 stereomicroscope. Pictures were taken using an Evolution MP digital microscope camera and ImagePro 6.0 software. Conidiophore structures were measured with ImagePro 6.0 and Nikon NIS-elements D V4.0. In species descriptions, average microscope measurements and its standard deviation are provided between square brackets.

Photoplates were prepared using Adobe® Photoshop® CS5. The healing brush tool was used for cleaning up images. Colony textures were captured using extended depth of view and processed in Helicon Focus 4.2 software. Line drawings were done using *Camera Lucida* on a Nikon Eclipse E800 light microscope.

Table 1. Media used for morphological characterization

Czapek yeast autolysate (CYA) agar (Pitt 1979)		**Yeast extract sucrose (YES) agar (Frisvad 1981)**		**Creatine sucrose (CREA) agar (Frisvad 1981)**	
Czapek concentrate	10 ml	Yeast extract	20 g	Sucrose	30 g
Sucrose	30 g	Sucrose	150 g	Creatine·1H_2O	3 g
Yeast extract	5 g	$MgSO_4 \cdot 7H_2O$	0.5 g	$K_3PO_4 \cdot 7H_2O$	1.6 g
K_2HPO_4	1 g	$CuSO_4 \cdot 5H_2O$	0.005 g	$MgSO_4 \cdot 7H_2O$	0.5 g
$CuSO_4 \cdot 5H_2O$	0.005 g	$ZnSO_4 \cdot 7H_2O$	0.001 g	KCl	0.5 g
$ZnSO_4 \cdot 7H_2O$	0.01 g	Agar	15 g	$FeSO_4 \cdot 7H_2O$	0.01 g
Agar	15 g	H_2O	1000 ml	$CuSO_4 \cdot 5H_2O$	0.005 g
H_2O	1000 ml			$ZnSO_4 \cdot 7H_2O$	0.01 g
				Bromocresol purple	0.05 g
Malt extract (MEA) agar (Blakeslee 1915)		**25% glycerol nitrate (G25N) agar (Pitt 1973)**		Agar	15 g
Malt extract	20 g	K_2HPO_4	0.75 g	H_2O	1000 ml
Peptone	1 g	Czapek concentrate	7.5 ml	*Adjust pH to 8.0 (±0.2) after autoclaving	
Glucose	20 g	Yeast extract	3.7 g		
$CuSO_4 \cdot 5H_2O$	0.005 g	Glycerol	250 g	**Czapek stock solution (100 ml) (Pitt 1979)**	
$ZnSO_4 \cdot 7H_2O$	0.01 g	$CuSO_4 \cdot 5H_2O$	0.005 g	$NaNO_3$	30 g
Agar	15 g	$ZnSO_4 \cdot 7H_2O$	0.01 g	KCl	5 g
H_2O	1000 ml	Agar	12 g	$MgSO_4 \cdot 7H_2O$	5 g
		H_2O	750 ml	$FeSO_4 \cdot 7H_2O$	0.1 g

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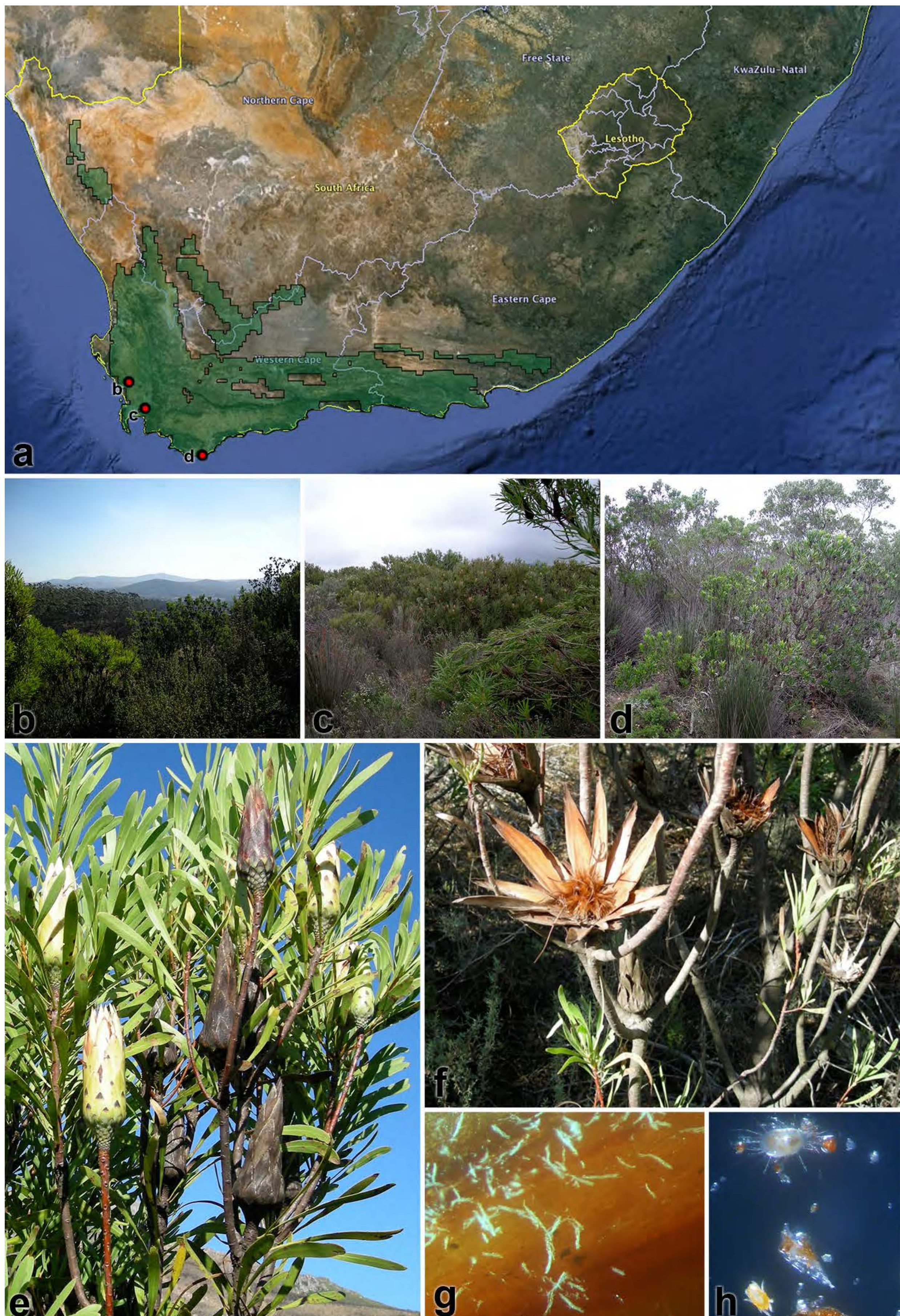

PLATE 4. Sampling done for this study. a. Three fynbos (indicated by green overlay) sites situated in the Western Cape. b. Stellenbosch mountain = Boland granite fynbos. c. Malmesbury, Riverlands Nature Reserve = Atlantis sandveld fynbos. d. Struisbaai = Agulhas sand fynbos. e. *Protea repens* bush containing inflorescences and woody infructescences. f. Open *Protea repens* infructescence after veld-fire. g. *Penicillium* conidiophores produced directly on *Protea repens* bracts. h. Mites typically found inside infructescences.

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Table 2. Primers used for amplification and sequencing

Locus	Primer name	Primer sequence (5'-3')	Reference
Internal Transcribed Spacer (ITS)	ITS1	TCC GTA GGT GAA CCT GCG G	White et al. 1990
	ITS4	TCC TCC GCT TAT TGA TAT GC	White et al. 1990
β-tubulin	Bt_2a	GGT AAC CAA ATC GGT GCT GCT TTC	Glass & Donaldson 1995
	Bt_2b	ACC CTC AGT GTA GTG ACC CTT GGC	Glass & Donaldson 1995
	T10	ACG ATA GGT TCA CCT CCA GAC	Glass & Donaldson 1995
	T11	AAT TGG TGC TGC TTT CTG GCA	Glass & Donaldson 1995
	T2	TAG TGA CCC TTG GCC CAG TTG	Glass & Donaldson 1995
	T224	GAG GGA ACG ACG GAG AAG GTG G	Glass & Donaldson 1995
Calmodulin	CMD5	CCG AGT ACA AGG ARG CCT TC	Hong et al. 2006
	CMD6	CCG ATR GAG GTC ATR ACG TGG	Hong et al. 2006
RNA polymerase II second largest subunit (RPB2)	5F	GAY GAY MGW GAT CAY TTY GG	Liu et al. 1999
	7CR	CCC ATR GCT TGY TTR CCC AT	Liu et al. 1999
	5F_Eur	GAY GAY CGK GAY CAY TTC GG	Houbraken et al. 2011a
	7CR_Eur	CCC ATR GCY TGY TTR CCC AT	Houbraken et al. 2011a
Elongation Factor 1α	EF6	CTT STY CCA RCC CTT GTA CCA	Peterson 2004
	EF1a	AAG ACA AGC AGC ACA TCA AC	Peterson 2004
	EF1b	CAC ATC AAC ATC GTC GTT AT	Peterson 2004
	EF1c	TCG TCG TTA TCG GCC ACG TC	Peterson 2004
	EF1d	GGC CAC GTC GAT TCC GG	Peterson 2004

<u>Phylogeny</u>

DNA extractions — Representative strains for each species were grown on MEA and DNA was extracted using the ZR Fungal/Bacterial DNA Kit (Zymo Research, California, USA).

PCR amplification — Subsequent amplification of target genes was set up using Kapa ReadyMix (Kapa Biosystems, Woburn, USA). Reactions had a final volume of 25 µl, which consisted out of 12.5 µl ReadyMix, 10.5 µl MiliQ H_2O, 1 µl DNA and 0.5 µl of both the forward and reverse primers (20 µM). Primers used for this study are summarized in TABLE 2. The thermal cycle for ITS, β-tubulin and Calmodulin amplifications had an initial denaturing step of 94 °C for 5 min, 36 cycles of 94 °C for 45 sec, 56 °C for 45 sec, 72 °C for 60 sec, followed by a final elongation step at 72 °C for 10 min. For some species, annealing temperatures was dropped down to 52 °C, to allow for amplification. The Elongation Factor-1α gene was amplified using the thermal cycle profile with an initial denaturing step of 96 °C for 2 min, 42 cycles of 96 °C for 30 sec, 51 °C for 30 sec, 72 °C for 90 sec, followed by a final elongation step at 72 °C for 5 min.

Sequencing — Sequencing reactions were set up using the 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 and 25 cycles at 94 °C for 10 sec, 50 °C for 10 sec, 60 °C for 4 min and sequence determined on an ABI PRISM 310 genetic analyzer.

Phylogenetic analysis — Sequence contigs were set up using CodonCode Aligner v4.0.1 (CodonCode Corporation). A sequence database was set up mainly using sequences published on GenBank for ex-type cultures. Additional sequences for identification were obtained from personal sequence databases of Dr. Keith Seifert from the Eastern Cereal and Oilseed Research Centre (ECORC) and Prof. Rob Samson, Jos Houbraken & Neriman Yılmaz from the Centraal Bureau voor Schimmelcultures (CBS). Strains used for sequence comparisons are summarized in TABLE 4 added at the end of this chapter. These sequences are not available on GenBank, although it will be deposited in future, and were only used for identification of Fynbos species. Alignments were done in MAFFT v6.850b (Katoh *et al*. 2009) using the L-INS-I option for ITS and the G-INS-I option for the other genes. To allow for reproduction of results by others, alignments were not adjusted manually. Nexus files were analyzed in PAUP* v4.0b10 (Swofford 2000) using the BioNJ option (Gascuel 1997), with confidence levels in nodes determined using a bootstrap analysis of 1000 replicates.

Results

<u>Isolations</u>

Isolations resulted in 2481 fungi isolated, 778 from the Stellenbosch mountain site, 811 from the Riverland Nature Reserve site and 892 from the Struisbaai site. Isolates included 2 *Alternaria*, 58 *Aspergillus*, 250 *Cladosporium*, 14 *Fusarium*, 1 *Paecilomyces*, 1671 *Penicillium*, 213 *Talaromyces*, 23 *Torulomyces* and 250 unidentified strains. In total 61 *Penicillium* and 15 *Talaromyces* species were isolated. The distribution and ecology of Fynbos *Penicillium* and *Talaromyces* species are treated in CHAPTER 4.

<u>The sections of *Penicillium*</u>

Amplification of the ITS region resulted in amplicons of ±600 bp. Fynbos *Penicillium* spp. were included into a dataset containing mostly ex-type sequences of previously described species. Species included in the phylogeny and their GenBank accession numbers are included in TABLE 4. A clade of *Talaromyces* species was used as outgroup. The aligned dataset was 558 bp long. The phylogeny (FIGURE 1a–j) resolved the Fynbos *Penicillium* spp. in 13 sections. Although there often were no

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bootstrap support for clades, morphological similarities of species was used for confirmation of clades. Sections represented in the Fynbos, include sections *Exilicaulis*, *Torulomyces*, *Lanata-Divaricata*, *Fasciculata*, *Chrysogena*, *Penicillium*, *Brevicompacta*, *Canescentia*, *Sclerotiora*, *Aspergilloides*, *Citrina*, *Charlesia* and *Ramigena*. Amplification of β-tubulin, Calmodulin, RPB2 and Elongation Factor 1α resulted in amplicons of ±500 bp, ±600 bp, ±900 bp and ±750 bp, respectively. These genes were used for more focused phylogenies relating to each section. Each section is treated in the following segments of the manuscript.

Section *Exilicaulis* — Five genes was used for phylogenetic analysis of section *Exilicaulis.* Aligned datasets for ITS (FIGURE 2, p. 42), RPB2 (FIGURE 2) and Elongation Factor 1-α (FIGURE 5, p. 44) were 517, 877 and 638 bp long, respectively. Section *Torulomyces* species was chosen as outgroups for the ITS and RPB2 phylogenies and *P. restrictum* used as outgroup for Elongation Factor 1-α. For β-tubulin and Calmodulin, the phylogenies were divided into the *P. corylophilum* (FIGURE 3, p. 43) and *P. restrictum* (FIGURE 4, p. 44) clades. For the *P. corylophilum* clade, *P. toxicarium* was used as outgroup and the aligned datasets was 454 and 410 bp long for β-tubulin and Calmodulin. For the *P. restrictum* clade, the *P. menonorum* clade was used as outgroup and the aligned datasets was 440 and 417 bp long for β-tubulin and Calmodulin

Section *Lanata-Divaricata* — Five genes were used for phylogenetic analysis of the section *Divaricata-Lanata*. Genes analyzed include ITS (FIGURE 6, p. 74), β-tubulin (FIGURE 6), Calmodulin (FIGURE 7, p. 75), Elongation Factor 1-α (FIGURE 7) and RPB2 (FIGURE 7). Aligned datasets were 519, 513, 587, 808 and 699 bp long, respectively. *Penicillium glabrum* were chosen as outgroup for the ITS and β-tubulin phylogenies, *P. oxalicum* for the Calmodulin and RPB2 phylogenies and *P. annulatum* as outgroup in Elongation Factor 1-α.

Section *Sclerotiora* — Four genes were used for the phylogenetic analysis (FIGURE 8, p. 96). These include ITS, RPB2, β-tubulin and Calmodulin, with aligned datasets that were respectively 514, 891, 484 and 478 bp long. *Penicillium levitum* was chosen as outgroup.

Section *Aspergilloides* — The ITS, RPB2, β-tubulin and Calmodulin genes were used in the phylogenetic analysis of section *Aspergilloides*. *Penicillium corylophilum* was chosen as outgroup for the ITS and RPB2 (FIGURE 9, p. 112, p. 113) phylogenies and was 487 and 851 bp long, respectively. For β-tubulin and Calmodulin, the phylogenies were divided into the *P. glabrum* (FIGURE 10) and *P. fuscum* (FIGURE 11, p. 114) clades. For the *P. glabrum* clade, the aligned datasets were 431 and 486 bp long. For the *P. fuscum* clade alignments were 430 and 471 bp long.

Subgenus *Penicillium* (including sections *Fasciculata*, *Chrysogena*, *Penicillium*, *Brevicompacta*) — Four genes were used for this clade. The aligned datasets for ITS (FIGURE 12, p. 144), RPB2 (FIGURE 13, p. 145), β-tubulin (FIGURE 12) and Calmodulin (FIGURE 13) were, respectively 499, 704, 380 and 401 bp long. *Penicillium canescens* and *P. jensenii* were chosen as outgroups for the phylogenies.

Section *Canescentia* — ITS (FIGURE 14, p. 170), β-tubulin (FIGURE 15, p. 171), Calmodulin (FIGURE 15), RPB2 (FIGURE 16, p. 172) and Elongation Factor 1-α (FIGURE 16) were used for phylogenetic analysis of section *Canescentia* species. The aligned datasets were 513, 409, 400, 686 and 689 bp long, respectively. *Penicillium brevicompactum* was used as outgroup in the ITS and RPB2 phylogeny, *P. swieckii* as outgroup for β-tubulin and Calmodulin, with *P. pseudoantarcticum* as outgroup for Elongation Factor 1-α.

Section *Torulomyces* — Four genes were used for phylogenetic analysis in this clade (FIGURE 17, p. 193). The aligned datasets for ITS, RPB2, β-tubulin and Calmodulin were 513, 686, 409 and 400 bp long, respectively. *Penicillium toxicarium* was used as outgroup.

Section *Citrina* — The ITS (FIGURE 18, p. 200), β-tubulin (FIGURE 19, p. 201) and Calmodulin (FIGURE 18) genes were used for phylogenetic analysis of section *Citrina*. The aligned datasets were 529, 475 and 650bp long, respectively. *Penicillium corylophilum* was chosen as outgroup.

Section *Ramigena* — Four genes were used for this clade (FIGURE 20, p. 231). The aligned datasets for ITS, RPB2, β-tubulin and Calmodulin was 524, 877, 456 and 532 bp long. *Penicillium charlesii* was used as outgroup.

Section *Charlesia* — Four genes were used for this clade (FIGURE 21, p. 237). The aligned datasets for ITS, RPB2, β-tubulin and Calmodulin was 542, 894, 474 and 565 bp long. *Penicillium ochrosalmoneum* was used as outgroup.

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Keys to *Penicillium* species occurring in the Fynbos biome

Table 3. Index to *Penicillium* species

		Description	Plates
Section *Exilicaulis*			
1.	*P. atrolazulinum*	p. 45	5 ,6, 19a, b
2.	*P. consobrinum*	p. 48	7, 19c
3.	*P. corylophilum*	p. 50	8, 19d
4.	*P. cravenianum*	p. 52	9, 19e
5.	*P. hemitrachum*	p. 54	10, 19f
6.	*P. melinii*	p. 56	11, 19g
7.	*P. pagulum*	p. 58	12, 19h
8.	*P. restrictum*	p. 60	13, 14, 19i-l
9	*P. rubefaciens*	p. 63	15, 16, 19m-p
10.	*P. toxicarium*	p. 66	17, 19q
11.	*P. xanthomelinii*	p. 68	18, 19r
Section *Lanata-Divaricata*			
12.	*P. annulatum*	p. 76	20, 21, 32a
13.	*P. brachycaulon*	p. 79	22, 23, 32b
14.	*P. cremeogriseum*	p. 82	24, 25, 32c
15.	*P. malacosphaerula*	p. 85	26, 27, 32d
16.	*P. oxalicum*	p. 88	28, 29, 32e
17.	*P. skrjabinii*	p. 91	30, 31, 32f
Section *Sclerotiora*			
18.	*P. bilaiae*	p. 97	33, 34, 41a
19.	*P. compactum*	p. 100	35, 36, 41b
20.	*P. hirayamae*	p. 103	37, 38, 41c
21.	*P. sclerotiorum*	p. 106	39, 40, 41d
Section *Aspergilloides*			
22.	*P. brunneoconidia*	p. 115	42, 55a
23.	*P. caseidecus*	p. 117	43, 55b
24.	*P. clavistipa*	p. 119	44, 55c
25.	*P. cumulacinatum*	p. 121	45, 55d
26.	*P. flavosclerotia*	p. 123	46, 55e
27.	*P. fuscum*	p. 125	47, 55f
28.	*P. glabrum*	p. 127	48, 55g
29.	*P. infra-aurantiacum*	p. 129	49, 55h
30.	*P. malmesburiensis*	p. 131	50, 55i
31.	*P. purpuroides*	p. 133	51, 55j
32.	*P. thomii morphogroup 1*	p. 135	52, 55k
33.	*P. thomii morphogroup 2*	p. 137	53, 55l
34.	*P. vagum*	p. 139	54, 55m
Subgenus *Penicillium*			
35.	*P. aurantiogriseum*	p. 146	56, 57, 70a
36.	*P. brevicompactum*	p. 149	58, 59, 70b
37.	*P. crustosum*	p. 152	60, 61, 70c
38.	*P. expansum*	p. 155	62, 63, 70d
39.	*P. griseofulvum*	p. 158	64, 65, 70e
40.	*P. melanoconidium*	p. 161	66, 67, 70f
41.	*P. rubens*	p. 164	68, 69, 70g
Section *Canescentia*			
42.	*P. fynbosense*	p. 173	71, 72, 83a
43.	*P. novae-zeelandiae*	p. 176	73, 74, 83b
44.	*P. pseudoantarcticum*	p. 179	75, 76, 83c
45.	*P. pseudoatrovenetum*	p. 182	77, 78, 83d
46.	*P. pseudocanescens*	p. 185	79, 80, 83e
47.	*P. radiatolobatum*	p. 188	81, 82, 83f
Section *Torulomyces*			
48.	*P. austricola*	p. 194	84, 86a
49.	*P. parviverrucosum*	p. 196	85, 86b
Section *Citrina*			
50.	*P. cairnsense*	p. 202	87, 88, 105a
51.	*P. citrinum*	p. 205	89, 90, 105b
52.	*P. pancosmium*	p. 208	91, 92, 105c
53.	*P. pasqualense*	p. 211	93, 94, 105d
54.	*P. sanguifluum*	p. 214	95, 96, 105e
55.	*P. sizovae*	p. 217	97, 98, 105f
56.	*P. sucrivorum*	p. 220	99, 100, 105g
57.	*P. sumatrense*	p. 223	101, 102, 105h
58.	*P. ubiquetum*	p. 226	103, 104, 105i
Section *Ramigena*			
59.	*P. cyaneum*	p. 232	106, 107, 108
Section *Charlesia*			
60.	*P. charlesii*	p. 238	109, 110, 113a
61.	*P. fellutanum*	p. 241	111, 112, 113b

Conidiophores monophialidic (section *Torulomyces*)

1. Brown soluble pigment and reverse pigmentation on all media .... ***P. austricola***
1. Brown colors missing; colonies on CYA at 37°C >5mm .... ***P. parviverrucosum***

Conidiophores monoverticillate

1. Stipes non-vesiculate .... 1.
1. Stipes vesiculate .... 10.
2. Colonies on CYA at 30°C <20 mm .... 3.
2. Colonies on CYA at 30°C >20 mm .... 7.
3. Conidia spheroid .... 4.
3. Conidia not spheroid .... 6.
4. Conidia heavy rough walled >3.5µm .... ***P. brunneoconidia***
4. Conidia lightly rough walled, <3.5µm .... 5.
5. Orange brown exudates produced, reverse red; yellow sclerotia absent .... ***P. sanguifluum***
5. Orange brown exudates absent, reverse yellow to pale; yellow sclerotia present .... ***P. flavosclerotia***
6. Conidia smooth; colonies on CYA <20mm .... ***P. cyaneum***
6. Conidia lightly rough; colonies on CYA >20mm .... ***P. fuscum***
7. Conidia broadly ellipsoid .... ***P. brachycaulon***
7. Conidia spheroid .... 8.

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8. Colonies on MEA <20mm; conidia <2μm .... ***P. toxicarium***
8. Colonies on MEA >20mm; conidia >2μm .... 9.
9. Colonies golden yellow to orange on CYA and MEA, abundant orange sclerotia/cleistothecia produced .... ***P. hirayamae***
9. Colonies not as above, white on CYA .... ***P. restrictum***
10. Stipes rough walled .... 11.
10. Stipes smooth, at most finely rough walled .... 13.
11. Colonies on CYA <20mm; conidia spheroid .... ***P. clavistipa***
11. Colonies on CYA >20mm; conidia ellipsoid .... 12.
12. Sclerotia produced pale; CYA at 30°C >20mm; strong acid produced on CREA .... ***P. thomii morphogroup2***
12. Sclerotia brownish orange; CYA at 30°C <20mm; acid not produced on CREA .... ***P. thomii morphogroup1***
13. Bright orange mycelia and sclerotia produced on CYA and MEA; conidia ellipsoid .... ***P. sclerotiorum***
13. Bright orange mycelia and sclerotia absent; conidia spheroid to subspheroid .... 14.
14. Stipes <30μm .... ***P. caseidecus***
14. Stipes >30μm .... 15.
15. Conidia heavy and spiny .... 16.
15. Conidia not heavy and spiny .... 17.
16. Colonies on MEA <20mm .... ***P. vagum***
16. Colonies on MEA >20mm .... ***P. purpuroides***
17. Colonies on YES <30mm .... ***P. flavosclerotia***
17. Colonies on YES >30mm .... 18.
18. Growth at 37°C .... 19.
18. No growth at 37°C .... 20.
19. Colonies on CYA <30mm, CYA at 30°C <30mm .... ***P. compactum***
19. Colonies on CYA >30mm, CYA at 30°C >30mm .... ***P. bilaiae***
20. Colonies at 30°C <15mm .... 21.
20. Colonies at 30°C >15mm .... 22.
21. Colonies on CYA at 25°C >30mm, at 30°C >5mm, orange reverse lacking; Conidia subspheroid .... ***P. malmesburiensis***
21. Colonies on CYA at 25°C <30mm, at 30°C <5mm, orange reverse color; Conidia spheroid .... ***P. infra-aurantiacum***
22. Colonies on CREA >20mm; Sclerotia not produced on CYA and MEA .... ***P. glabrum***
22. Colonies on CREA <20mm; Sclerotia produced on CYA and MEA .... ***P. cumulacinatum***

Conidiophores typically biverticillate

1. Biverticillate divaricate, without terminal verticil of metulae .... 2.
1. Biverticillate with terminal verticil of metulae .... 6.
2. Stipes <20μm; conidia broadly ellipsoid .... ***P. brachycaulon***
2. Stipes >20μm; conidia spheroid to subspheroid .... 3.

3. Colonies on CYA at 25°C <25mm, at 37°C no growth .... 4.
3. Colonies on CYA at 25°C >25mm, at 37°C >30mm .... 5.
4. Colonies on CYA 30°C >12mm; conidia a light green (26D4–26D6–26E6) .... ***P. fellutanum***
4. Colonies on CYA 30°C <12mm; conidia darker (25E7–25F7) .... ***P. charlesii***
5. Colonies on CYA and MEA <35mm; yellow cleistothecia/sclerotia produced on MEA .... ***P. malacosphaerula***
5. Colonies on CYA and MEA >35mm; yellow cleistothecia/sclerotia absent .... ***P. cremeogriseum***
6. Colonies on CYA <20mm .... ***P. pagulum***
6. Colonies on CYA >20mm .... 7.
7. Black sclerotia produced embedded in media on CYA, MEA and YES .... ***P. novae-zeelandiae***
7. Black sclerotia absent .... 8.
8. Conidia ellipsoid, 4-6μm in length .... ***P. oxalicum***
8. Conidia not ellipsoid, <4μm .... 9.
9. Colonies on CYA at 25°C >50mm, at 30°C >60mm .... ***P. hemitrachum***
9. Colonies on CYA at 25°C <50mm, at 30°C <60mm .... 10.
10. Colonies on MEA <25mm and CYA at 30°C <10mm .... ***P. ubiquetum***
10. Colonies on MEA >25mm or CYA at 30°C >10mm .... 11.
11. Colonies on CYA at 30°C >40mm .... 12.
11. Colonies on CYA at 30°C <40mm .... 13.
12. Colonies on CYA >45mm, MEA <40mm, CYA at 37°C >10mm; acid moderate on CREA .... ***P. annulatum***

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12. Colonies on CYA <45mm, MEA >40mm, CYA at 37°C <10mm; acid not produced on CREA ... ***P. skrjabinii***
13. Conidia smooth, at most finely rough walled ... 14.
13. Conidia rough walled ... 24.
14. Colonies on MEA <20mm ... 15.
14. Colonies on MEA >20mm ... 16.
15. Conidiophore stipes rough walled; acid not produced on CREA ... ***P. cravenianum***
15. Conidiophores stipes smooth walled, moderate acid produced on CREA ... ***P. citrinum***
16. Colony reverse pigmentation on CYA and MEA greyish to dark green ... ***P. atrolazulinum***
16. Colony reverse pigmentation not as above ... 17.
17. Colonies on CYA and MEA >40mm ... ***P. cairnsense***
17. Colonies on CYA or MEA <40mm ... 18.
18. Conidia smooth ... 19.
18. Conidia finely rough walled ... 22.
19. Colonies on MEA <30mm ... 20.
19. Colonies on MEA >30mm ... 21.
20. Colonies on CYA at 30°C >20mm and YES >45mm ... ***P. pancosmium***
20. Colonies on CYA at 30°C <20mm and YES <45mm ... ***P. sumatrense***
21. Colonies on CYA at 25°C <35mm, at 30C <20mm, CREA <20mm ... ***P. corylophilum***
21. Colonies on CYA at 25°C >35mm, at 30C >20mm, CREA >20mm ... ***P. pseudoantarcticum***
22. Colonies on CYA at 30°C <20mm ... ***P. sucrivorum***
22. Colonies on CYA at 30°C >20mm ... 23.
23. Stipes never rough walled; no growth at 37°C; Colonies on CREA >20 ... ***P. sizovae***
23. Stipes rough walled, sometimes smooth; growth at 37°C; Colonies on CREA <20mm ... ***P. fynbosense***
24. Sclerotia produced on CYA or MEA ... ***P. pasqualense***
24. Sclerotia absent ... 25.
25. Colonies on MEA >45mm ... ***P. consobrinum***
25. Colonies on MEA <45mm ... 26.
26. Colonies on MEA <25mm ... 27.
26. Colonies on MEA >25mm ... 28.
27. Yellow soluble pigment on CYA, MEA, YES; abundant yellow brown exudate on CYA; yellow reverse pigmentation on YES ... ***P. pseudoatrovenetum***
27. Soluble pigment absent; clear exudate when produced; dark brown reverse coloration on CYA and YES ... ***P. pseudocanescens***
28. Colony reverse pigmentation on CYA or YES greyish to dark green ... ***P. rubefaciens***
28. Colony reverse pigmentation not greyish to dark green ... 29.
29. Conidia spinose ... 30.
29. Conidia finely rough to rough walled ... 31.
30. Colonies on CYA <30mm, on MEA <39mm; CYA reverse pigmentation brown ... ***P. melinii***
30. Colonies on CYA >30mm, on MEA >39mm; CYA reverse pigmentation yellow ... ***P. xanthomelinii***
31. Colonies on CREA <20mm; soluble pigment absent; metulae vesiculate ... ***P. fynbosense***
31. Colonies on CREA >20mm; soluble pigment yellow; metulae not vesiculate ... ***P. radiatolobatum***

Conidiophores typically terverticillate with terminal ramus produced (subgenus *Penicillium*)

1. Colonies MEA <20mm; Microcolonies on CYA 30°C; Conidiophores broad and big, metulae swollen at apex ... ***P. brevicompactum***
1. Colonies MEA >20mm; Conidiophores not as above ... 2.
2. Conidia *en masse* typically grey; Phialide length <6.5µm ... ***P. griseofulvum***
2. Conidia *en masse* not grey; Phialide length >6.6µm ... 3.
3. Colonies CYA 30°C >35mm, YES >60mm ... ***P. chrysogenum***
3. Colonies CYA 30°C and YES < than above ... 4.
4. Colonies CYA >40mm, YES >50mm ... 5.
4. Colonies CYA <40mm, YES <50mm ... 6.
5. Stipe smooth walled; Colonies YES >60mm, G25N <20mm ... ***P. expansum***
5. Stipe rough walled; Colonies YES <60mm, G25N >20mm ... ***P. crustosum***
6. Colonies YES <30mm; Conidia greyish green to turquoise, length 3.3±0.19µm ... ***P. aurantiogriseum***
6. Colonies YES >30mm; Conidia dark green, length 3±0.13µm ... ***P. melanoconidium***

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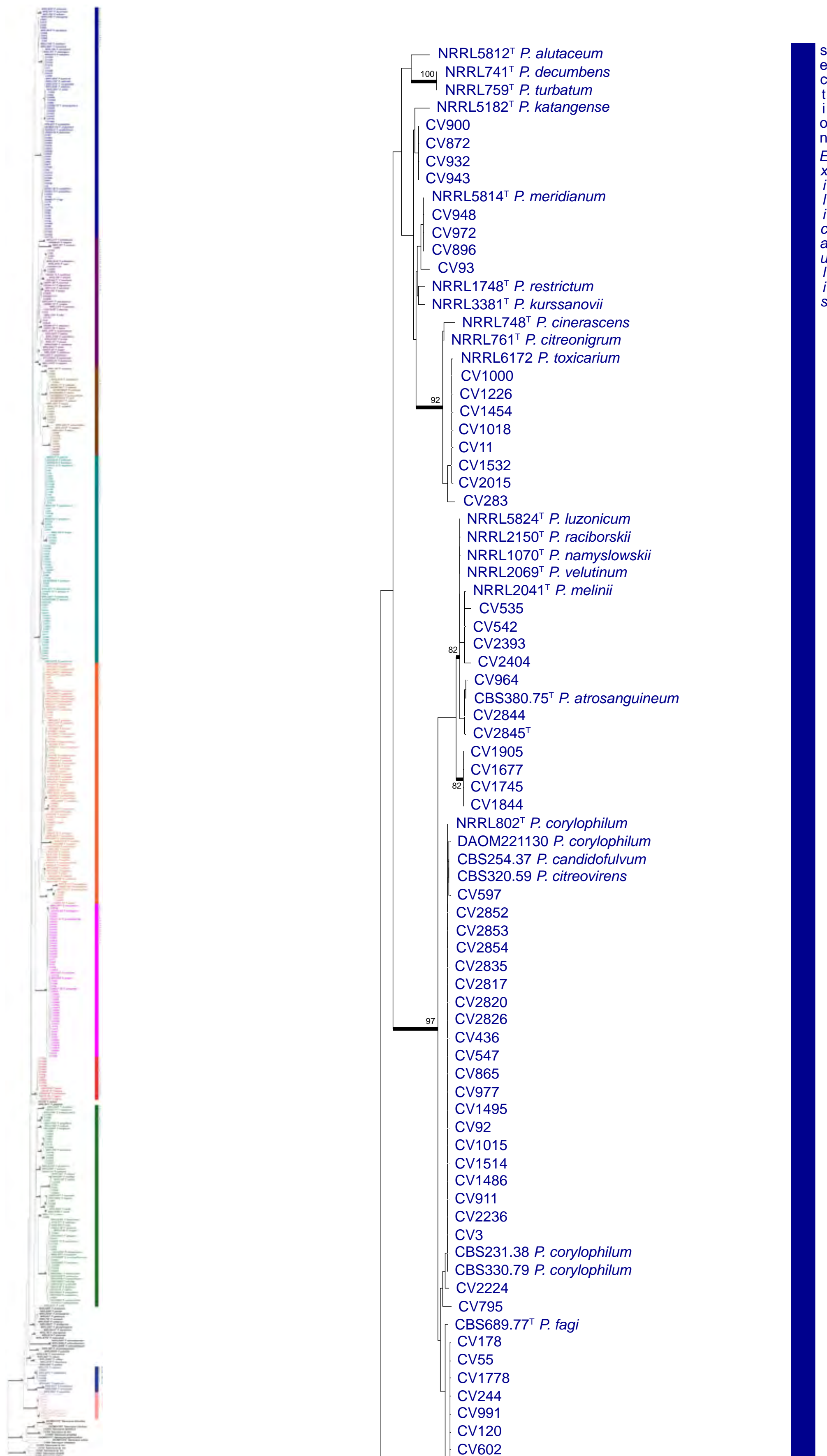

FIGURE 1a. Phylogenetic tree, based on the ITS barcodes, showing relationship between Fynbos *Penicillium* spp. and other members of the genus. The Fynbos species resolved in 10 major clades, which correspond with sections delimited by Houbraken & Samson (2011). Members of the genus *Talaromyces* were chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type strain; colors correspond to the sectional classification). The tree continues to FIGURE 1b.

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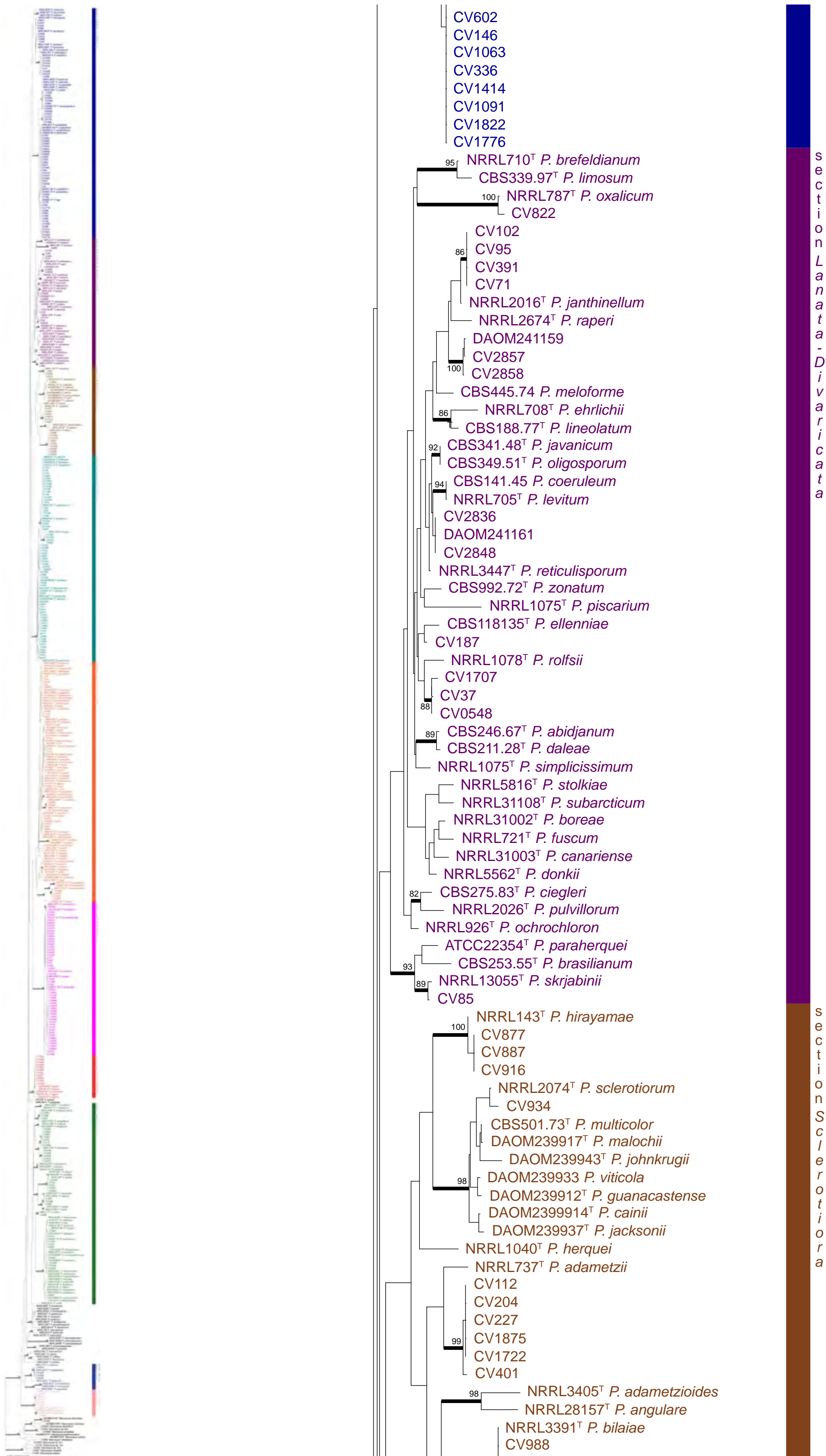

FIGURE 1b: Tree continues to FIGURE 1c.

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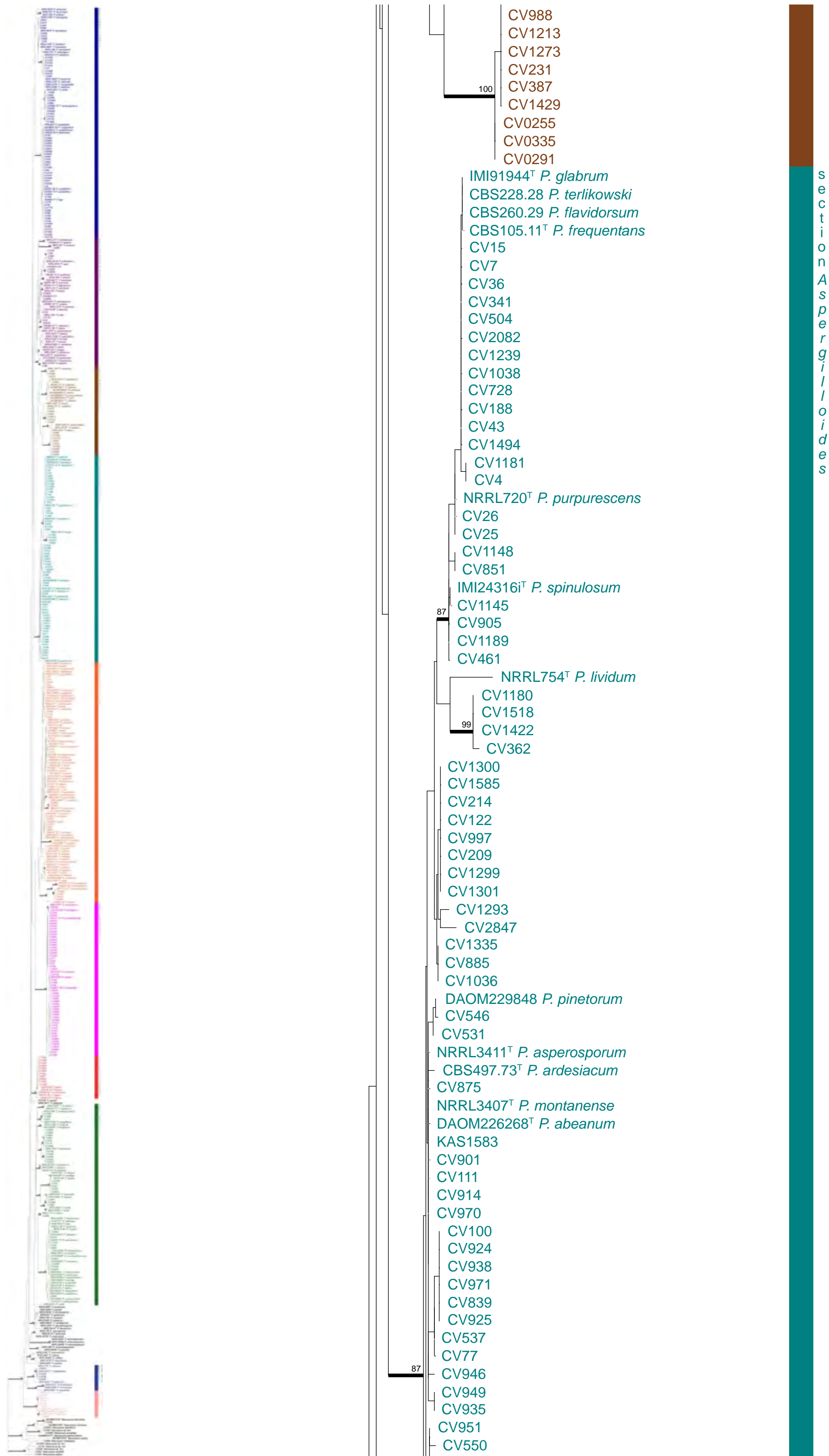

FIGURE 1c: Tree continues to FIGURE 1d.

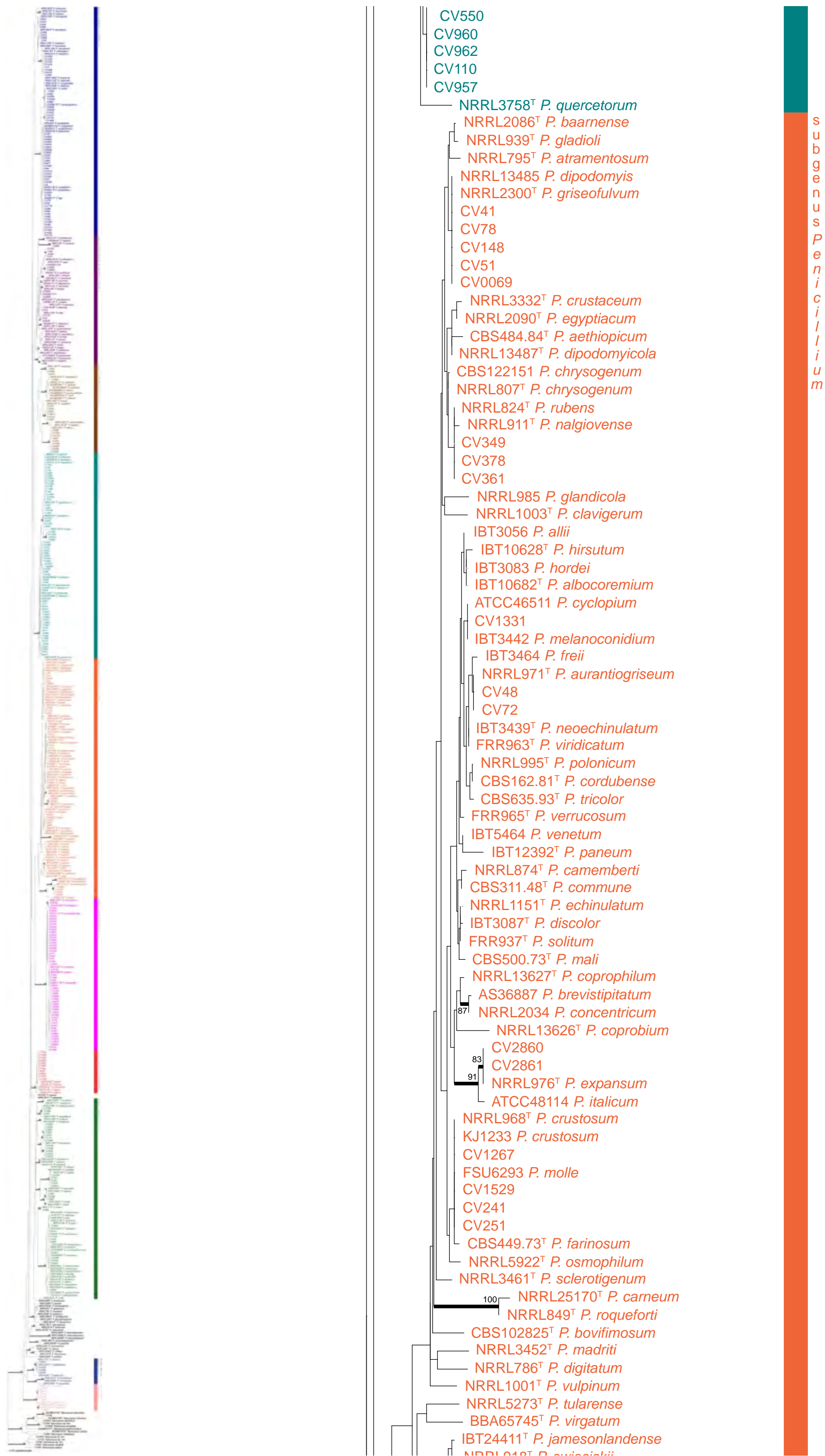

FIGURE 1d: Tree continues to FIGURE 1e.

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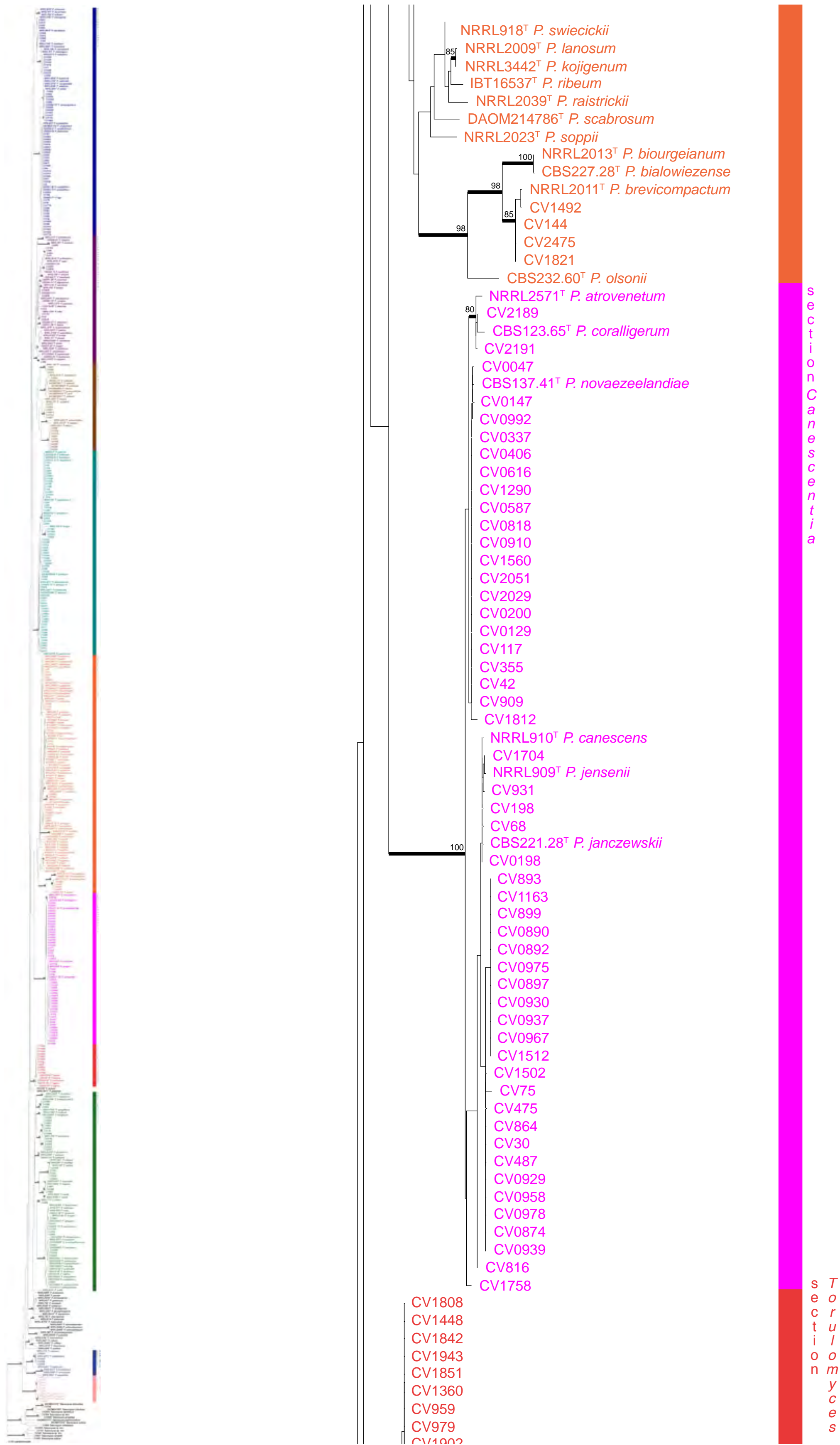

FIGURE 1e: Tree continues to FIGURE 1f.

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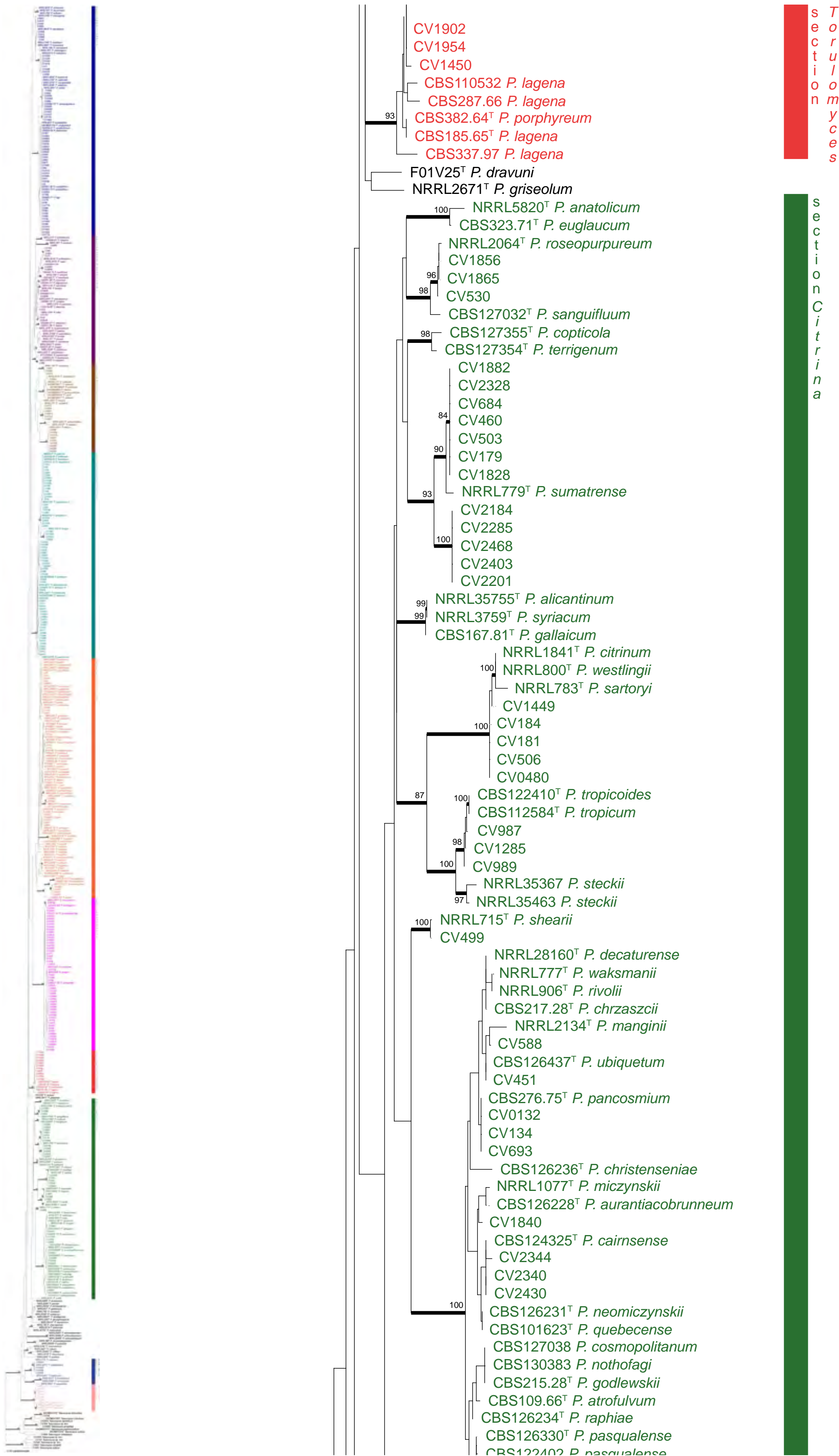

FIGURE 1f: Tree continues to FIGURE 1g.

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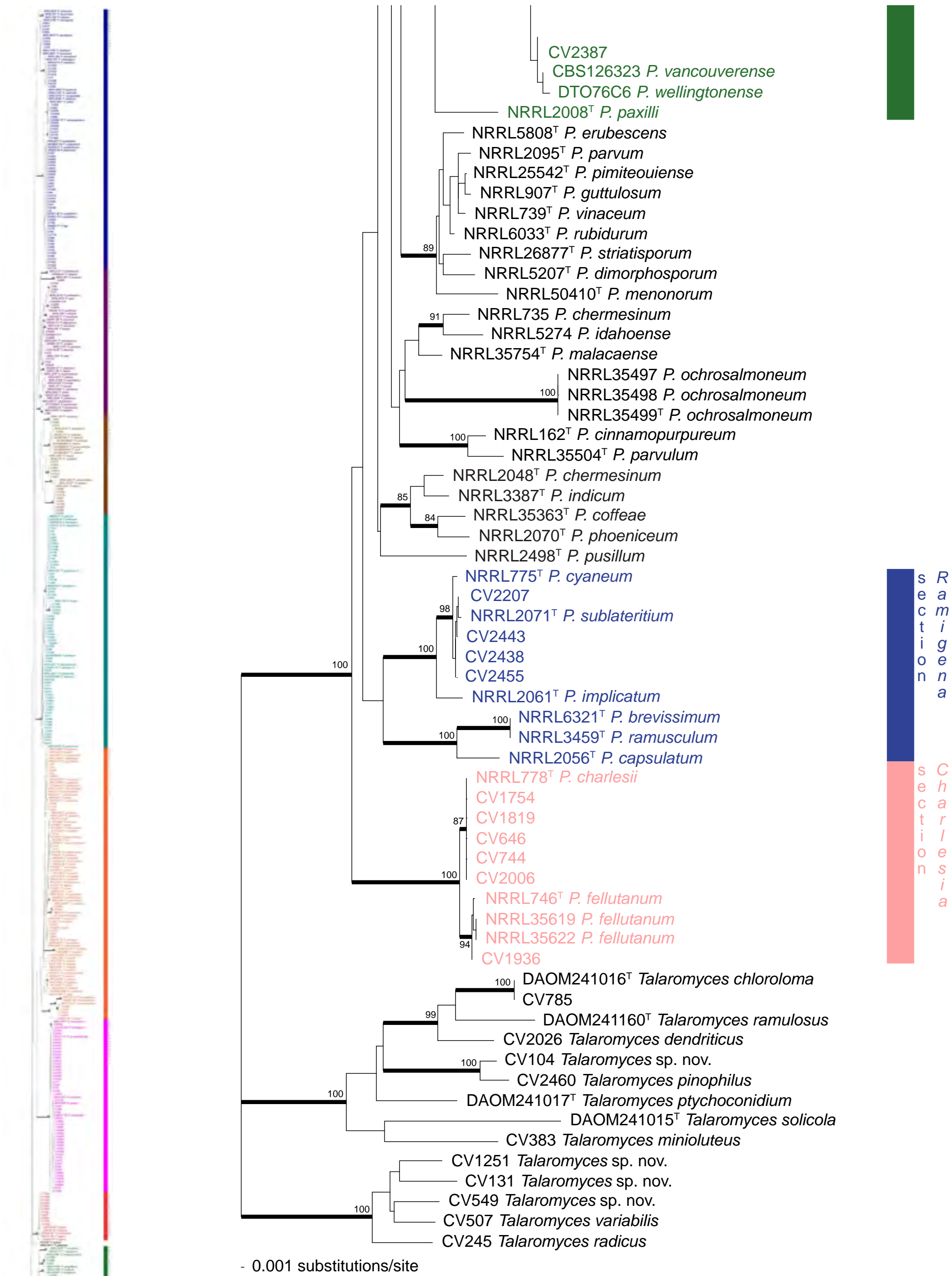

FIGURE 1g: Tree ends.

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Taxonomy

The section *Exilicaulis* Pitt

The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*: 205. 1979.

TAXONOMIC NOVELTIES: *Penicillium atrolazulinum* prov. nom., *P. consobrinum* prov. nom., *P. cravenianum* prov. nom., *P. hemitrachum* prov. nom., *P. pagulum* prov. nom., *P. xanthomelinii* prov. nom.

SPECIES TREATED: *Penicillium corylophilum, P. melinii*, *P. restrictum*, *P. rubefaciens*, *P. toxicarium*

Pitt (1979) introduced section *Exilicaulis* for monoverticillate species that do not produce terminal swellings. In the recent reclassification of *Penicillium* using multigene phylogenies, Houbraken & Samson (2011) added typically biverticillate species such as *P. melinii, P. corylophilum* and *P. raciborskii* into the section. The section *Exilicaulis* corresponded to clade 4 of the Peterson (2000) phylogeny. The biverticillate species resolved in a clade separate from the section's monoverticillate species, such as *P. restrictum*. The morphological connection between these two groups of species is difficult to detect. However, interestingly all seems to produce similar compact colonies on CYA. Also, for the biverticillate species, stipes were distinctly rough walled with only *P. corylophilum* as exception and species commonly produce green, blue or brown colony reverse pigmentations. The monoverticillate species form two major clades, one that contain *P. menonorum*, studied by Peterson *et al*. (2011), and the other *P. restrictum*. Species from both these clades commonly produce conidiophores with short stipes, often as short as 10 µm.

Isolations from air, soil and *Protea repens* infructescences resulted in the isolation of 11 section *Exilicaulis* species. They were identified as *P. corylophilum, P. melinii, P. restrictum, P. rubefaciens* and *P. toxicarium*. Six species could not be identified using morphology or gene sequences and are considered to be novel. These species were common in all samples collected from Fynbos, especially *P. atrolazulinum* and *P. toxicarium*. However, considerable variation was observed amongst strains studied, which created problems with species definition. As such, a bias was placed on the genealogical concordance between strains that aided in species delineation.

One of the most commonly isolated species in this study is the newly described *P. atrolazulinum*. Considerable morphological variation was observed in *P. atrolazulinum* colonies (PLATE 5). Conidiophores are typically borne from dark green-pigmented mycelia and sometimes itself also contain the green pigment, with stipes that is rough walled to almost warted. Phylogenetically, strains show very little sequence variation across the five genes studied. As such, it is accepted here as a distinct species.

Strains identified as *P. rubefaciens* displayed minor morphological variations amongst strains, as well as sequence variation. Although clades were consistent and possibly represent distinct species, the position of strain CV1015 in this complex is questionable. Also, bootstrap support for clades was not observed for the Calmodulin (FIGURE 3) or Elongation Factor 1-α (FIGURE 5) phylogenies. Strains CV597 and CV1558 are consistently resolved with the ex-type sequences for *P. rubefaciens* (CBS145.83). Morphologically, however, these strains differ from the original description by Quintanilla (1982), growing faster and not producing reddish colors in colonies. As such, an extensive review of this clade is needed in order to revise the circumscription of *P. rubefaciens* and determine the taxonomic position of the Fynbos strains that belong in this complex.

Similarly, an extensive review of *P. restrictum* and its close relatives is needed. *Penicillium restrictum* resolved in a clade together with *P. arabicum, P. kurssanovii, P. cineroatrum, P. heteromorphum, P. phillipense* (= *Eupenicillium phillipense*), *P. meridianum* (= *Eupenicillium meridianum*) and *P. katangense* (= *Eupenicillium katangense*). Conidiophores observed for the strains from the Fynbos showed similar morphological features to those described by Pitt (1979) for these species (PLATE 14). Colony morphology also varies between the different strains studied (PLATE 13). However, these morphological variations are not consistent and no concordance with phylogenetic data was observed. Conidial walls, for instance, in one strain varied from smooth walled to spiny. Phylogenetically, variation is also observed amongst strains (FIGURES 4, 5). However, between the different genes studied, the clades formed with Fynbos and ex-type strain sequences were not consistent. Also there was no bootstrap support for many of the clades observed (FIGURES 4, 5). This suggests that these species need to be synonymized with *P. restrictum*. Extensive reviews that include additional strains typical of the species within this complex are, therefore, necessary to confirm this.

The remaining species from section *Exilicaulis* species from Fynbos were not considered problematic. The species described as new in this section displayed unique morphological characters, which was confirmed with the multigene phylogenies.

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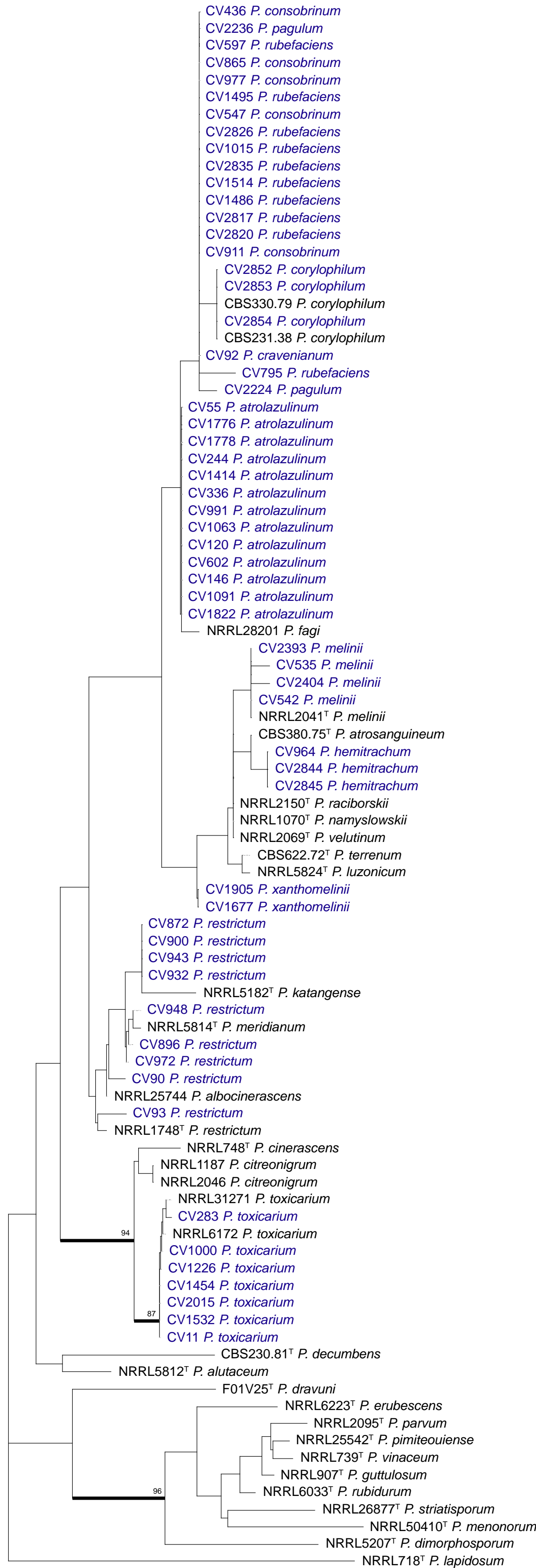

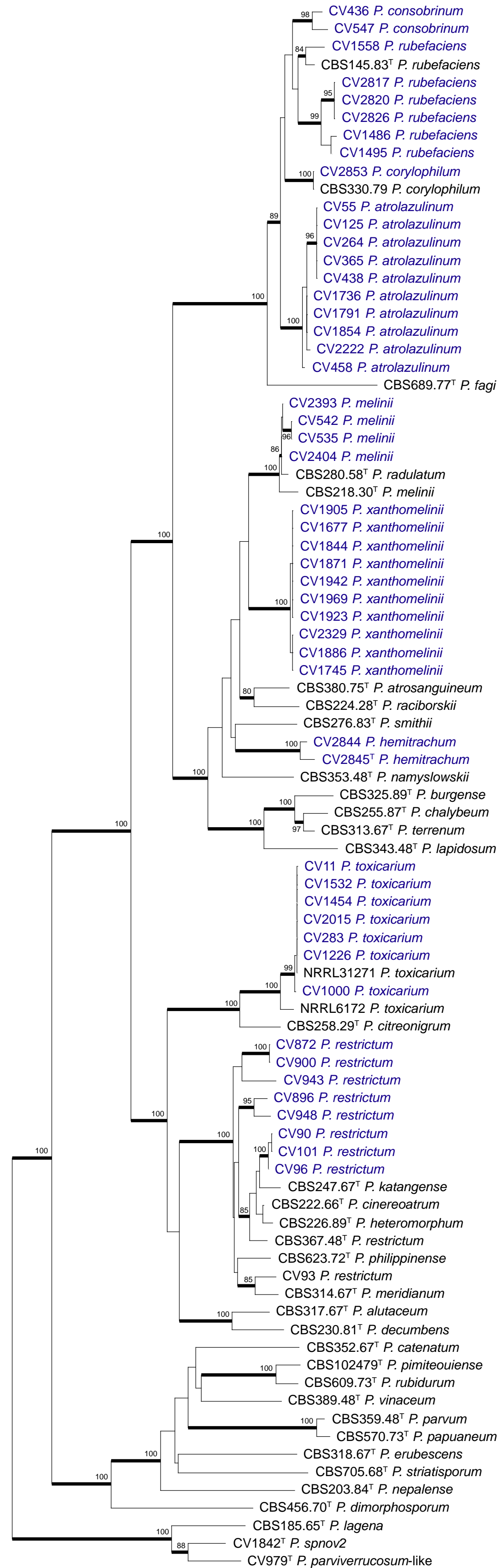

FIGURE 2: Phylogenetic trees based on ITS and RPB2, showing relationship of species in the section *Exilicaulis*. *Penicillium lapidosum* was chosen as outgroup in the ITS phylogeny and species from section *Torulomyces* as outgroup for RPB2. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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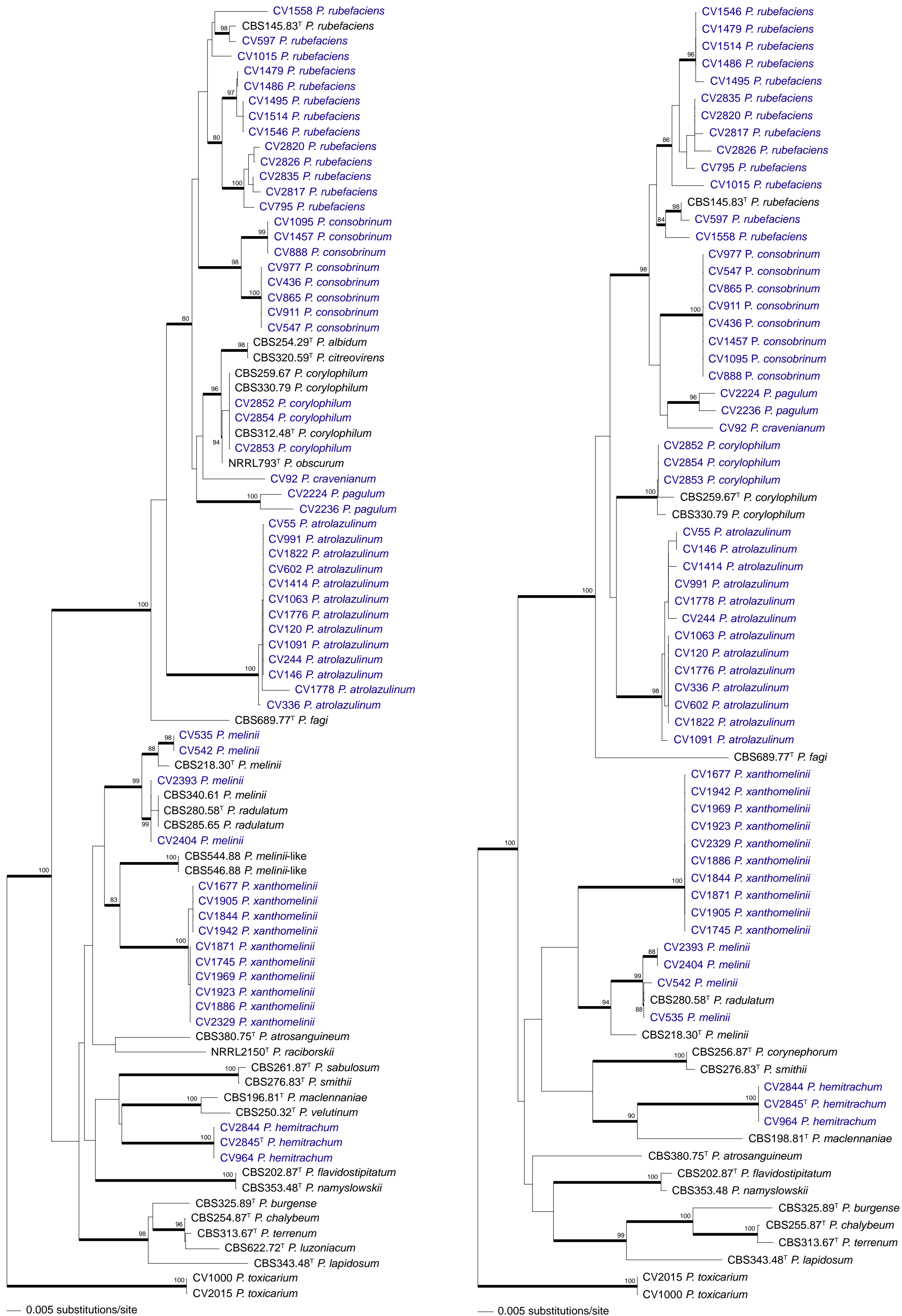

FIGURE 3: Phylogenetic trees based on β-tubulin and Calmodulin, showing relationship of biverticillate species in the section *Exilicaulis*. *Penicillium toxicarium* was chosen as outgroup in both phylogenies. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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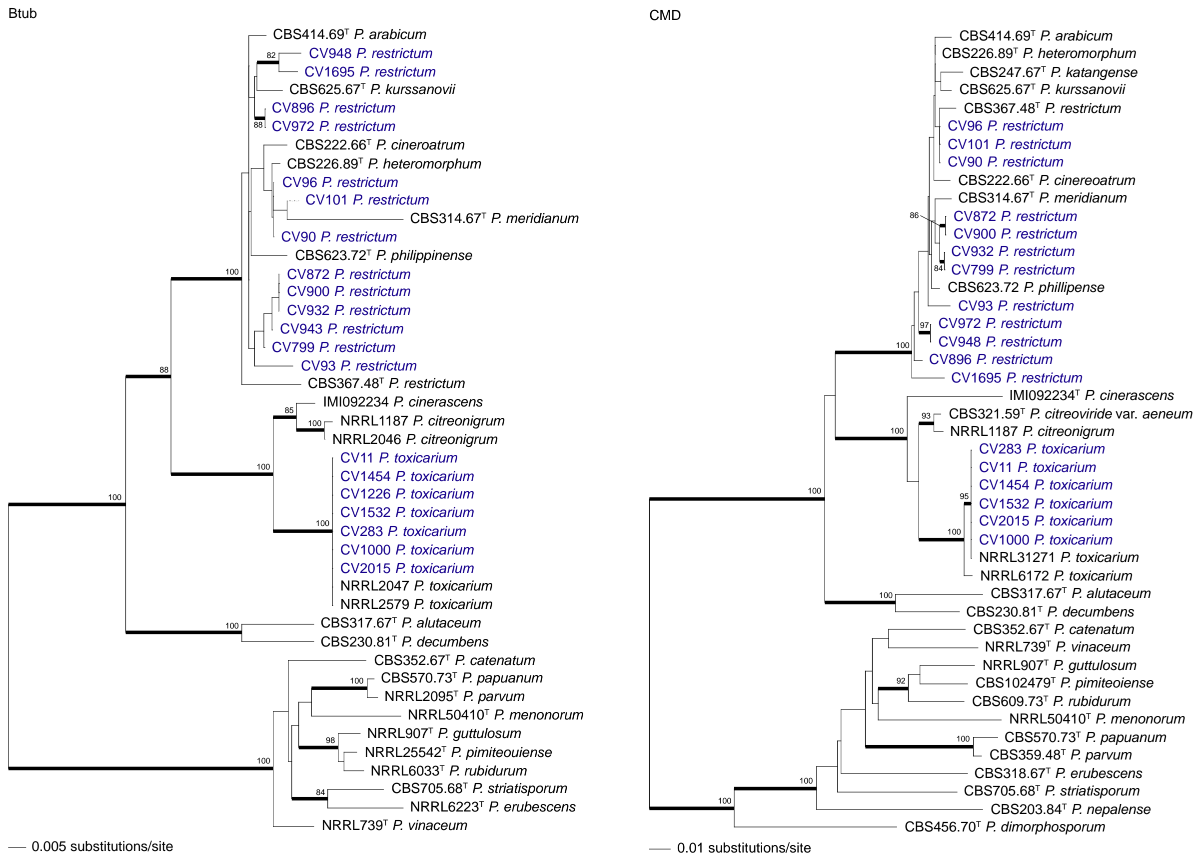

FIGURE 4: Phylogenetic trees based on β-tubulin and Calmodulin, showing relationship of monoverticillate species in the section *Exilicaulis*. *Penicillium menonorum* and its closely related species were chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

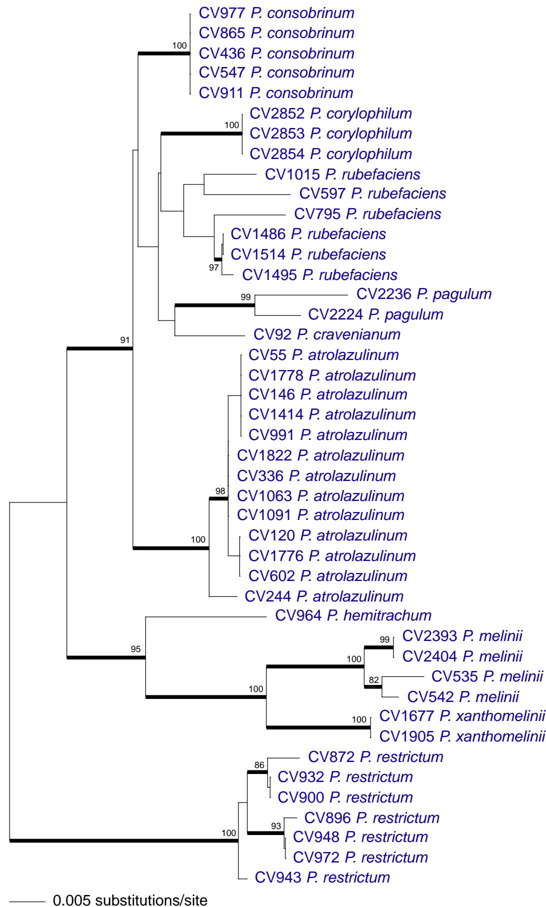

FIGURE 5: Phylogenetic tree based Elongation Factor 1-α, showing relationship of species in the section *Exilicaulis* isolated from Fynbos. The *P. restrictum* species complex was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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1. *Penicillium atrolazulinum* Visagie **prov. nom.**

PLATES 5, 6, 19e, 19a, b

ETYMOLOGY: Latin, *atrolazulinum* = meaning dark blue; named after the dark blue colony reverse pigmentation
EX-TYPE: CV55 = DTO180H4 = KAS4155 = DAOM241083
TYPE ISOLATED FROM: Air sample, Stellenbosch
ADDITIONAL SPECIMENS EXAMINED: CV1063, CV1091, CV120, CV125, CV1414, CV146, CV1736, CV1776, CV1778, CV1791, CV1822, CV1854, CV2222, CV244, CV264, CV336, CV365, CV438, CV458, CV602, CV991.
ISOLATED FROM: Air, soil, mites and bracts from *Protea repens* infructescences, Malmesbury, Stellenbosch, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 20–50 mm, low to moderately deep, plane to very lightly sulcate; margins low, narrow to wide (1–5 mm), entire to somewhat irregular; mycelia white; texture velutinous to floccose in some regions; sporulation moderately dense, conidia *en masse* dull to greyish green (24D425D6–24D625D6–25D6–25D4–25E4–26E4); exudate clear to almost a hazy yellow, sometimes absent, soluble pigment yellowish orange in some strains, absent in others, reverse pigmentation dark blue to dark turquoise (23F8–24F8), pale yellow (1A3–2A3) at margin, some strains dark green (27F6–27F7) at centre, pastel yellow (3A4) at margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colony characters similar to CYA at 25 °C, except diameter 12–27 mm.

CYA, 37 °C, 7d: Mostly no germination, sometimes colonies up to 5 mm, conidia *en masse* similar to CYA.

MEA, 25 °C, 7d: Colonies 30–50 mm, low, plant; margins low to almost subsurface, wide (4–5 mm), entire; mycelia white; texture velutinous, some floccose mycelia present; sporulation dense to moderately dense, conidia *en masse* dull green to greyish green (26E4–26E6) at centre, greyish green (25D6) near margin, some strains greyish turquoise (24E4–24E5) fading into (24C6) towards margin; exudate absent, soluble pigment absent, reverse pigmentation dark blue to dark turquoise (24F8–25F8), greyish green (29C3) near margin, some strains dark green (28F6–28F7) at centre, (30C5) near margin.

YES, 25 °C, 7d: Colonies 28–47 mm, low, to moderately deep, having a large number of random furrows and ridges; margins low, wide, regular, entire; mycelia white; texture velutinous, with funiculose mycelia present; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, some strains yellowish orange, reverse pigmentation dark blue to dark turquoise (24F8–25F8), pale yellow (1A3–2A3) at margin, some strains dark green (25F8) at centre greyish yellow to yellow (3B5–3B8) near margin.

G25N, 25 °C, 7d: Colonies 6–15 mm, moderately deep, plane to lightly sulcate; margins low, very narrow, entire; mycelia white; sporulation absent to moderately dense, conida *en masse* similar to CYA; soluble pigment absent, exudate absent, reverse pigmentation white to greyish green (1D3).

CREA, 25 °C, 7d: Colonies 18–25 mm, no acid produced.

Micromorphology — Conidiophores mostly biverticillate, terverticillate present, monoverticillate side branches sometimes present, typically borne from green pigmented mycelia, sometimes green pigmented; stipes very short to very long, typically rough walled, minor proportion smooth to finely rough walled, 40–400 × 2.5–4 µm; branches when present 2, 13–39.5 × 2.5–4 µm; metulae mostly 3–5, sometimes only 2 per stipe, divergent, 38–69° [52.8±9.2°], 11–23 × 2.5–4 [15.4±2.7 × 3.1±0.4] µm, vesicle 3.5–6 [4.7±0.5] µm; phialides ampulliform, 6–9, sometimes up to 16 per metula, 7–9.5 × 2.5–4 [8.3±0.6 × 3±0.29] µm, vesicle 3.3–6 [4.7±0.5] µm; conidia smooth walled, subspheroid, 2–3 × 2–2.5 [2.3±0.2 × 2.2±0.14] µm, average width/length = 0.95±0.05, n = 32.

Notes — *Penicillium atrolazulinum* conidiophores are characteristically rough walled, and are often borne from greenish blue-pigmented mycelia. Strains display variation in color and diameter especially on CYA at 25 °C. A large number of strains produce yellowish orange soluble pigments, which masks the characteristic dark turquoise reverse pigmentation. Based on all the genes analyzed, strains have very little variation in their sequences. The reason for variation is not clear. Its closest relative is *P. fagi*, which also produce the dark blue to turquoise reverse pigmentations (Ramirez 1982). This species is known only from its type strain. Based on the original description (Matinez & Ramirez 1978), both species have very similar conidiophores. *Penicillium atrolazulinum*, however, does to some degree produce longer stipes and metulae. The morphological variation seen in colonies for *P. atrolazulinum* makes colony characters difficult to use for comparing these two species. Phylogenetically, *P. fagi* resolves in a clade distinct from *P. atrolazulinum* (FIGURES 2, 3).

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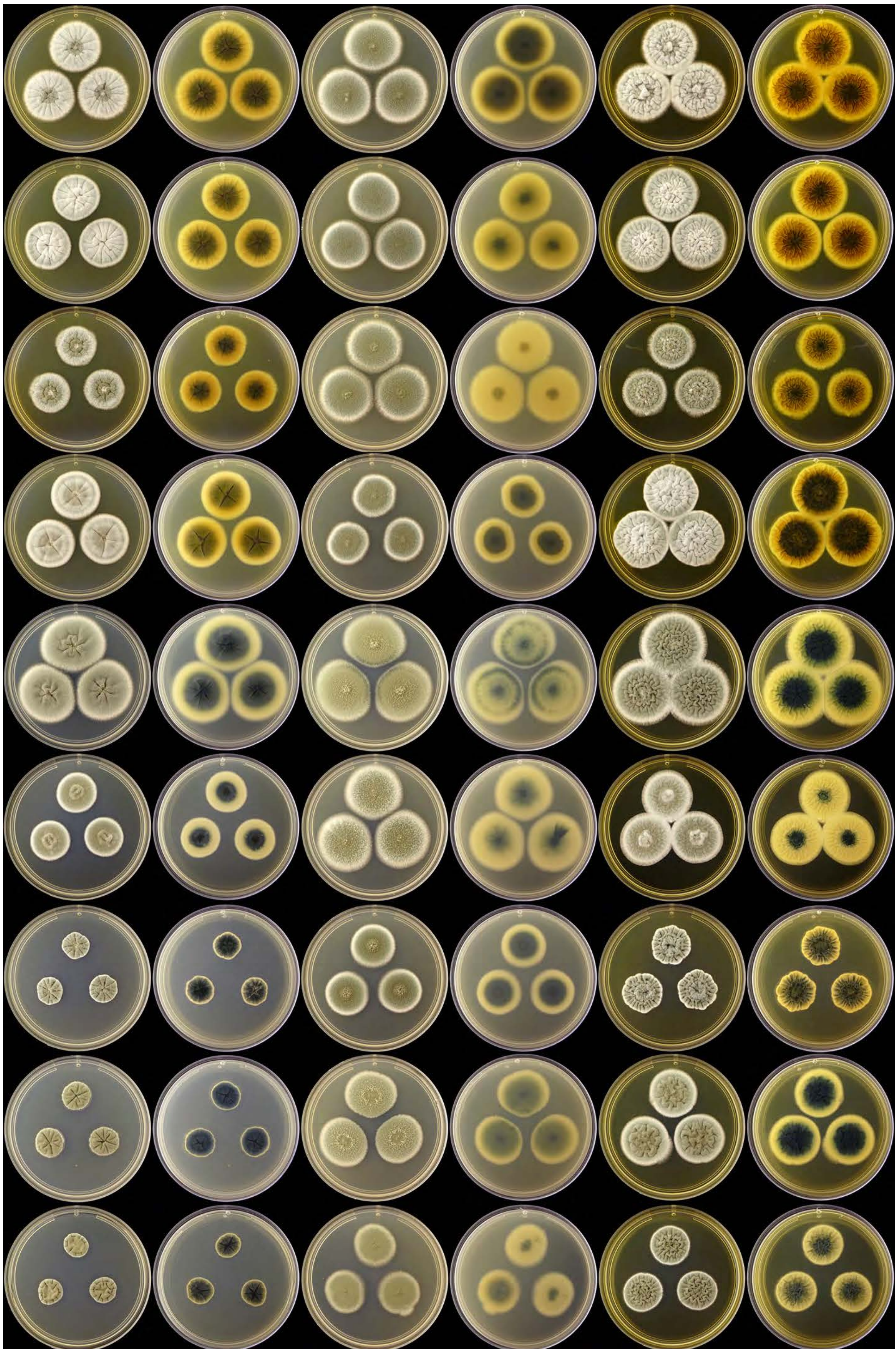

PLATE 5. *Penicillium atrolazulinum* strains that show variation in colony morphology. Colonies from left to right on CYA (obv.), CYA (rev.), MEA (obv.), MEA (rev.), YES (obv.), YES (rev.). Rows from top to bottom CV1091, CV1822, CV120, CV336, CV55, CV244, CV991, CV1778, CV146.

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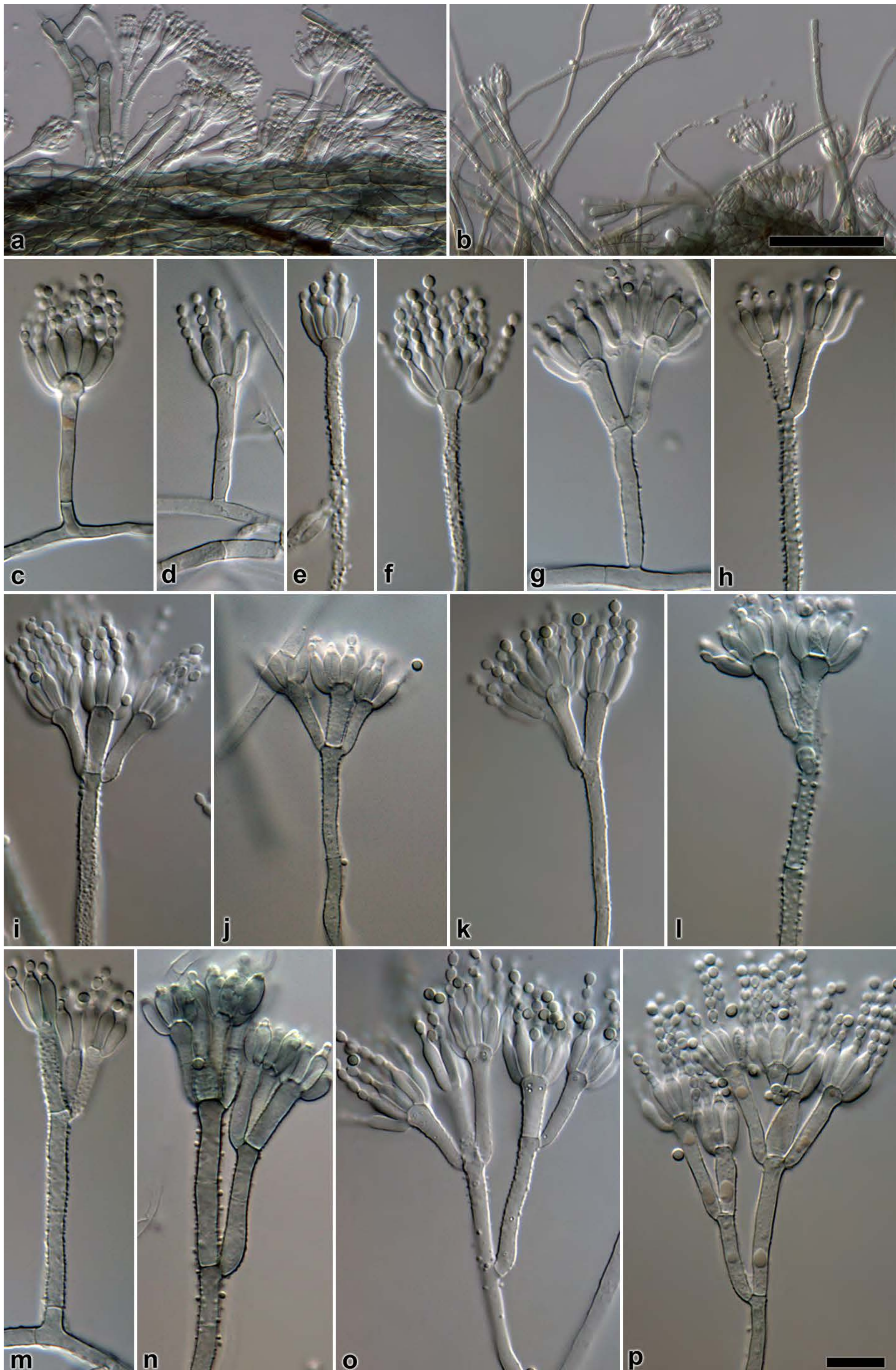

PLATE 6. *Penicillium atrolazulinum* microscopic characters. a, d, g, j, o. CV1778. b, c, i, m, p. CV120. e, k, n. CV55. f, h, l. CV244. (— Scale bar in b = 50µm, applies to a, b; — Scale bar in p = 10µm, applies to c–p).

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2. *Penicillium consobrinum* Visagie **prov. nom.**

PLATES 7, 19c

ETYMOLOGY: Latin, *consobrinum* = meaning the cousin
EX-TYPE: CV547 = DTO181H9 = KAS4152 = DAOM241072
TYPE ISOLATED FROM: Soil, Stellenbosch
ADDITIONAL SPECIMENS EXAMINED: CV436, CV1095, CV1457, CV865, CV888, CV911, CV977.
ISOLATED FROM: Soil, Air, Mites and Bracts from *Protea repens* infructescens, Malmesbury, Stellenbosch, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 29–34 mm, low to moderately deep, lightly radially sulcate, often having sterile areas at centre, giving colony a greyish green colour, having an greyish green colour at centre; margins low, very narrow (1 mm), entire; mycelia white; texture velutinous, floccose at colony centre; sporulation moderately dense in some regions, conidia *en masse* greyish green (25E6–25E7) and greyish turquoise (24B3–24B4); exudate mostly absent, sometimes clear exudate present, soluble pigment absent, reverse pigmentation brown (5F7–6F7) at centre, becoming pale yellow (4A3) near margin.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 25–32 mm, low, lightly radially sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous, floccose elsewhere; sporulation sparse to moderately dense areas, conidia *en masse* dull to dark green (26E4–26F4) in dense areas, greyish green (25C4); exudate clear, soluble pigment absent, reverse pigmentation dark green (29F6) at centre, greyish green (29C3–30C3) near greenish white (30A2) margin.

CYA, 37 °C, 7d: No germination to sometimes germination.

MEA, 25 °C, 7d: Colonies 48–52 mm, low, plane; margins low to subsurface, wide (4 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish green (25E6–25E7); exudate absent, soluble pigment absent, reverse pigmentation (2B5) at centre, fading into greyish green to greyish yellow (30B3–30C3–30C4).

YES, 25 °C, 7d: Colonies 40–45 mm, low to moderately deep, radially and lightly concentrically sulcate, random furrows present; margins low, narrow (1 mm), entire; mycelia white; texture velutinous, floccose mycelia present; sporulation moderately dense to dense in regions, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation dark green (25F8) at centre, fading into greyish green (28C4) to greyish yellow (1B4) near margin.

G25N, 25 °C, 7d: Colonies 11–15 mm, raised at centre, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25E6) to dull to greyish green (26E4–26E6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B4) at centre, light green (27A4–27A5) elsewhere.

CREA, 25 °C, 7d: Colonies 17–20 mm, no acid produced.

Micromorphology — Conidiophores biverticillate with minor proportion terverticillate and having subterminal branches; stipes rough walled, 75–400 × 2.5–3.5 µm; branches when present 2, divergent, 16–70 × 2.5–3.5 µm; metulae 2–5, divergent, sometimes slightly appressed, 25–76° [46±10.4°], 12–25 × 2.5–3.5 [17±2.3 × 2.9±0.3] µm, vesicle 3.5–5.5 [4.5±0.5] µm; phialides ampulliform, 12–16 per metula, 6.5–10 × 2–3.5 [8.3±0.6 × 2.8±0.27] µm; conidia rough walled, spheroid, 2–3 × 2–3 [2.3±0.2 × 2.3±0.2] µm, average width/length = 0.97±0.02, n = 79.

Notes — *Penicillium consobrinum* typically produces conidiophores that is rough walled and colonies that produce dark green reverse pigmentation on MEA and YES, but brownish on CYA. Colony characters resemble those of *P. rubefaciens* species complex and *P. corylophilum*. *Penicillium corylophilum*, however, produce conidiophores that is smooth walled. *Penicillium consobrinum* typically grows faster than strains from the *P. rubefaciens* species complex on most media, especially on YES. Colonies on CYA is also more compact. All of the genes resolved strains of *P. consobrinum* separate from all other species in section *Exilicaulis* (FIGURES 2, 3, 5).

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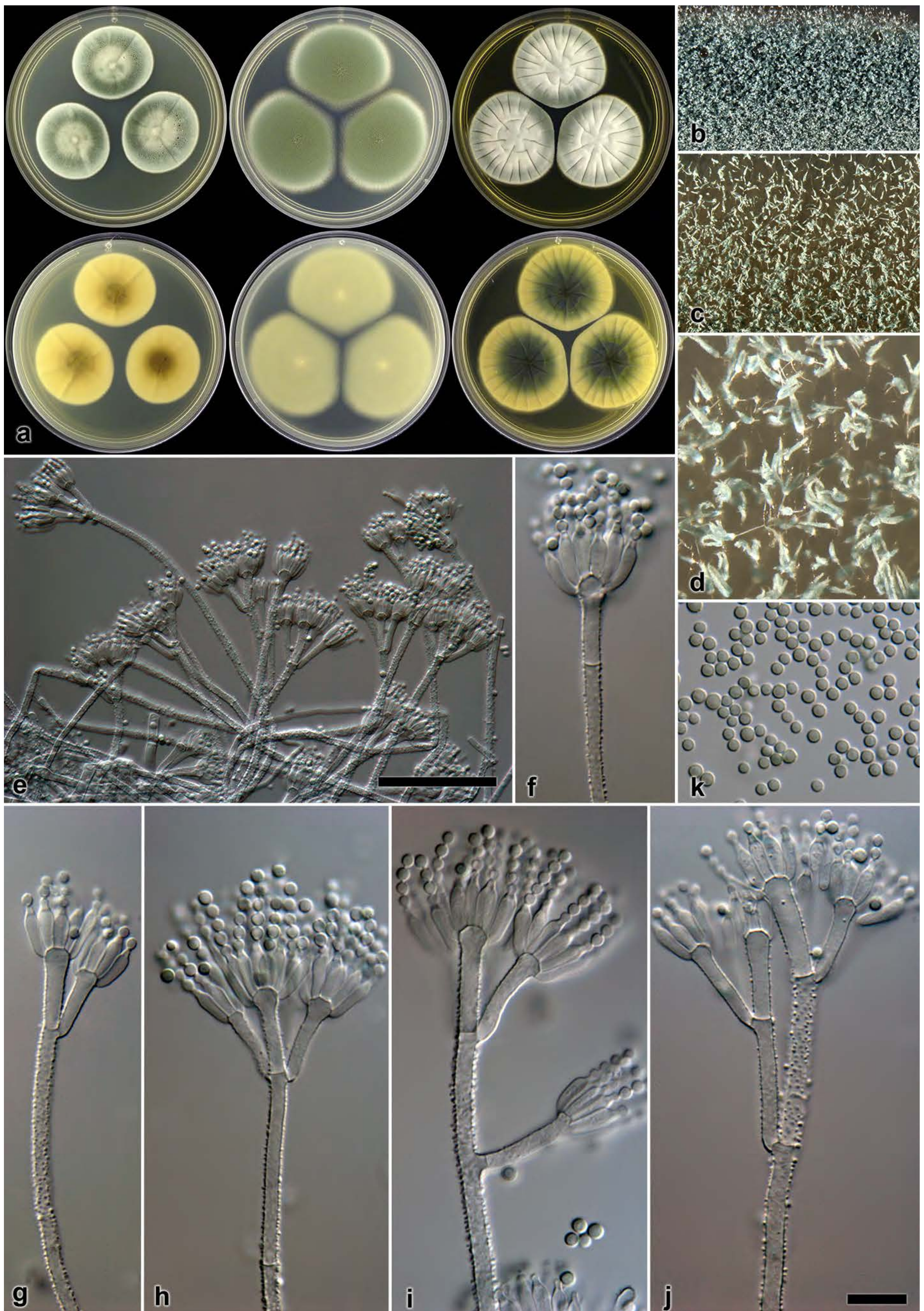

PLATE 7. *Penicillium consoborinum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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3. ***Penicillium corylophilum*** Dierckx

PLATES 8, 19d

Annales de la societe scientifique de bruxelles 25: 86. 1901.

REPRESENTATIVE STRAINS: CBS312.48 = NRRL802 = IMI39754
TYPE ISOLATED FROM: Unrecorded source
ADDITIONAL SPECIMENS EXAMINED: NRRL802, CV2852, CV2853, CV2854.
ISOLATED FROM: Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 32-35 mm, low, lightly radially sulcate, having a brownish colour at colony centre; margins low, narrow (1 mm); mycelia white; texture velutinous; sporulation sparse, moderately dense only near margin, conidia *en masse* greyish to dark green (25D5–25F5); exudate clear, soluble pigment absent, reverse pigmentation dark green (5F4) at centre, sometimes yellowish brown (5E5) at centre, pale yellow (4A3) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 15–20 mm, raised at centre, radially and concentrically sulcate, having brownish color at centre; margins low, narrow (< 1 mm), entire; mycelia white; texture velutinous; sporulation sparse, conidia *en masse* greyish green (25C4); exudate absent, soluble pigment absent, reverse pigmentation olive brown (4E7) at centre, pale at margin.

CYA, 37 °C, 7d: Colonies 4–8 mm, consisting of white mycelial mass.

MEA, 25 °C, 7d: Colonies 35-40 mm, low, plane; margins low to subsurface, narrow (2 mm), entire; mycelia white; texture velutinous, some floccose mycelia present near centre; sporulation dense, conidia *en masse* dull green to greyish green (26E4-26E7); exudate absent, soluble pigment absent, reverse pigmentation dark green (25F8) at centre, fading into greyish green (27C3).

YES, 25 °C, 7d: Colonies 45-47 mm, low to moderately deep, radially sulcate, slightly raised towards centre where grooves are rather random; margins low, narrow (1-2 mm), entire; mycelia white; texture velutinous, some floccose mycelia present; sporulation sparse to moderately dense in colony areas facing each other, conidia *en masse* greyish green (25B3-26B3), darker greyish green (26E6) in colony areas facing each other; exudate absent, soluble pigment absent, reverse pigmentation dull green (26E3) centrally, light yellow to dull yellow (3A5-3B5) near margin.

G25N, 25 °C, 7d: Colonies 9–12 mm, low, plane; margin low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25D5); exudate absent, soluble pigment absent, reverse pigmentation greyish green (28C4) to pale at margin.

CREA, 25 °C, 7d: Colonies 13–15 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, minor proportion monoverticillate and tervertillate; stipes smooth walled, 100–250 × 2.5–3.5 µm; branches when present 2, 18–27 × 2.5–3.5 µm; metulae 2–5 per stipe, divergent, 33–60° [42±10°], 10.5–28.5 × 2–3 [17.7±3.9 × 2.55±0.5] µm, vesicle 3–5 [4.0±0.5] µm; phialides ampulliform, mostly 6–10 per metula, 7–8.5 × 2.5–3.5 [7.8±0.4 × 3±0.25] µm; conidia smooth walled, spheroid to subspheroid, 2.5–3 × 2.5–3 [2.8±0.2 × 2.76±0.2] µm, average width/length = 0.98±0.03, n = 50.

Notes — *Penicillium corylophilum* typically produces biverticillate smooth walled conidiophores. This character distinguishes it from other species resolved as close relatives. This group of species does, however, share the character of producing dark green colony reverse pigmentation on most media.

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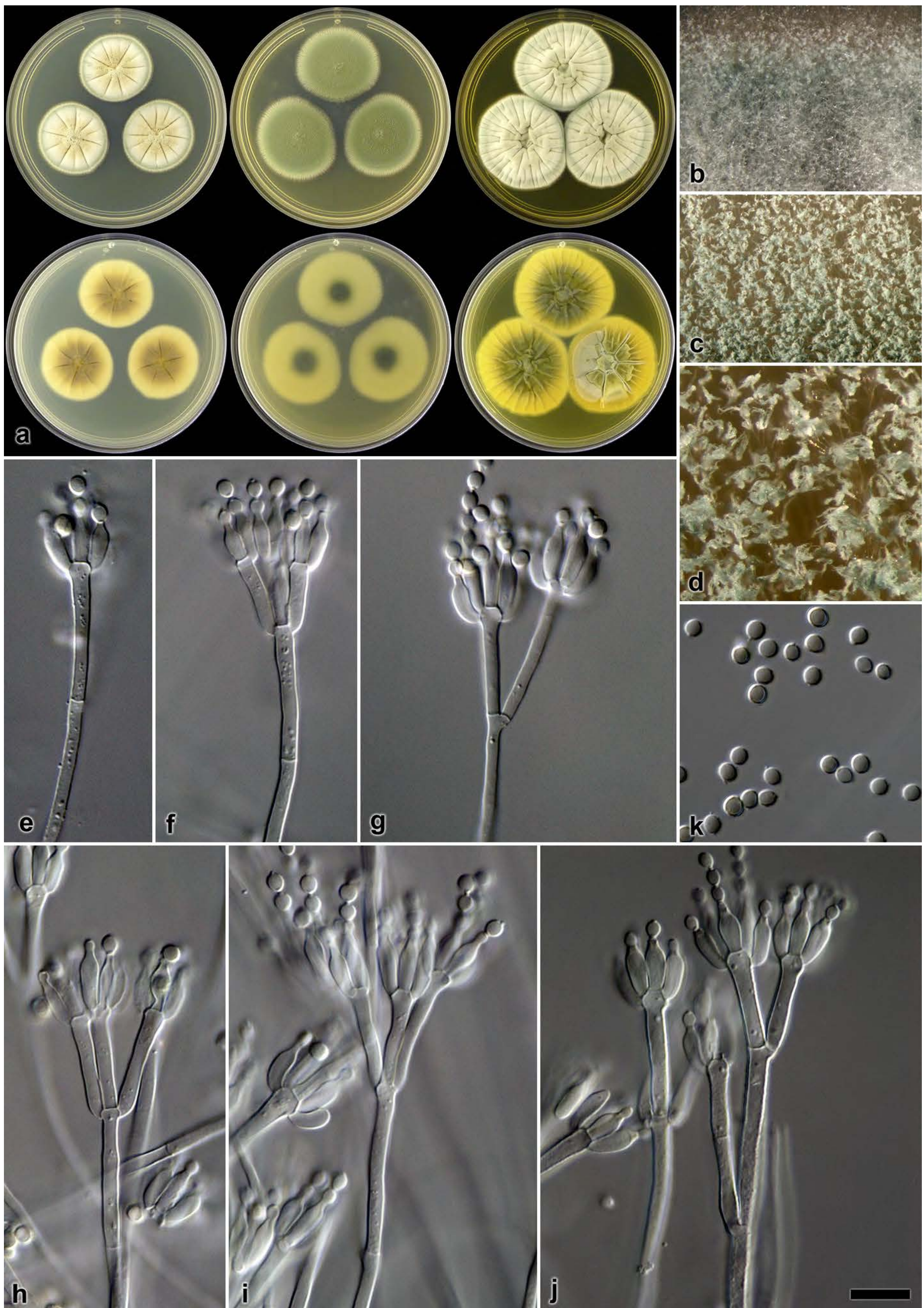

PLATE 8. *Penicillium corylophilum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in j = 10 µm, applies to e–k).

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4. *Penicillium cravenianum* Visagie prov. nom.

PLATES 9, 19e

ETYMOLOGY: Latin, *cravenianum* = Named after Dr. Danie Craven, former president of the South African Rugby Board and ex-student at Stellenbosch University; this species was isolated from the mountain next to the Danie Craven Rugby stadium.
EX-TYPE: CV92 = DTO180I5 = KAS4202 = DAOM241082
TYPE ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 28-30 mm, low to moderately deep, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation sparse to sometimes moderate, conidia *en masse* dull green (30E3–30E4) at centre, dull green to greyish green (25D4–25D5) elsewhere; exudate absent, soluble pigment absent, reverse pigmentation dark green to olive (30F5–30F8–1F5–1F8) at centre, pale yellow (2A3–3A3) near edge.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 25–28 mm, low, radially sulcate, slightly raised at centre; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous, with some floccose areas near margin; sporulation moderately dense, conidia *en masse* greyish green (29C4) at centre, dark green (26F6–26F8) near margin; exudate clear, soluble pigment absent, reverse pigmentation similar to CYA at 25°C.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 16–20 mm, sometimes up to 35 mm, low, plane; margins subsurface, narrow (<1 mm), entire; mycelia white; texture velutinous, floccose near centre; sporulation dense, conidia *en masse* dark green (25F8–26F8); exudate absent, soluble pigment absent, reverse pigmentation yellow (3B8) at centre, fading into dull yellow (3B4) margin.

YES, 25 °C, 7d: Colonies 28–34 mm, low, radially and concentrically sulcate, with randomly raised furrows present; margins narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation spares to sometimes moderately dense, conidia *en masse* similarly coloured as CYA; exudate absent, soluble pigment absent, reverse colouration greyish green (28D6–28E6) at centre, light to greyish yellow (3A5–3B5) margin.

G25N, 25 °C, 7d: Colonies 8–11 mm, raised at centre, lightly radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25E5) occurring in patches, to a lighter greyish green (25C5) elsewhere; exudate absent, soluble pigment absent, reverse pigmentation pale yellow (2A3) at centre and margin, greyish yellow (2B3) elsewhere.

CREA, 25 °C, 7d: Colonies 19–22 mm, no acid produced.

Micromorphology — Conidiophores mostly biverticillate, with proportion terverticillate; stipes heavy rough walled, 120–325 × 2.5–4 µm; rami/branches when present 2 per stipe, 16–48 × 2.5–4 µm; metulae 2–6, slightly divergent, 31–73° [51±10.3°], 11–22 × 2.5–4.5 [15.4±2.1 × 3.1±0.4] µm, vesicle 3.5–5.5 [4.7±0.5] µm; phialides ampulliform, 8–12 per metula, 7–9.5 × 2.5–3.5 [8.1±0.6 × 3±0.23] µm; conidia finely rough walled, spheroid to broadly ellipsoidal, 2.5–3 × 2–3 [2.6±0.18 × 2.4±0.14], average width/length = 0.93±0.04, n = 112.

Notes — *Penicillium cravenianum* characteristically produce conidiophores with rough walls, with colonies that display restricted growth on MEA. This distinguishes *P. cravenianum* from its close relatives, *P. corylophilum* and *P. pagulum*.

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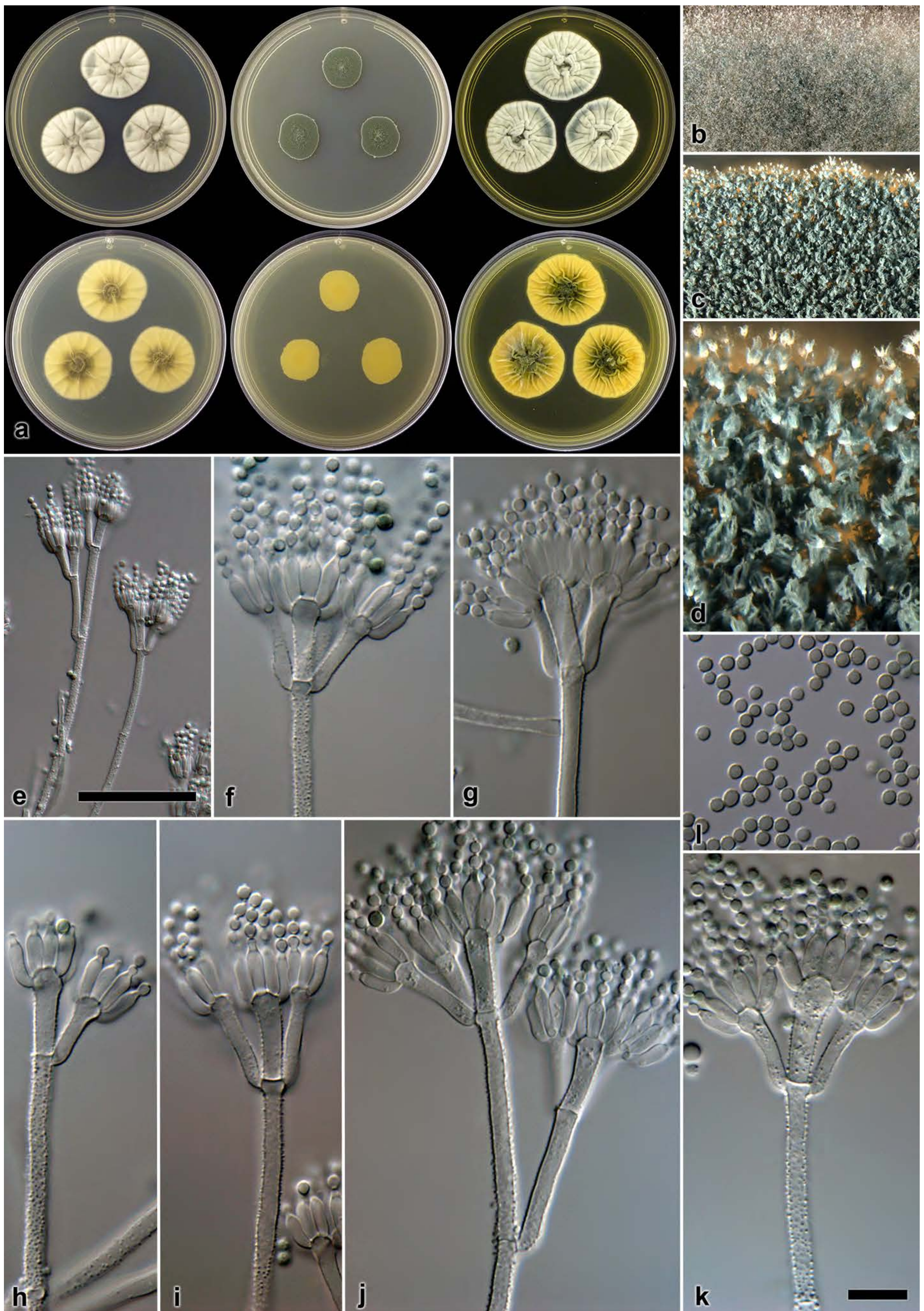

PLATE 9. *Penicillium cravenianum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 50 µm; — Scale bar in k = 10 µm, applies to f–l).

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5. *Penicillium hemitrachum* Visagie **prov. nom.**

PLATES 10, 19f

ETYMOLOGY: Latin, *hemitrachum* = meaning half rough walled; named after the rough and smooth walled metulae produced on the same conidiophore
EX-TYPE: CV2845 = DTO180D8 = KAS3942 = DAOM241098
TYPE ISOLATED FROM: Soil, Malmesbury
ADDITIONAL SPECIMENS EXAMINED: CV2844, CV964.
ISOLATED FROM: Soil Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 54-58 mm, low, radially sulcate, concentrically sulcate in fresh cultures, grey sterile hyphae present overlaying some conidial areas; margins low, narrow (2-3 mm), entire; mycelia white; texture velutinous, with some floccose mycelia present; sporulation dense, conidia *en masse* dull to greyish green (26E3-26E6), a lighter dull green (26D3) near margin; exudate absent, soluble pigment yellow sometimes produced in low concentrations, reverse pigmentation olive (3F6-3F7) in central areas, dull green (28D4-28E4) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 60–62 mm, sometimes only 40 mm, low, radially sulcate, raised at centre; margins low, wide (2–4 mm), entire; mycelia white; texture velutinous, with some floccose regions; sporulation dense, conidia *en masse* similar to CYA at 25°C; exudate absent, soluble pigment yellow, absent in CV964, reverse pigmentation similar to CYA at 25°C.

CYA, 37 °C, 7d: Colonies 4–9 mm, consisting out of white mycelia; reverse pigmentation greyish yellow (4B4).

MEA, 25 °C, 7d: Colonies 68-70 mm, low, plane; margins low to subsurface, wide (4 mm); mycelia white; texture velutinous, some floccose mycelia present; sporulation dense, conidia *en masse* dull green to greyish green (28F4-28F6); exudate absent, soluble pigment yellow, reverse pigmentation greyish yellow (1B6) at point of inoculation, greyish green (1D6) elsewhere.

YES, 25 °C, 7d: Colonies 68-70 mm, low, randomly sulcate; margins low, narrow (2 mm); mycelia white; texture velutinous, floccose mycelia present near centre; sporulation dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation similar to CYA reverse.

G25N, 25 °C, 7d: Colonies 10–18 mm, raised at centre, plane; margins narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (29E5–29E6); exudate absent, soluble pigment absent, reverse pigmentation greyish green (29E5), in some isolates greyish green (30C5).

CREA, 25 °C, 7d: Colonies 20–25 mm, no acid produced.

Micromorphology — Conidiophores mostly biverticillate, sometimes monoverticillate, having a brownish to green pigment, on same conidiophore smooth and rough walled metulae present; stipes rough walled, often conidiophores with short stipes smooth, 20–180 × 2–3.5 µm; metulae 2–4, divergent, 38–67° [52±8.8°], 9.5–18 × 2–3.5 [13.6±1.9 × 2.9±0.26] µm, vesicle 3–6 [4.2±0.6] µm; phialides ampulliform, mostly 8–12, but sometimes only 4–6 per metula, 7–9 × 2–3.5 [7.7±0.5 × 2.8±0.2] µm; conidia finely rough walled, spheroid, 2–2.5 × 2–2.5 [2.4±0.09 × 2.4±0.1] µm, average width/length = 0.98±0.01, n = 108.

Notes — *Penicillium hemitrachum* characteristically produce fast growing colonies, a character not observed for other strains studied in this clade. Also, conidiophores have a green pigmentation and are commonly borne from green-pigmented mycelia. Interestingly, this species produce conidiophores that have smooth and rough walled metulae on the same conidiophore. Phylogenetically this species is distinct, closely related to *P. velutinum, P. maclennaniae* and *P. smithii* (FIGURES 2, 3). The fast growth rate of *P. hemitrachum* and *P. maclennaniae* distinguishes it from other species in this clade. *Penicillium maclennaniae*, however, produces conidiophores lacking pigment and produces larger conidia (3.8–4.0 µm) than *P. hemitrachum* (Yip 1981).

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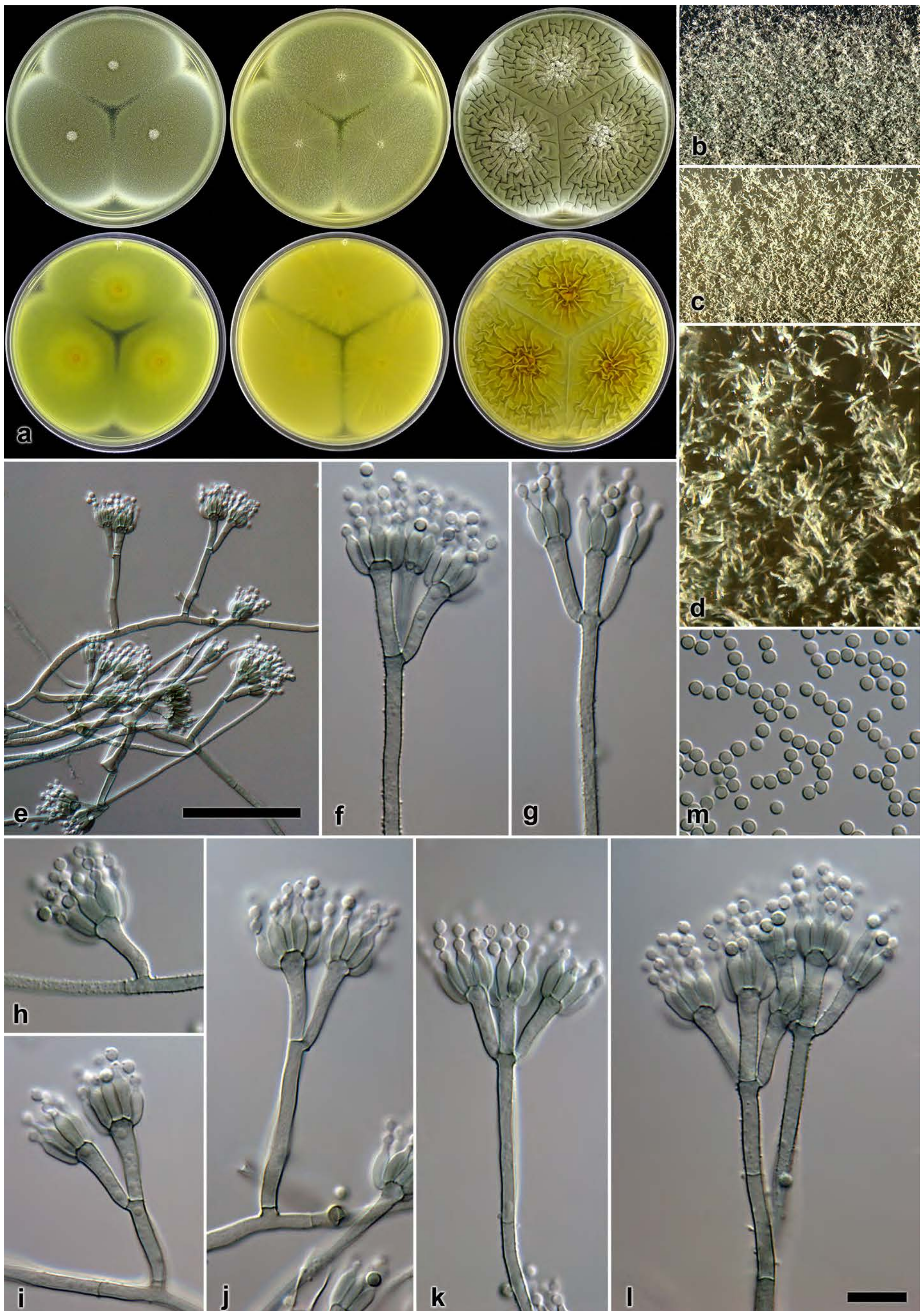

PLATE 10. *Penicillium hemitrachum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–l. Conidiophores. m. Conidia (— Scale bar in e = 50 µm; — Scale bar in l = 10 µm, applies to f–m).

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6. *Penicillium melinii* Thom

PLATES 11, 19g

The Penicillia: 273. 1930.

EX-TYPE: CBS218.30 = NRRL2041 = IMI040216
TYPE ISOLATED FROM: Forest soil, USA
ADDITIONAL SPECIMENS EXAMINED: CV535, CV542, CV2393, CV2404.
ISOLATED FROM: Soil from Stellenbosch, Bracts from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 24–29 mm, low, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous, some floccose mycelia present; sporulation sparse to moderate, sometimes only in colony areas facing each other, conidia *en masse* in most areas greyish green to dull green(25C4-25D4), dark green (25F5–25F6) in denser areas of sporulation; exudate sometimes clear, most isolates having a reddish orange (7A8) colour, soluble pigment visible in isolates producing coloured exudates, then also reddish orange, reverse pigmentation brown (5F5–5F8) at centre, fading into pale yellow (4A3) near margin, the brown less pronounced in isolates not producing exudate, then olive brown (4E4) at centre, greyish yellow (4C3) elsewhere, some isolates dark brown (6F8) at centre fading into orange (6B8).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 16–26 mm, low, raised at centre, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous with some floccose areas; sporulation sparse to moderately dense, conidia *en masse* turquoise white to greyish turquoise (24A2–24B3) dark green (25F4) in dense areas; exudate clear to light brown, soluble pigment absent is some isolates, brownish orange in others, reverse pigmentation brown (6E8) to brownish orange (6C8) at centre, fading to greyish yellow (3C5) at margin, in isolates lacing soluble pigments, greyish green to dull green (30C3–30D4).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 35–39 mm, sometimes only reaching 26 mm, low, plane; margins subsurface, wide (4 mm), entire; mycelia white; texture velutinous, some floccose mycelia present; sporulation dense, conidia *en masse* dark green (25F6–25F7); exudate absent, soluble pigment absent, some isolates produce a yellowish orange exudate in very low concentration, reverse pigmentation dull yellow (3B4) near centre, fading into greyish yellow (3C3) towards margin, CV542 more orange fade to reverse colouration.

YES, 25 °C, 7d: Colonies 32–37 mm, low to moderately deep, radially and concentrically sulcate, some random grooves also present, sometimes some isolates have a yellowish grey (4B2) colour; margins low, narrow (1 mm), entire; mycelia white; texture velutinous, some floccose mycelia; sporulation sparse to moderately dense in some isolates, conidia *en masse* greyish green (25D5–25D6), darker greyish green (25E6-25E7) when more dense; exudate clear, reddish brown in some isolates, exudate however not produced by older cultures, soluble pigment absent, reverse pigmentation brown (6E8) at centre, brown (7E6) in some strains, although these brownish colours are not produced by older cultures, more generally greyish yellow (3B5-4B5) fading into a pastel yellow (3A4) margin.

G25N, 25 °C, 7d: Colonies 5–12 mm, raised at centre, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish turquoise to greyish green (24B4–25C4); exudate absent, soluble pigment typically absent, yellow to brown in some strains, reverse pigmentation greyish green (30E5–30E6).

CREA, 25 °C, 7d: Colonies 15–18 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, with subterminal branching common, could sometimes be interpreted as being terverticillate; stipes rough walled to warted, 75–270 × 2–4 μm; branches 11–66 × 2–4 μm; metulae 2–4, divergent, 23–71° [44.1±10.6°], 9.5–36 × 2–3 μm [16.5±4.3 × 2.3±0.2] μm, vesicle 2–4.5 [3.1±0.5] μm; phialides rough walled, ampulliform, 6–12 per metula, 6.5–10 × 2.5–3 [7.6±0.7 × 2.7±0.2] μm; conidia spinose, spheroid, 2.5–3.5 × 2.5–3.5 [3±0.2 × 3±0.2] μm, average width/length = 0.98±0.02, n = 77.

Notes — *Penicillium melinii* characteristically produces rough walled to warted, divergent and irregular conidiophores and has spinose conidia. *Penicillium xanthomelinii*, described in this study, is its closest relative. However, *P. melinii* grows more restricted on most media. In addition, *P. melinii* produce brown colors in colonies that are absent in *P. xanthomelinii*. Conidia on average are slightly smaller in *P. xanthomelinii* (2.8±0.1 × 2.8±0.1 μm).

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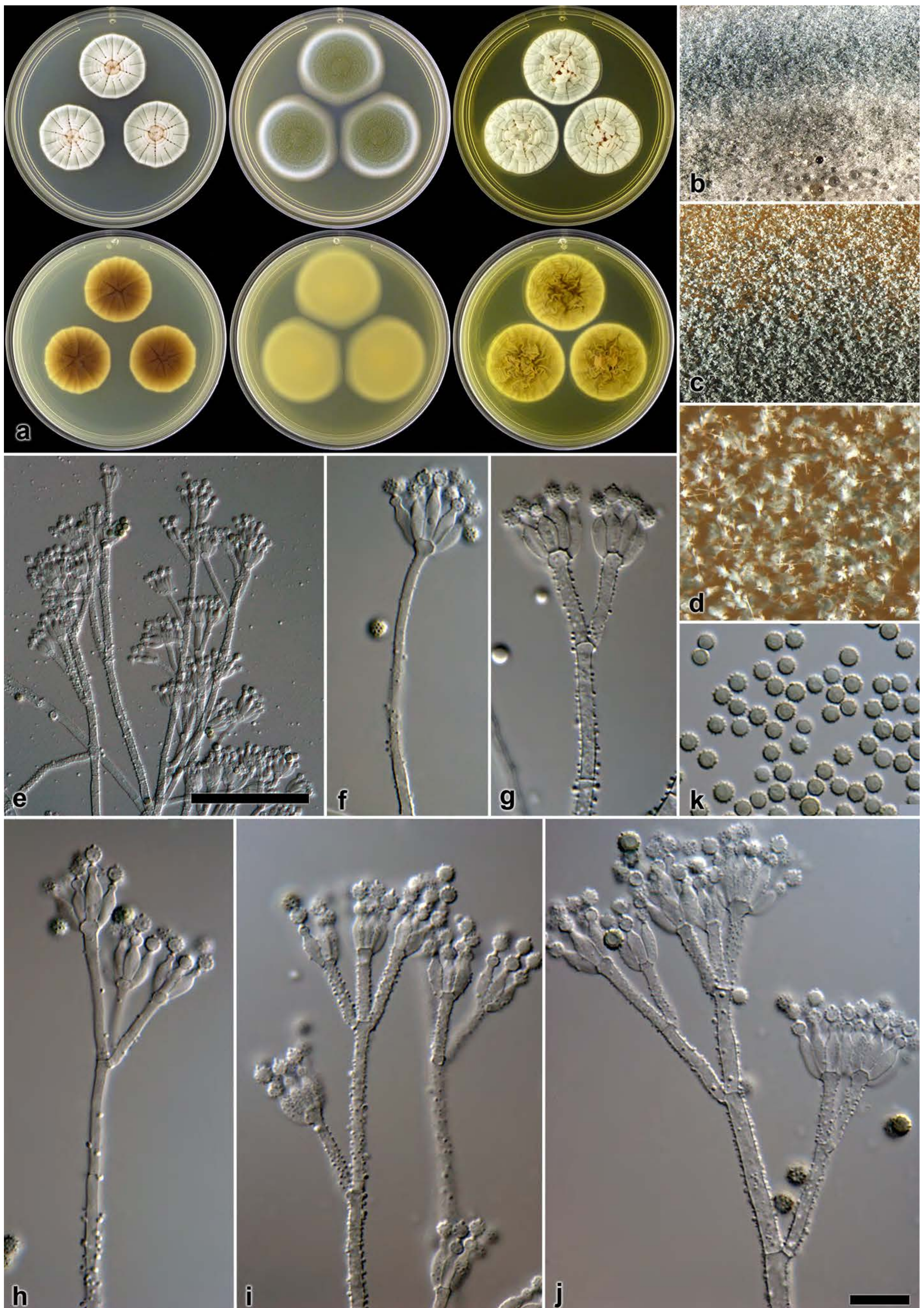

PLATE 11. *Penicillium melinii* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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7. ***Penicillium pagulum*** Visagie **prov. nom.**

PLATES 12, 19h

ETYMOLOGY: Latin, *pagulum* = meaning small village; named after the small colonies produced on CYA
EX-TYPE: CV2224 = DTO183H2 = KAS4076 = DAOM241069
TYPE ISOLATED FROM: Bract from *Protea repens* infructescens, Struisbaai
ADDITIONAL SPECIMENS EXAMINED: CV2236
ISOLATED FROM: Bract from *Protea repens* infructescens, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 15–20 mm, low, radially sulcate; margins low, narrow, entire; mycelia white; texture velutinous with some floccose areas; sporulation sparse, conidia *en masse* greyish turquoise (24b5–24E5); exudate absent, soluble pigment absent, reverse pigmentation (2F5–3F5).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colony characters similar to CYA °C, except diameter 12–15 mm.

CYA, 37 °C, 7d: Colonies 1–5 mm, consisting of white mycelial mass.

MEA, 25 °C, 7d: Colonies 25–32 mm, low, plane; margins subsurface, wide (4 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish to dark green (25E5–25F5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3B7) near centre becoming greyish green (30C5) near margin.

YES, 25 °C, 7d: Colonies 26–34 mm, low, radially and concentrically sulcate; margins low, narrow, entire; mycelia white; texture velutinous with some floccose areas; sporulation moderately dense, conidia *en masse* greyish turquoise (24B3) in some regions, greyish turquoise (24E5) in others; exudate absent, soluble pigment absent, in one isolate CV535 deep orange, reverse pigmentation dark green (25F8), orange (6B7) at margin

G25N, 25 °C, 7d: Colonies 5–10 mm, consisting of white mycelial mass, no sporulation.

CREA, 25 °C, 7d: Colonies 19–24 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, with minor proportion terverticillate; stipes finely rough walled, 100–250 × 2.5–4 µm; branches when present 2 per stipe, 21–32 × 2.5–4 µm; metulae 2–5, divergent, 28–62° [41±8.4°], 13–24 × 2.5–4 [16.8±2.5 × 3.1±0.4] µm, vesicle 3.5–5 [4.3±0.45] µm; phialides ampulliform, 9–14 per metula, 8–10.5 × 2.5–3.5 [9.3±0.55 × 3±0.2] µm; conidia finely rough walled, spheroid to somewhat subspheroid, 2–3 × 2–3 [2.6±0.2 × 2.5±0.18] µm, average width/length = 0.97±0.03, n = 35.

Notes — *Penicillium pagulum* displays restricted growth on CYA, which makes it distinct from all closely related species. Conidiophores are typically rough walled. Phylogenetically, it is resolved closely related to *P. cravenianum* and *P. corylophilum* (FIGURES 2, 3). Both its close relatives, however, grow faster on CYA. Additionally, *P. corylophilum* produce conidiophores with smooth walls. *Penicillium cravenianum* also displays restricted growth on MEA compared to *P. pagulum*.

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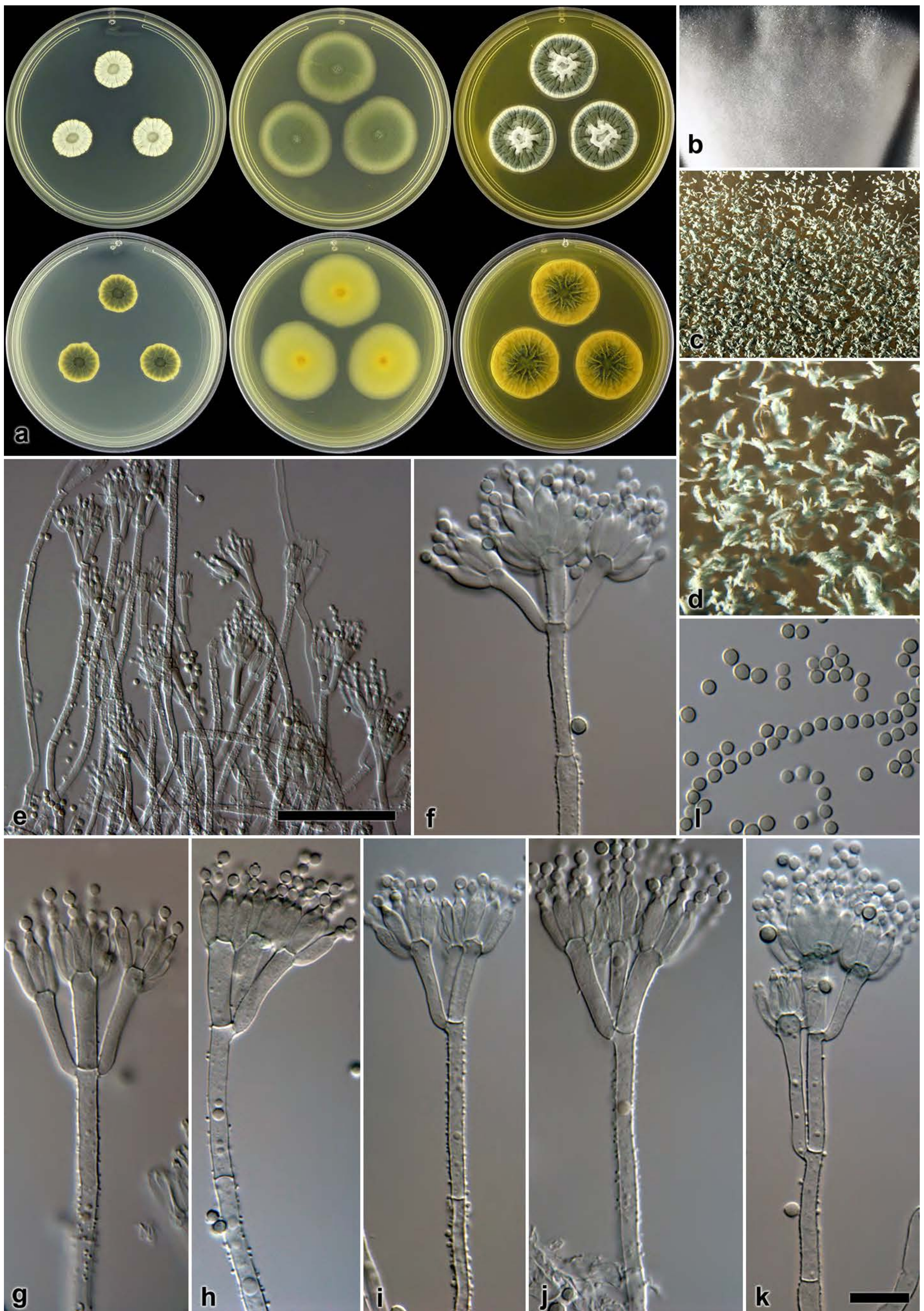

PLATE 12. *Penicillium pagulum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 50 µm; — Scale bar in k = 10 µm, applies to f–l).

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8. *Penicillium restrictum* Gilman & Abbott PLATES 13, 14, 19i–l

Iowa State College Journal of Science 1: 297. 1927.

EX-TYPE: CBS367.48 = NRRL1748
TYPE ISOLATED FROM: Soil, Honduras
ADDITIONAL SPECIMENS EXAMINED: CV101, CV1695, CV799, CV872, CV896, CV90, CV900, CV93, CV932, CV943, CV948, CV96, CV972.
ISOLATED FROM: Air and Soil, Stellenbosch, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 20–25 mm, moderately deep, lightly radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation mostly absent, but moderately dense in some isolates, conidia *en masse* light turquoise (24A4); exudate clear, red in strain CV932, soluble pigment absent, reverse pigmentation white to yellowish white (1A2–2A2), light yellow (4A5) in some isolates.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colony characters similar to CYA 25 °C, dimensions 20–30 mm.

CYA, 37 °C, 7d: Colonies 10–22 mm, consisting of white mycelia, sporulation absent; clear exudate produced in some isolates, reverse pigmentation greyish to light yellow (3B4–4A4), sometimes yellowish white (3A2).

MEA, 25 °C, 7d: Colonies 24–35 mm, low to moderately deep, plane, very soft small sclerotia observed in some isolates, these never matured into cleistothecia; margin low, narrow, entire; mycelia white and yellow; texture floccose; sporulation sparse to moderately dense, conidia *en masse* dull green (25D4–26D4), greyish green (25B3–25C3) in less dense sporulating areas; exudate absent, soluble pigment absent, reverse pigmentation generally pale yellow (2A3), some isolates greyish green (30B3–30C3), some isolates reddish yellow (4A6).

YES, 25 °C, 7d: Colonies 25–35 mm, moderately deep, radially and concentrically sulcate, some isolates have beige color near centre; margin low, narrow, entire; mycelia white, yellow mycelia in some isolates; sporulation absent, very sparse in some isolates, conidia *en masse* turquoise grey (24B2); exudate clear, soluble pigment absent, reverse pigmentation light yellow (4A4) at centre, pale yellow (3A4) elsewhere.

G25N, 25 °C, 7d: Colonies 8–12 mm, consisting of white mycelial mass, some isolates sparse sporulation.

CREA, 25 °C, 7d: Colonies 12–20 mm, isolates produce variable strengths of acid, absent in some.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 14–140 [52±30] × 1.5–2.5 µm, vesicle 2.5–5 µm; phialides ampulliform, 5.5–8 × 2–3.5 [6.9±0.6 × 2.8±0.3] µm; conidia smooth to heavy rough walled, spheroid, 2–4 × 2–4 [2.6±0.5 × 2.6±0.5] µm, average width/length = 0.98±0.02, n = 88.

Notes — *Penicillium restrictum* is characterized by restricted growth on CYA, with most strains examined showing week sporulation. Colonies are typically compact often consisting of dense white mycelia. Conidiophores are typically smooth, with stipes mostly very short and producing either small smooth walled conidia or larger heavy rough walled conidia. Under the genealogical concordance species concept, this clade has to be considered a single species. This would mean the synonymizing of *P. arabicum, P. heteromorphum, P. katangense, P. kurssanovii, P. cinereoatrum, P. meridianum* and *P. phillipense*. However, a large degree of variation is observed in morphological features, as well as some sub-clades that are formed in the phylogenies. *Penicillium restrictum* is thus considered here to represent a species complex and a full review of the complex is suggested.

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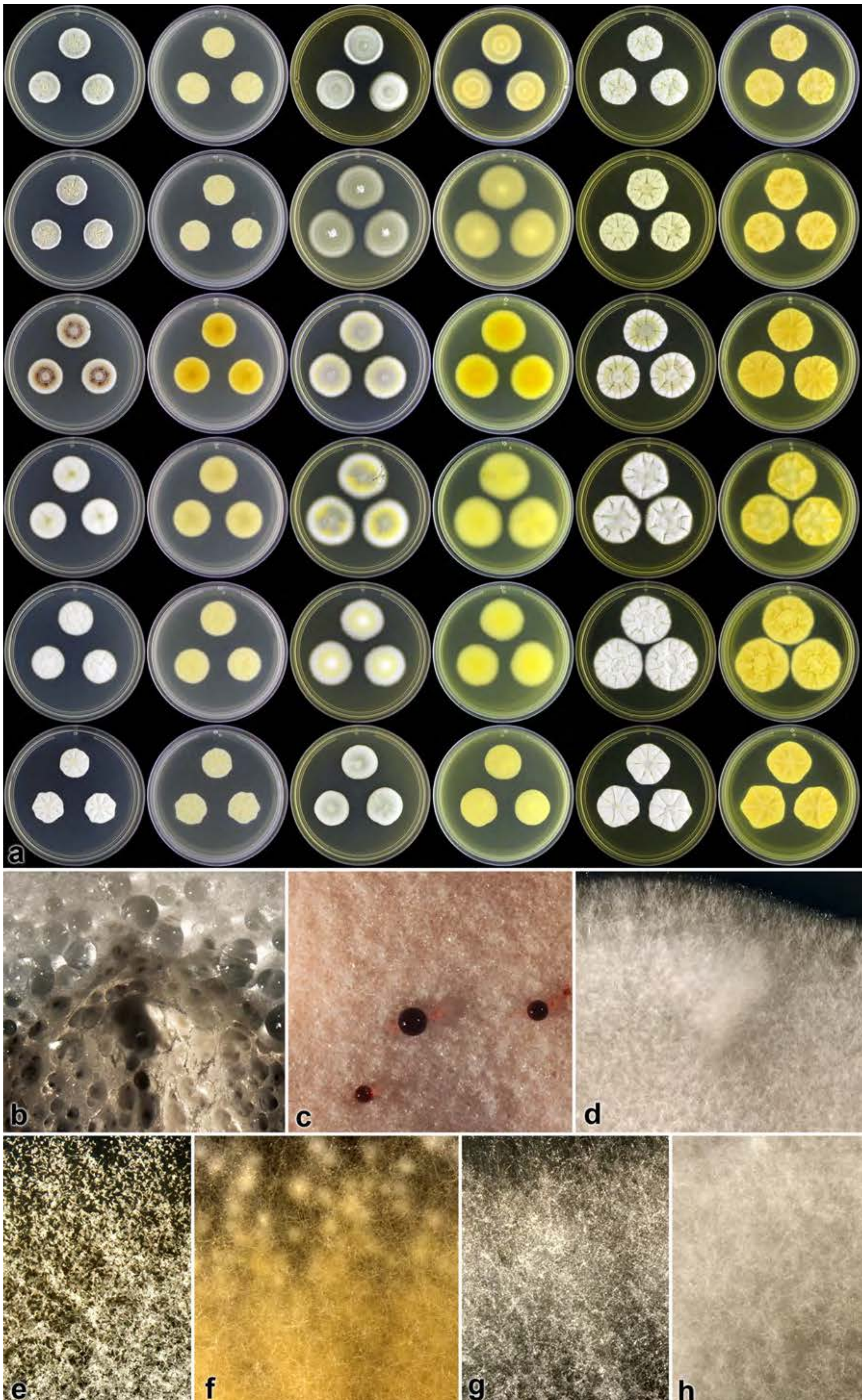

PLATE 13. *Penicillium restrictum* species complex strains that show morphological variation within complex. a. Colonies from left to right on CYA (obv.), CYA (rev.), MEA (obv.), MEA (rev.), YES (obv.), YES (rev.). Rows from top to bottom CV90, CV96, CV932, CV943, CV93, CV948. b–d. Colony textures on CYA: b. CV90. c. CV932. d. CV948. e–h. Colony textures on MEA: e. CV90. f. CV932. g. CV943. h. CV948.

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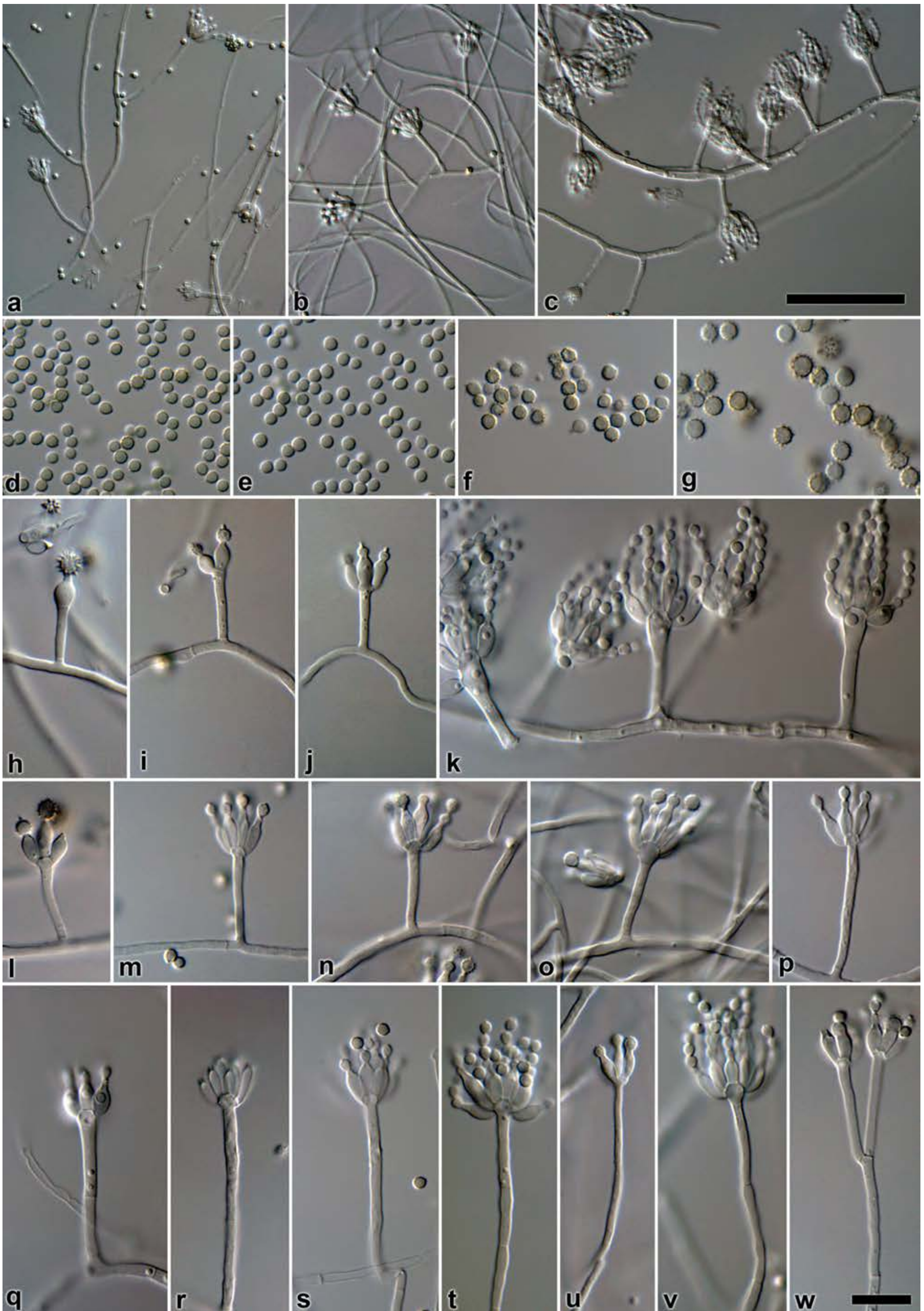

PLATE 14. *Penicillium restrictum* species complex conidiophores and conidia produced in culture. a, d, m, p, r, s, w. CV90. b, f, n, o, u, . CV932. c, e, k, q, t, v. CV896. g–j, l. CV948. (Scale bar in — c = 50µm, applies to a–c; — Scale bar in w = 10µm, applies to d–w).

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9. *Penicillium rubefaciens* Quintanilla PLATES 15, 16, 19m–p

Mycopathologia 80: 73. 1982.

EX-TYPE: CBS145.83

TYPE ISOLATED FROM: Soil, Valladolid, Spain

ADDITIONAL SPECIMENS EXAMINED: CV1546, CV1479, CV1514, CV1486, CV1495, CV2835, CV2820, CV2817, CV2826, CV795, CV1015, CV597, CV1558.

ISOLATED FROM: Air sample from Malmesbury, Mites and bracts from *Protea repens* infructescences, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 23–32 mm, low to moderately deep, radially and concentrically sulcate, white sterile mycelia present at colony centre, giving colony a greyish green (26D3) to orange beige (4C3) colour; margins low, narrow to wide (1–3 mm), somewhat irregular; mycelia white; texture mostly floccose and velutinous; sporulation moderately dense in fresh isolates, less dense in older cultures, conidia *en masse* dark green (25F7–26F7), sometimes lighter, then pale green (26A3–26A4); exudate clear to orange brown, but not always produced, soluble pigment mostly absent although in CV2820 a pinkish soluble pigment was produced, reverse pigmentation greyish green to dark green (25E7–25F7) at centre, (26A2–27A2) at margin, sometimes dark green to olive green (30F8–3F8).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 20–28 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture floccose with some velutinous areas; sporulation moderately dense near margin, conidia *en masse* greyish green (26F5); exudate clear droplets sometimes produced, soluble pigment absent, sometimes very light in isolate CMV51, reverse pigmentation (25F7–25F8) at centre, (26B3) near margin, isolate CMV51 brown (7E7) at centre.

CYA, 37 °C, 7d: Colonies 7–15 mm, sometime no growth, raised at centre; sporulation absent to sometimes sparse.

MEA, 25 °C, 7d: Colonies 30–43 mm, low, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous, with floccose areas present; sporulation dense, conidia *en masse* greyish green (27E5–27E7); exudate absent, soluble pigment absent, reverse pigmentation sometimes olive brown (4F8) and sometimes greyish to dull yellow (2B3–3B3) at centre, dull green (28D4–30D4) elsewhere, sometimes dark green (27F7) at centre, greyish green (27B3) elsewhere.

YES, 25 °C, 7d: Colonies 28–38 mm, low to moderately deep, radially and concentrically sulcate, random furrows also present, greyish purple color sometimes present; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation ranging from moderately sparse to moderately dense, conidia *en masse* similarly coloured as CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish green to dark green (26E7–28F7–28F8) at centre, pale yellow (2A3) at margin.

G25N, 25 °C, 7d: Colonies 10–22 mm, raised at centre, plane; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25E6) to dull to greyish green (26E4–26E6); exudate absent, soluble pigment absent, reverse pigmentation yellowish grey (2B2) at centre, light green (27A4–27A5) elsewhere.

CREA, 25 °C, 7d: Colonies 15–17 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, minor proportion monoverticillate, one or two terverticillate; stipes rough walled to warted, 45–270 × 2.5–3.5 µm; branches when present 2, 20–30 × 2.5–3.5 µm; metulae 2–5, divergent, sometimes slightly appressed, 27–100° [58.7±15°], 12–21 × 2.5–4 [16±1.8 × 3±0.39] µm, vesicle 4–6.5 [5.1±0.7] µm; phialides ampulliform, 10–12 per metula, 7–9.5 × 2.5–3.5 [8.3±0.7 × 2.9±0.3] µm; conidia rough walled, spheroid, 2–3 × 2–3 [2.36±0.16 × 2.35±0.15] µm, average width/length = 0.97±0.02, n = 69.

Notes — Morphologically, strains isolated from the Fynbos does not match the original description of *P. rubefaciens* (Quintanilla 1982). The species was described as displaying more restricted growth with reddish brown reverse pigmentations compared to the isolates obtained from the Fynbos. Microscopically, the conidiophore and its dimensions are very similar. Morphologically the Fynbos strains show minor variations. Phylogenetically, the strains were not resolved satisfactorily across all genes, mainly due to single strains, such as CV1015, that shifts tree topologies (FIGURES 2, 3, 5). Strains are thus considered here as part of *P. rubefaciens*. It seems, however, that this species might represent a species complex and a more detailed study examining additional strains is necessary.

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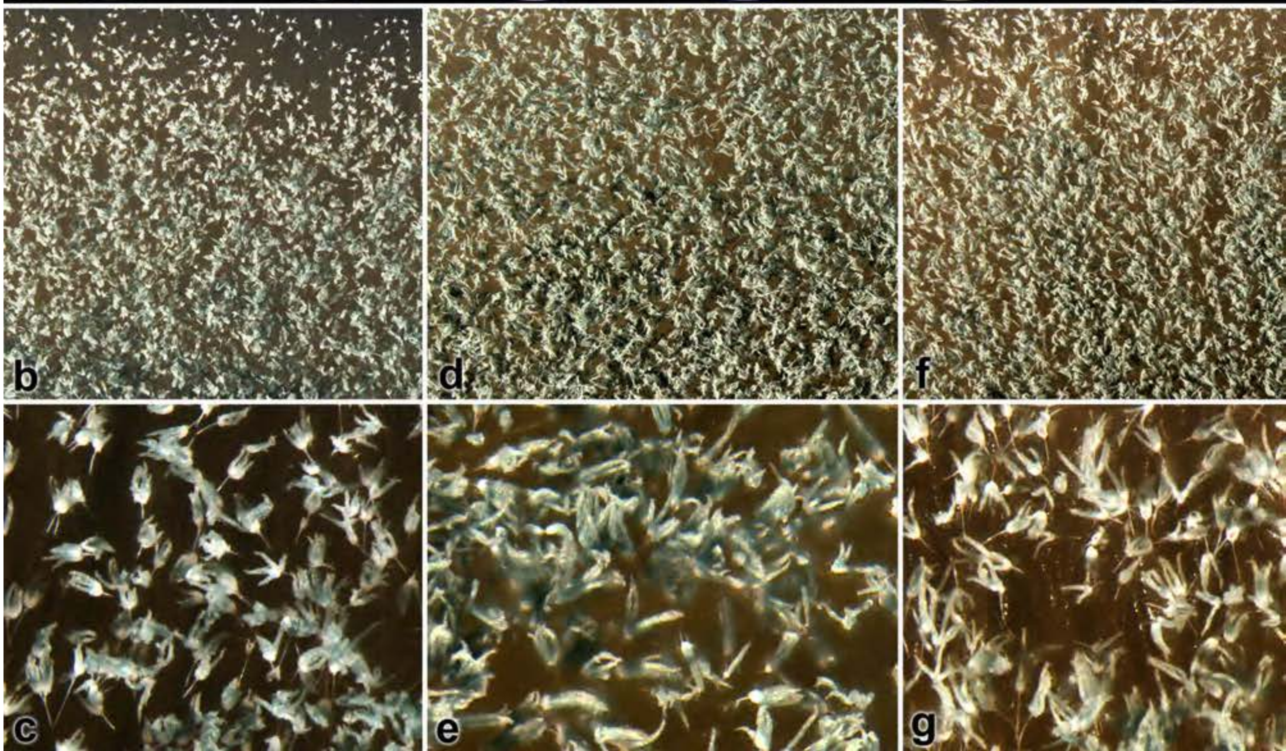

PLATE 15. *Penicillium rubefaciens* species complex strains that show morphological variation within complex. a. Colonies from left to right on CYA (obv.), CYA (rev.), MEA (obv.), MEA (rev.), YES (obv.), YES (rev.). Rows from top to bottom CV1479, CV795, CV1015, CV597, CV2826. b–g. Colony texture on MEA: b, c. CV1015. d, e. CV1479. f, g. CV795.

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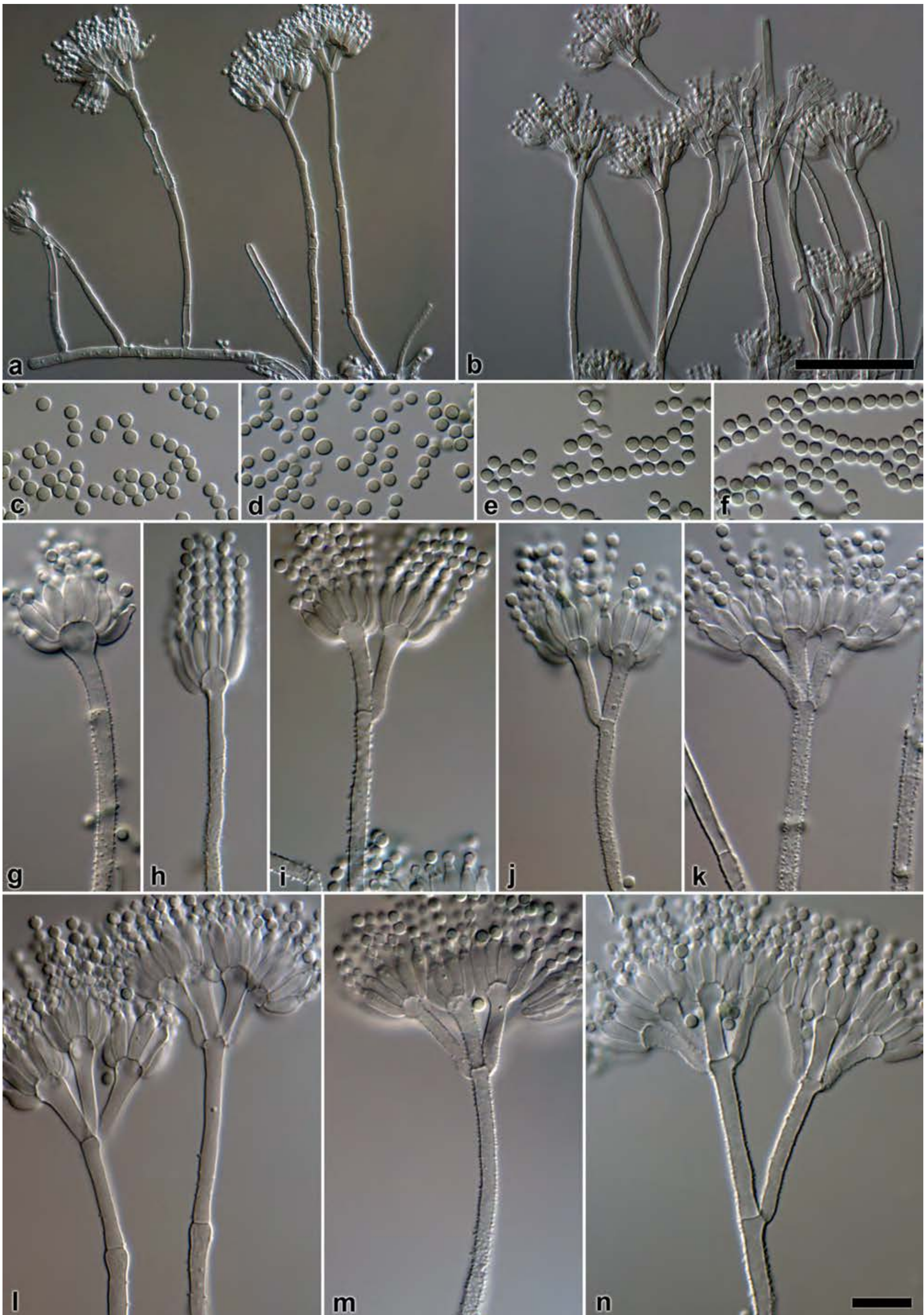

PLATE 16. *Penicillium rubefaciencs* species complex conidiophores and conidia produced in culture. a, c, g, i, l. CV795. b, e, j, k, n. CV1015. d, h, m. CV1479. f. CV1479. (Scale bar in — b = 50µm, applies to a, b; — Scale bar in n = 10µm, applies to c–n).

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10. *Penicillium toxicarium* Miyake PLATES 17, 19q

Rep. Res. Inst. Rice Improvement 1: 1. 1940.

EX-TYPE: CBS351.51 = FRR841
TYPE ISOLATED FROM: Rice, Japan
ADDITIONAL SPECIMENS EXAMINED: CV11, CV1454, CV1226, CV1532, CV283, CV1000, CV2015.
ISOLATED FROM: Air, Soil, Mites and Bracts from *Protea repens* infructescence, Stellenbosch, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 20–25 mm, radially and concentrically sulcate, moderately deep; margins low, narrow (1 mm), entire; mycelia yellow; texture floccose; sporulation sparse to moderately deep, conidia *en masse* (25D6–25F6) to (26E6); exudate clear, mostly absent, soluble pigment yellow, reverse pigmentation yellow (3A6–3A8) to orange yellow (4B8).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 20–25 mm, all characters similar to CYA at 25°C.

CYA, 37 °C, 7d: Colonies 3–7 mm, consisting out of white mycelia; reverse pigmentation pale yellow (3A3) to light yellow (2A4).

MEA, 25 °C, 7d: Colonies 15–18 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white at margins, yellow mycelia present at centre; texture floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25E4–26E5); exudate absent, soluble pigment yellow, reverse pigmentation greenish yellow (1A6) at point of inoculation, fading into greyish yellow (1B6–2B6).

YES, 25 °C, 7d: Colonies 27–33 mm, low to almost moderately deep, radially and concentrically sulcate; margins low, very narrow (<1 mm), entire; mycelia white at edges, yellow centrally; texture floccose; sporulation sparse, conidia *en masse* greenish white (27A2), dull green (27E4) when more dense; exudate absent, soluble pigment absent, reverse pigmentation light yellow to yellow (3A4–3A6) to deep yellow (4A8) in some isolates.

G25N, 25 °C, 7d: Colonies 12–15 mm, low, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia yellow; texture floccose; sporulation moderately dense near centre, conidia *en masse* greyish turquoise (24D5–24E5), greyish green (27C4) when less dense between yellow mycelia; exudate absent, soluble pigment yellow, reverse pigmentation orange yellow (4A8) at centre, light yellow to greenish yellow (1A5–1A8) elsewhere.

CREA, 25 °C, 7d: Colonies 10–13 mm, no acid produced.

Micromorphology — Conidiophores mostly monoverticillate with very short stipes although these might be interpreted as subterminal branches, with biverticillate conidiophores not uncommon; stipes/branches smooth walled, when terminal 70–290 × 2–2.5, borne subterminally 18–90 × 2–2.5 µm; metulae mostly 2, sometimes 3, divergent, 8–29 × 1.5–2.5 [18±4.5 × 2.1±0.2] µm, vesicle 2.5–4 [3.4±0.3] µm; phialides ampulliform, 5–10 per metula, 5–9 × 2–3 [6.7±0.9 × 2.4±0.2] µm; conidia smooth walled, spheroid, 1.5–2 × 1.5–2 [1.8±0.08 × 1.8±0.08] µm, average width/length = 0.98±0.02, n = 50.

Notes — *Penicillium toxicarium* is distinguished by its compact yellow colonies and soluble pigments produced on most media. Its conidiophores are typically smooth walled, small and slender and can be interpreted as either monoverticillate with very short stipes or as irregularly biverticillate. For strains examined, most conidiophores were monoverticillate in the strict sense. *Penicillium toxicarium* is closely related to *P. citreonigrum.* Although morphologically not distinct, Serra *et al.* (2008) considered them distinct based on multigene phylogenies.

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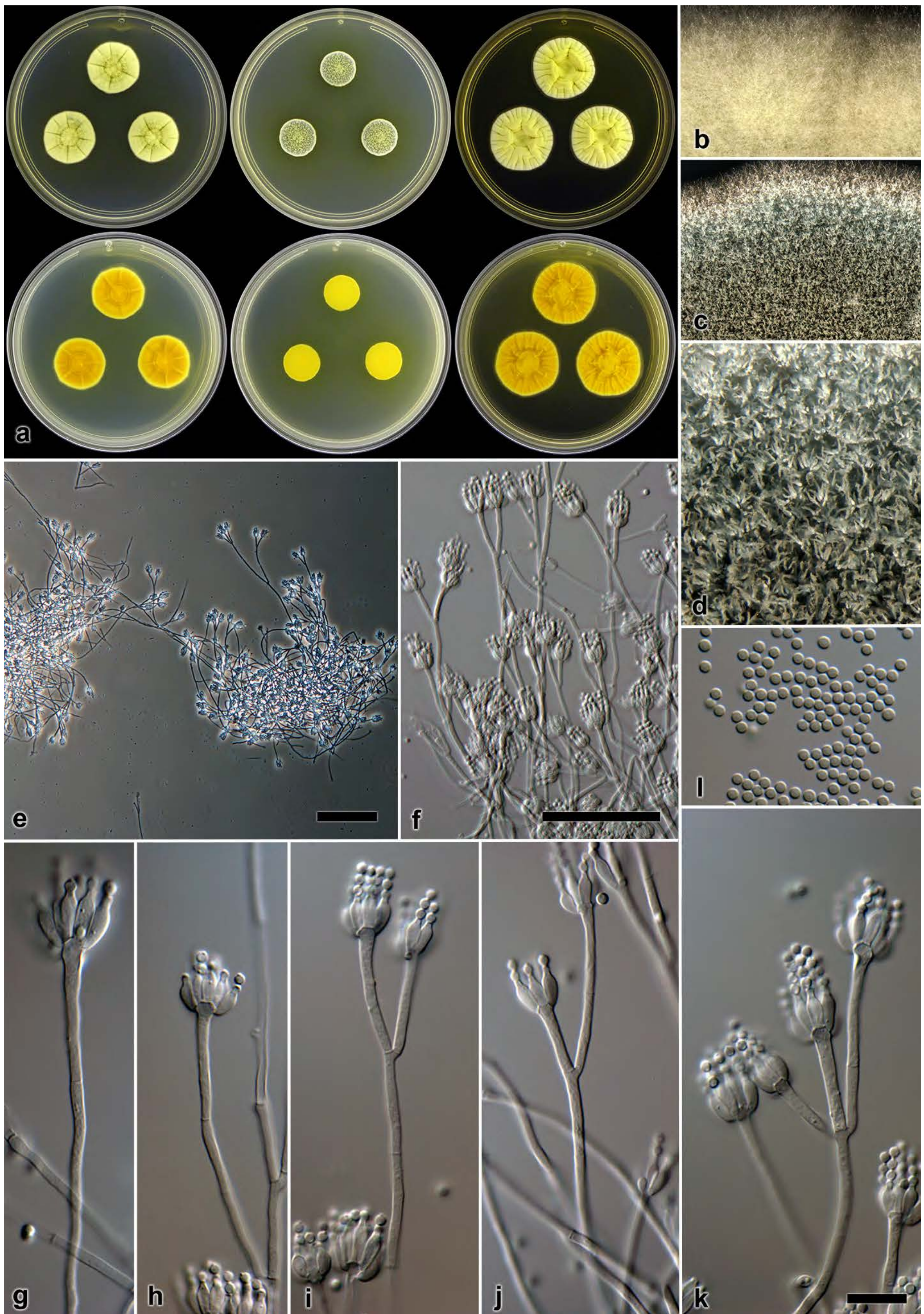

PLATE 17. *Penicillium toxicarium* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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11. ***Penicillium xanthomelinii*** Visagie **prov. nom.** PLATES 18, 19r

ETYMOLOGY: Latin, *xanthomelinii* = meaning yellow *melinii*; named after the bright yellow colony reverses and reflects closely relatedness to *P. melinii*
EX-TYPE: CV1677 = DTO183C7 = KAS4026 = DAOM241104
TYPE ISOLATED FROM: Soil, Struisbaai
ADDITIONAL SPECIMENS EXAMINED: CV1905, CV1844, CV1942, CV1871, CV1745, CV1969, CV1871, CV1745, CV1969, CV1923, CV1886, CV2329.
ISOLATED FROM: Soil, mites and bracts from *Protea repens* infrusctescences, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 30–33 mm, low, radially and concentrically sulcate; margins low, narrow (1 mm), narrow; mycelia white; texture velutinous at margin, but floccose areas near colony centre; sporulation moderately dense, conidia *en masse* colour showing variation, greyish green to dark green (24E5–24F5), and various shades of greyish green (25B3-25C3-24D5); exudate deep yellow to orange brown, less pronounced in some isolates, soluble pigment deep yellow to orange brown produced in low concentration, reverse pigmentation brown (7E7–7E8) at colony centre, greyish orange to orange (5C5–5C8) elsewhere, greyish yellow (2B3–2C3) near the yellowish white (2A2) margin.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 17–21 mm, craterform, radially and concentrically sulcate; margins low, narrow (<1 mm), entire; mycelia white, yellowish at centre; texture floccose and velutinous; sporulation sparse, conidia *en masse* greenish white (25A2); exudate absent, soluble pigment absent, reverse pigmentation olive brown (4E8) at centre, fading into yellow (3A8) at margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 39–45 mm, low, plane; margins low, narrow moderately wide (3 mm), entire; mycelia white; texture velutinous, with floccose mycelia present; sporulation moderately dense to dense, conidia *en masse* greyish green (25F4–25F5–25E5); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at centre, greyish green (1C6–1D6–1D7) elsewhere, margin greyish yellow (1B3).

YES, 25 °C, 7d: Colonies 31–38 mm, low moderately deep near centre, radially and concentrically sulcate, as well as random grooves present; margins low, narrow (1-2 mm), entire; mycelia white; texture velutinous, with floccose mycelia present near colony centre; sporulation moderately dense, conidia *en masse* dull green (27E3–27E4), in less dense sporulating regions greyish green (27C2–27C3); exudate absent in most isolates, although some producing minute droplets similarly coloured as on CYA, soluble pigment absent, reverse pigmentation Golden yellow to orange (5B7–5B8) at centre, orange yellow (4B8), becoming yellow (3B6) nearing the margin.

G25N, 25 °C, 7d: Colonies 7–10 mm, raised at centre, lightly sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3B6) at centre, olive (3D5) near margin.

CREA, 25 °C, 7d: Colonies 18–20 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, monoverticillate subterminal branches common; stipes rough walled to warted, 80–350 × 2–3 µm; branches divergent, 25–85 × 2–3 µm; metulae 2–4, divergent, 20–67° [36.5±8.9°], 14–31 × 2–3 [20.9±3.7 × 2.5±0.25] µm, vesicle 3–5 [4±0.5] µm; phialides ampulliform, 8–14 per metula, 6.5–10 × 2.5–3.5 [8.3±0.68 × 3±0.27] µm; conidia spinose to heavy and rough walled, spheroid, 2.5–3 × 2.5–3 [2.8±0.1 × 2.8±0.1] µm, average width/length = 0.98±0.02, n = 61.

Notes — *Penicillium xanthomelinii* characteristically produces rough walled to warted, divergent and irregular conidiophores and has spinose conidia. *Penicillium melinii*, also isolated from this study, is its closest relative. However, it grows slower on most media. In addition, *P. melinii* produce brown colors in colonies that are absent in *P. xanthomelinii*. Conidia on average are slightly smaller in *P. xanthomelinii*. Phylogenetically *P. xanthomelinii* strains form a coherent group separate from *P. melinii* and its previously assigned synonyms (FIGURE 2, 3).

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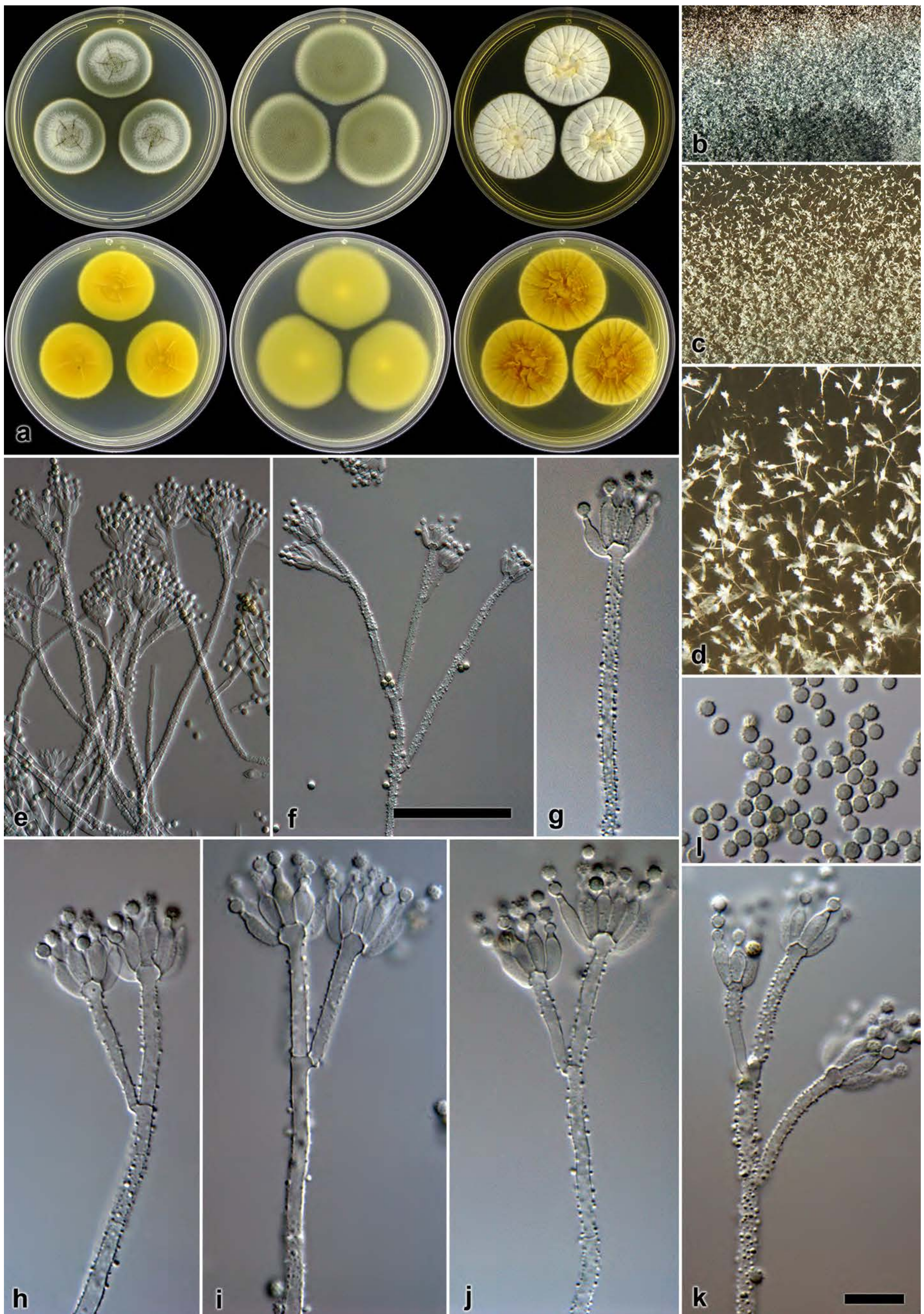

PLATE 18. *Penicillium xanthomelinii* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in f = 50 µm, applies to e, f; — Scale bar in k = 10 µm, applies to f–l).

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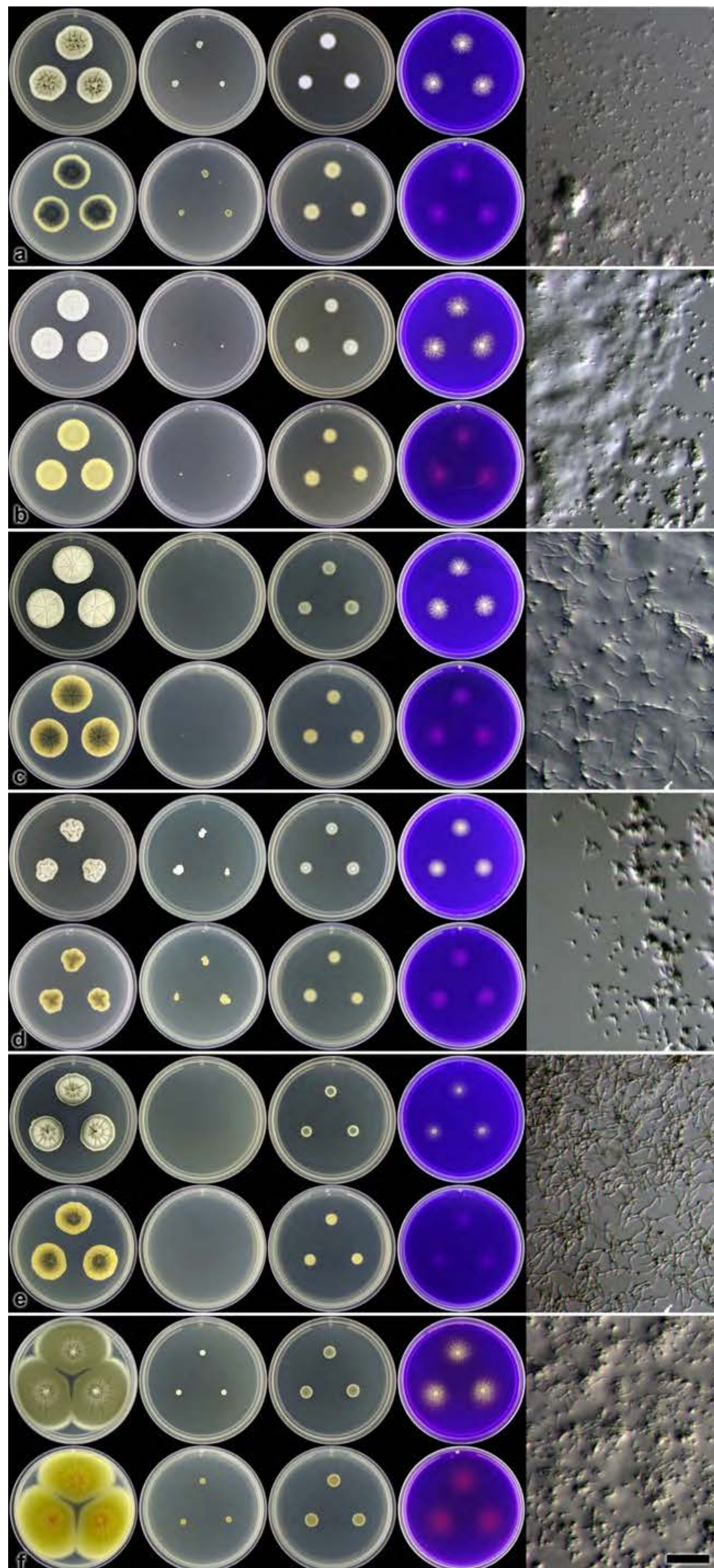

PLATE 19. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium atrolazulinum* CV244. b. *P. atrolazulinum* CV120. c. *P. consobrinum.* d. *P. corylophilum*. e. *P. cravenianum*. f. *P. hemitrachum*.

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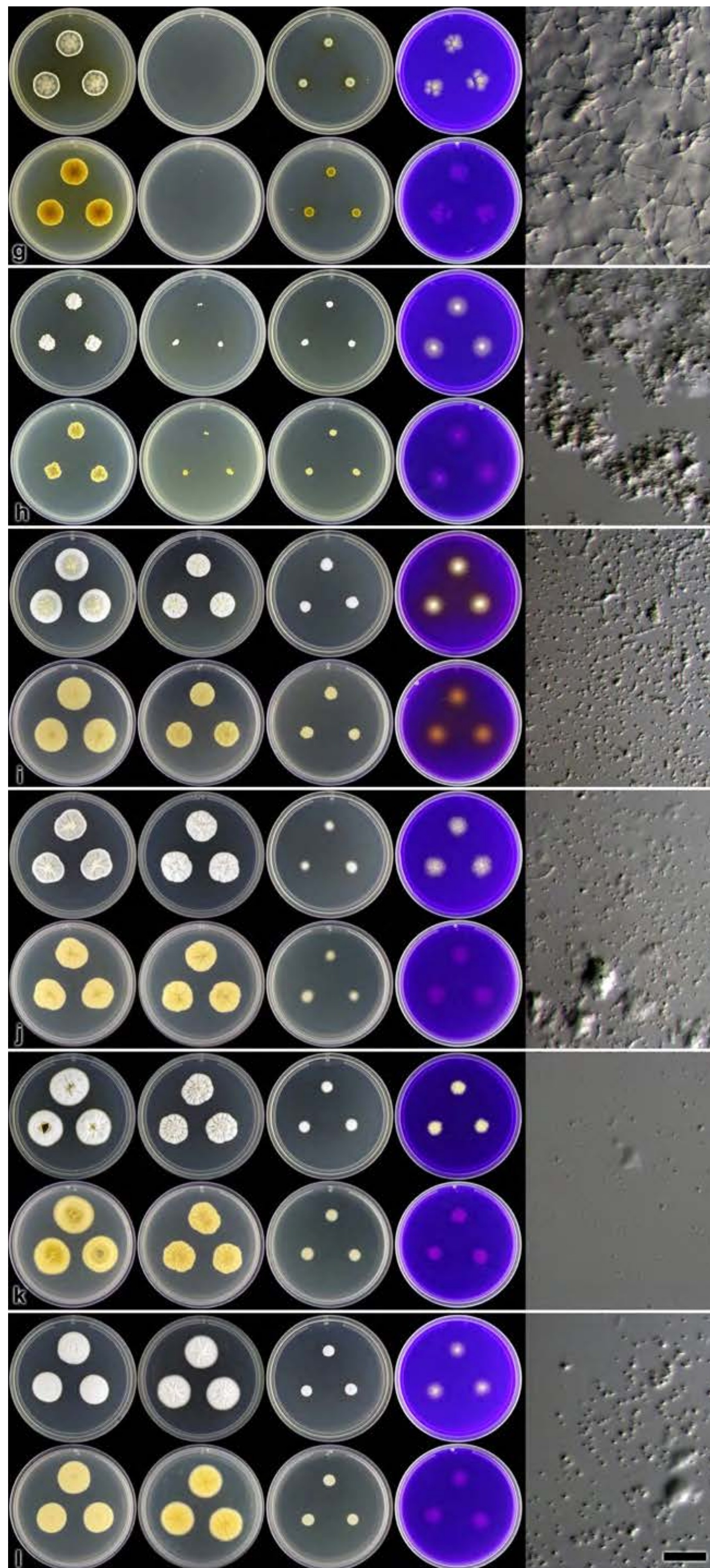

PLATE 19. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). g. *Penicillium melinii*. h. *P. pagulum*. i. *P. restrictum* CV90. j. *P. restrictum* CV93. k. *P. restrictum* CV932. l. *P. restrictum* CV948.

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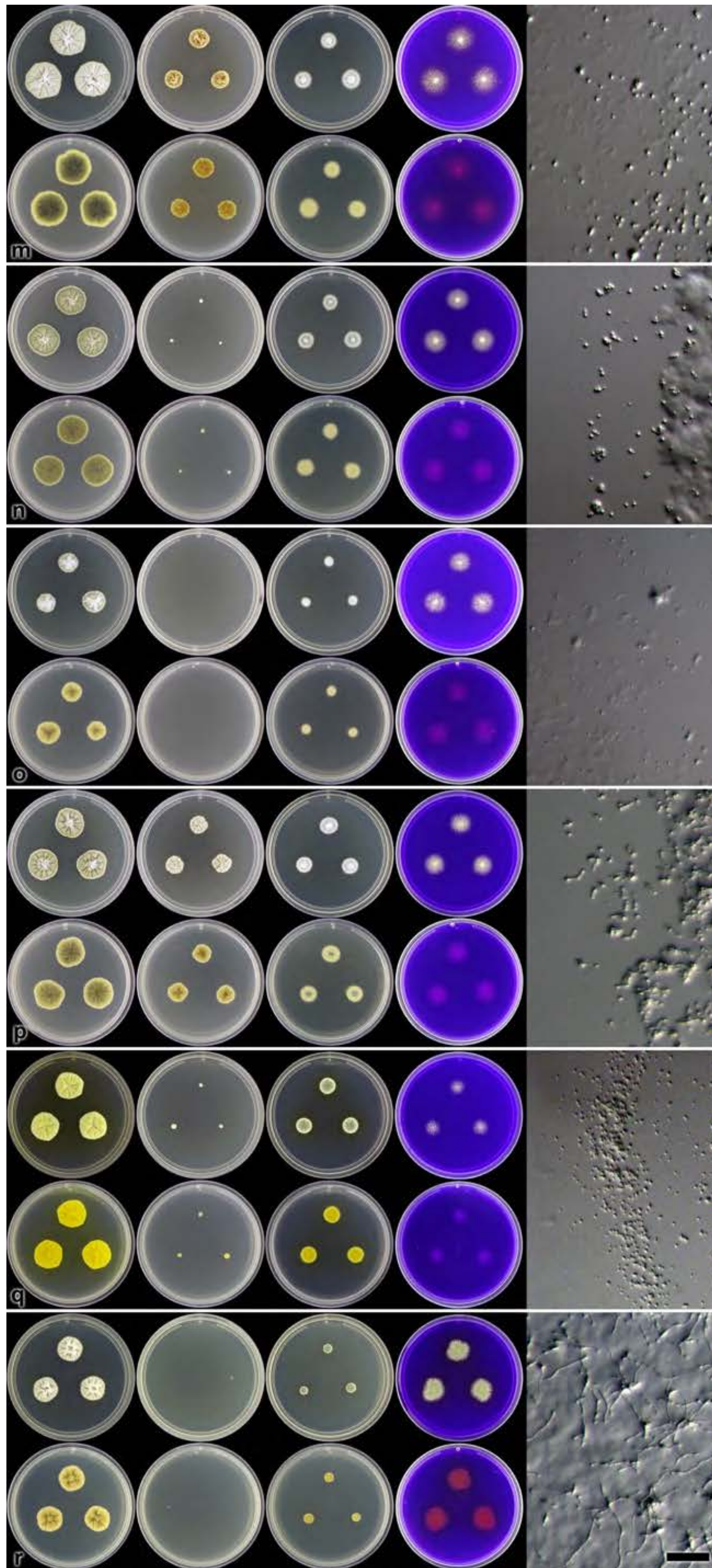

PLATE 19. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). m. *P. rubefaciens* CV1015. n. *P. rubefaciens* CV1495. o. *P. rubefaciens* CV597. p. *P. rubefaciens* CV795. q. *Penicillium toxicarium.* r. *P. xanthomelinii*.

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The section *Lanata-Divaricata* Thom

The Penicillia: 328. 1930.

TAXONOMIC NOVELTIES: *Penicillium annulatum* prov. nom., *P. brachycaulon* prov. nom., *P. malacosphaerula* prov. nom.

SPECIES TREATED: *Penicillium cremeogriseum, P. oxalicum, P. skrjabinii*

Thom (1930) introduced section *Lanata-Divaricata* to accommodate species that produce biverticillate conidiophores that usually contain a prolongation of the conidiophore's main axis and metulae that diverge from this axis to form an asymmetrical verticil. The conidiophores can thus often be interpreted as monoverticillate, although they are in most cases biverticilliate conidiophores with very divergent branched groups so that they appear monoverticillate. This group of species is commonly considered as soil fungi (Thom 1930, Raper & Thom 1949, Pitt 1979, Ramirez 1982, Christensen *et al*. 2000, Houbraken *et al*. 2011a), although sometimes members are encountered in rotting leaf litter (Houbraken *et al*. 2011a). *Penicillium janthinellum*, the type for the section, and *P. simplicissimum* are historically interesting species (Houbraken & Samson 2011). These species have broad species concepts, and Pitt (1979) synonymized nine species with *P. janthinellum* and ten with *P. simplicissimum.* Stolk & Samson (1983), what was later showed to be erroneous, synonymized *P. janthinellum* and *P. simplicissimum* and linked them and 24 other species to the teleomorph *Eupenicillium javanicum.* However, phylogenetic data have shown that this is a species complex that contains a number of unique species (Peterson 2000, Tuthill *et al*. 2001, Houbraken *et al*. 2011a, Houbraken & Samson 2011), with morphological differences that are very difficult to observe. All these species grow rapidly on most media and have typical floccose colony textures. Houbraken *et al*. (2011) accepted 38 species in section *Lanata-Divaricata,* including five new species isolated from Columbian forest leaf litter. They also proposed that a revision of the *P. janthinellum* species complex is necessary.

Unfortunately, complete datasets for Calmodulin and RPB2 is not available for this section. However, β-tubulin is here considered sufficient for conclusions on species identifications and delineation. Isolations from the Fynbos biome resulted in the isolation of six species from the section *Lanata-Divaricata*. Three of these species were identified as *P. oxalicum*, *P. skrjabinii* and *P. cremeogriseum*. Three of the species displayed unique characters, which could not be identified and are described here as new species. Phylogenetic analysis confirmed morphological observations, and resolved the three species in distinct clades. Phylogenetically, strains did not match any sequenced strains from other unpublished studies (Houbraken, unpubl.).

Penicillium annulatum, most closely resemble morphological characters of *P. janthinellum* and *P. simplicissimum*. Phylogenetically, it does not match any strains previously sequenced. Its closest relative is *P. rolfsii*, although this species grows much faster than the Fynbos species. *Penicillium rolfsii* also typically grows very well at 37 °C, reaching up to 70 mm (Pitt 1979), compared to P. *annulatum* reaching only 18 mm. Based on morphology the species resembles *P. simplicissimum* based on original descriptions of Oudemans & Konings (1902) and Jensen (1912), which is also characterized by the production the rings of sporulation. *Penicillium annulatum* typically produces rough-walled conidiophores, a character variably documented in literature for both *P. janthinellum* and *P. simplicissimum* (Thom 1930, Raper & Thom 1949, Pitt 1979, Ramirez 1982, Stolk & Samson 1983). The confusion in the latter two species makes it difficult to morphologically distinguish *P. annulatum*. However, based on phylogenetic data the species clearly represents a novel species (FIGURES 6, 7).

Penicillium malacosphaerula is closely related to *P. reticulisporum*. Their colony morphologies are similar. However, their sexual states are distinct. *Penicillium reticulisporum* produces hard cleistothecia of 80–250 µm in diameter (Pitt 1979, Stolk & Samson 1983). In comparison, the Fynbos species characteristically produce soft cleistothecia that are smaller than 150 µm. Also, *P. malacosphaerula* grows very well at 37 °C, compared to the weaker growth displayed in *P. reticulisporum*. Phylogenetically, *P. malacosphaerula* consistently resolved in a distinct clade from *P. reticulisporum* (FIGURES 6, 7).

Penicillium brachycaulon is another novel species from this group and is closely related to *P. janthinellum* and *P. raperi* based on phylogenetic characters. Morphologically, *P. brachycaulon* resemble *P. raperi* more closely than *P. janthinellum*. *Penicillium brachycaulon* display slow growth rates on both CYA (23–26 mm) and MEA (19–21 mm), compared to *P. janthinellum* that grows much faster on both media (>35 mm). *Penicillium brachycaulon* also has monverticillate conidiophores that are borne irregularly down the length of mycelia without a terminal verticil, compared to both *P. janthinellum* and *P. raperi* that produce terminal verticils. Growth rate at 37 °C also distinguishes *P. brachycaulon* and *P. raperi* from *P. janthinellum*. Compared to *P. brachycaulon*, *P. raperi* produce longer phialides and somewhat bigger conidia (Smith 1957). The latter species also showed faster growth rates on CYA and MEA (Ramirez 1982), compared to that of the Fynbos species. Phylogenetically, *P. brachycaulon* is confirmed as

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unique and distinct from all known species (FIGURES 6, 7).

The strains identified as *P. cremeogriseum*, displayed slight sequence variation from the ex-type culture (CBS223.66T). This was especially evident in the Elongation Factor 1-α phylogeny (FIGURE 7). However, the strains were morphologically similar to the ex-type of *P. cremeogriseum* (Chalabuda 1950). Strains identified as *P. oxalicum* and *P. skrjabinii* displayed no variation in morphology or sequence data compared to reference strains and were identified as such.

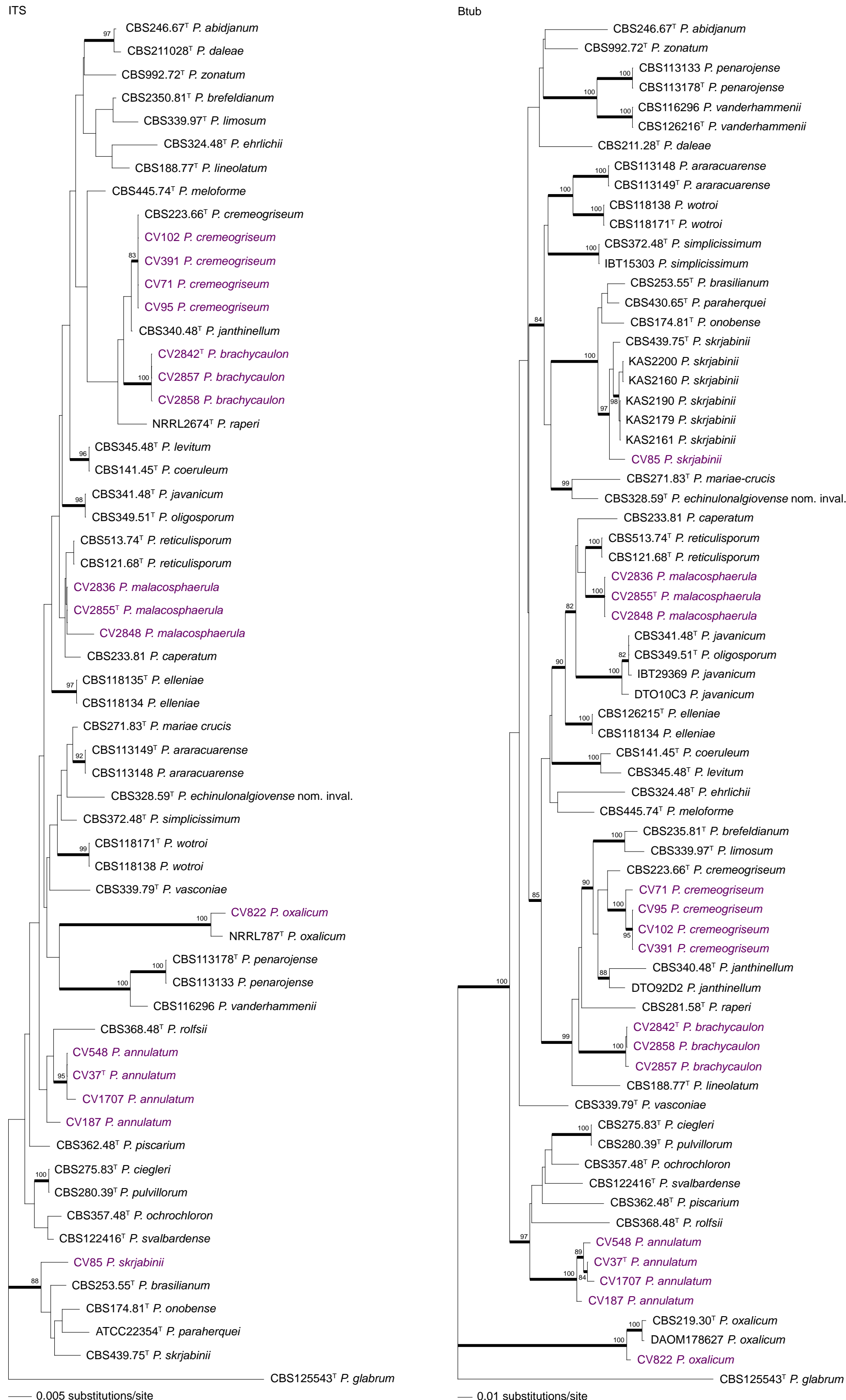

FIGURE 6: Phylogenetic trees based on ITS and β-tubulin, showing relationship of species in the section *Lanata-Divaricata*. *Penicillium glabrum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type). Colored names indicate strains isolated from Fynbos.

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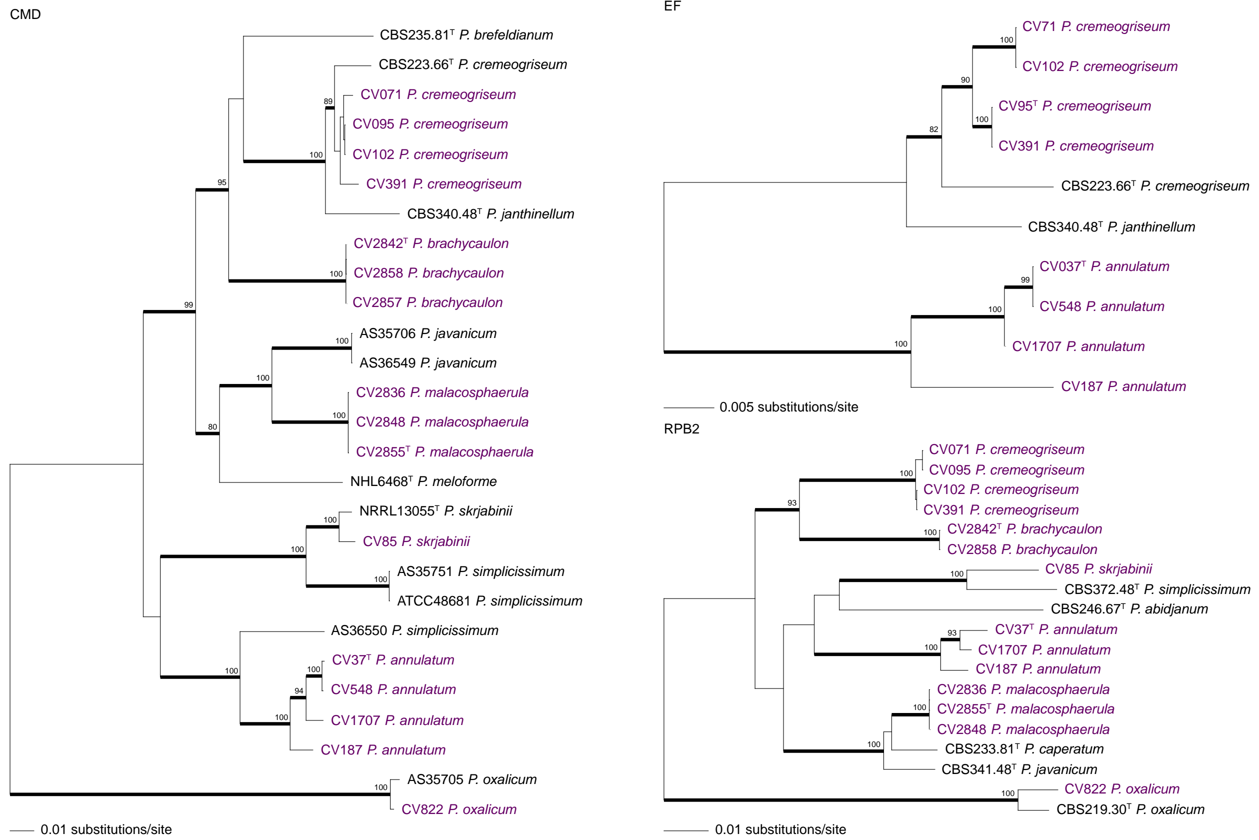

FIGURE 7: Phylogenetic trees based on Calmodulin, Elongation Factor 1α and RPB2 showing relationship of species in the section *Lanata-Divaricata*. *Penicillium oxalicum* was chosen as outgroup in the Calmodulin and RPB2 phylogenies, with *P. annulatum* the outgroup for Elongation Factor $1\text{-}\alpha$. Bootstrap values above 80% are indicated above thick branches. ([T] = ex-type). Colored names indicate strains isolated from Fynbos.

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12. ***Penicillium annulatum*** Visagie **prov. nom.**

PLATES 20, 21, 32a

ETYMOLOGY: Latin, *annulatum* = meaning surrounded by rings; named after the rings of sporulation observed in colonies
EX-TYPE: CV37 = DTO180G7 = KAS4119
TYPE ISOLATED FROM: Air sample, Stellenbosch
ADDITIONAL SPECIMENS EXAMINED: CV187, CV548, CV1707.
ISOLATED FROM: Soil, Stellenbosch and Struisbaai; *Protea repens* infructescence, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 45–48 mm, low to moderately deep, radially and concentrically sulcate, forming rings of areas where no sporulation and sporulation lies adjacent to each other; margins low, narrow (2 mm), entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* dull green (25E4–26E4); exudate dark red, soluble pigment absent, reverse pigmentation some isolates having dark ruby (12F8) areas, light to greyish orange (5A5–5B5–5B6).

CYA 5 °C, 7d: No germination.

CYA 30 °C, 7d: Colonies 45–55 mm, low, lightly radially sulcate, forming rings of areas where no sporulation and sporulation lies adjacent to each other; margin low, narrow (2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25E4–25E6), sometimes (26E4–25E6); exudate sometimes dark red droplets present, soluble pigment absent, reverse pigmentation dark ruby (12F8) in central areas for isolate CV37, otherwise greyish orange (5B4–5B6) fading into pale yellow (4A3) margin.

CYA 37 °C, 7d: Colonies 13–18 mm, deep, radially sulcate, sunken in at centre; margins moderately deep, narrow, irregular; mycelia white; sporulation absent; exudate absent, soluble pigment absent, reverse pigmentation brownish orange (5C4–5C6).

MEA, 25 °C, 7d: Colonies 29–38 mm, low to moderately deep, plane; margins low, narrow (2 mm), irregular; mycelia white; texture floccose; sporulation moderately dense to dense, conidia *en masse* greyish green (25E6–25E7); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B3–2B4).

YES, 25 °C, 7d: Colonies 47–52 mm, low, lightly radially sulcate, rings present, but less obvious than those on CYA; margins low, narrow (2 mm), entire; mycelia white; texture floccose; sporulation sparse to moderate, conidia *en masse* greyish green (25B4–25B5), greyish green (30D5) areas also present; exudate absent, soluble pigment absent, reverse pigmentation dull yellow to yellowish white (3B4–3B3–3A3).

G25N, 25 °C, 7d: Colonies 9–12 mm, low, lightly radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish green (25D5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3C4) at centre, margin yellowish white (2A2).

CREA 25 °C, 7d: Colonies 28–33 mm, moderate acid production in some isolates.

Micromorphology — Conidiophores bi-and terverticillate, with some degree of side braches produced; stipes rough walled, 180–750 × 3–4.5 µm; rami/branches divergent, 6–40 × 3–4.5 [19.8±6.73]; metulae 3–6 per stipe/branch, appressed to divergent, angle 28–80° [52±11.3°], great variation in length in the same conidiophore, 8–20 × 2.5–4.5 [11.5±1.89 × 3.5±0.43] µm, vesicle 3–4.5 [3.38±0.41] µm; phialides ampulliform, 4–5 per metula, 6–8 × 2–3.5 [7.1±0.5 × 2.8±0.25]; conidia rough walled, spheroid to some subspheroidal, 2.5–3 × 2–3 [2.5±0.12 × 2.4±0.13] µm, average width/length ± stdev = 0.93±0.04, n= 116.

Notes — *Penicillium annulatum* is characterized by fast growing colonies on most media. Varying degrees of sporulation are typically observed on CYA and YES, which form "rings" on colonies. The species consistently produce rough walled conidiophores with rough walled conidia. It closely resemble the morphology of species in the *P. janthinellum* / *P. simplicissimum* species complex. Phylogenetic data, however, confirms this as an undescribed species (FIGURES 6, 7).

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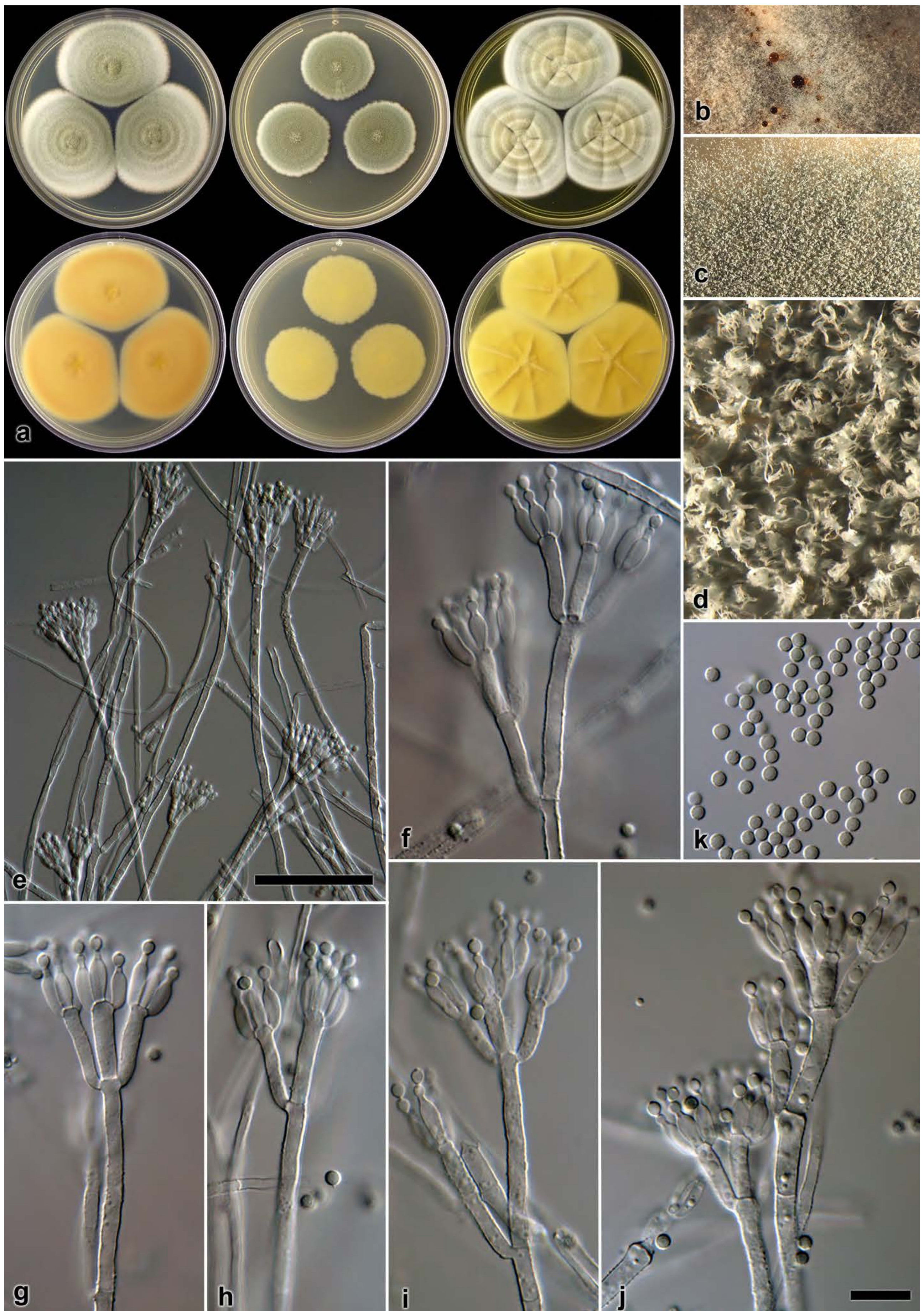

PLATE 20. *Penicillium annulatum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in e = 100 µm; — Scale bar in j = 10 µm, applies to f–k).

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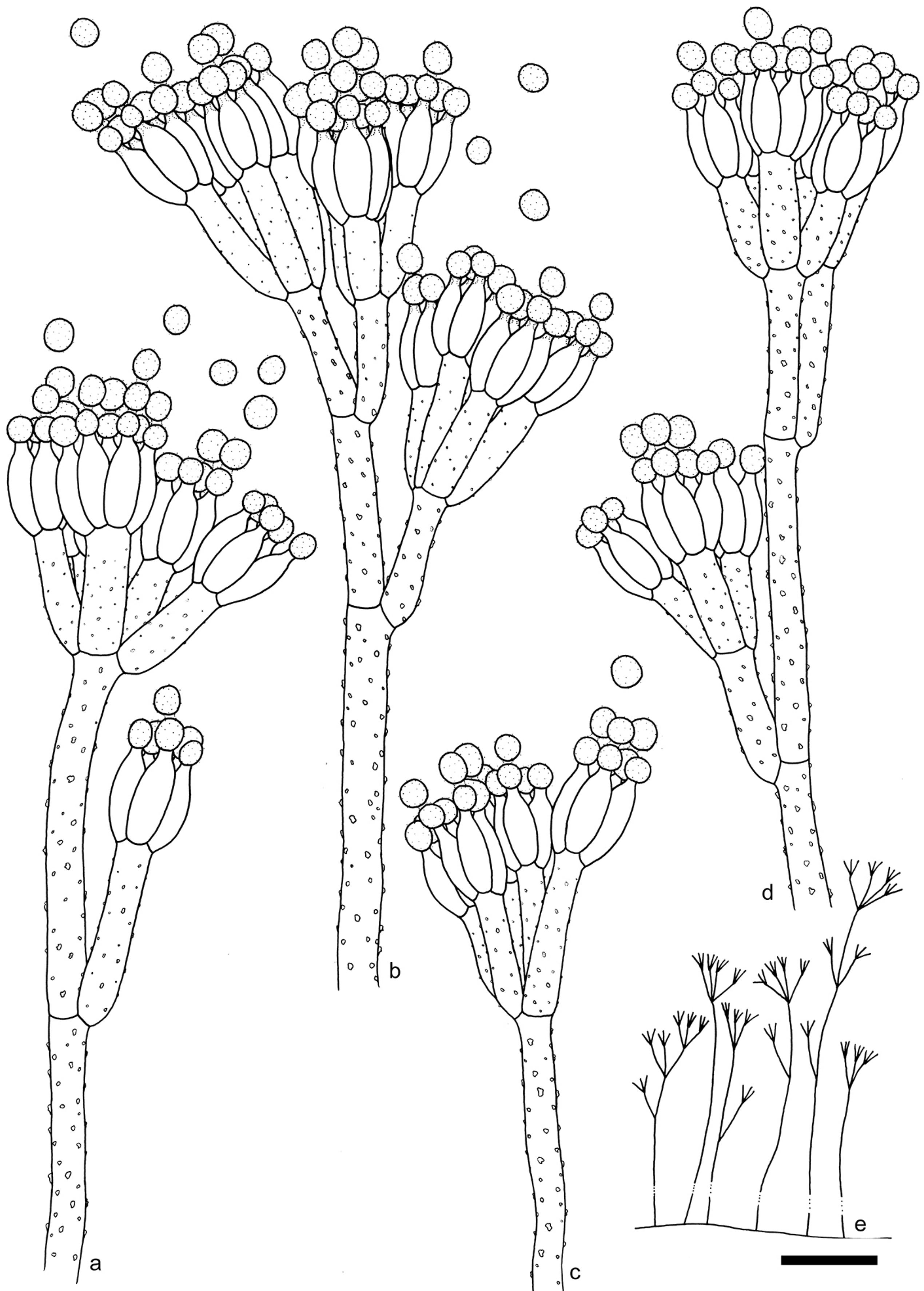

PLATE 21. Line drawing of *P. annulatum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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13. ***Penicillium brachycaulon*** Visagie **prov. nom.**

PLATES 22, 23, 32b

ETYMOLOGY: Latin, *brachycaulon*: *brachys* = short, *caulon* = stem; referring to the short stipes produced by this species
TYPE: PREM60045
EX-TYPE: CV2842 = DTO180D3 = DAOM241159 = KAS3937
TYPE ISOLATED FROM: Soil, Malmesbury, South Africa
ADDITIONAL SPECIMENS EXAMINED: CV2857, CV2858.
ISOLATED FROM: Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 23–26 mm, low to moderately deep, radially sulcate, randomly furrowed as well; margins low, narrow (2 mm), yellowish olive color; mycelia white; texture floccose; sporulation sparse to moderate, conidia *en masse* dull green (25D3–25D4); exudate absent, soluble pigment absent, reverse pigmentation olive yellow to olive to olive brown (3D7–3D8–4D8–4D7).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 27–31 mm, low to moderately deep, irregular furrows present; margins low, narrow (1–2 mm), having a yellowish olive color, entire; mycelia white; texture floccose; sporulation sparse, conidia *en masse* greyish turquoise (25C3); exudate absent, soluble pigment absent, reverse pigmentation brown (6E7–6E8) at centre, olive brown (4D8) and greenish yellow (1A7) elsewhere.

CYA, 37 °C, 7d: Microcolonies.

MEA, 25 °C, 7d: Colonies 19–21 mm, low, plane, slightly raised at centre; margins low, narrow (2–3 mm); mycelia white; texture floccose, loosely funiculose; sporulation moderately dense, conidia *en masse* greyish to dull green (25C3–25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation olive to yellowish brown (4E4–5E4) at centre, margin greyish yellow (3B6).

YES, 25 °C, 7d: Colonies 30–35 mm, low, radially sulcate, randomly furrowed as well; margins low, narrow (1–2 mm), entire, yellowish olive color; mycelia white; texture floccose; sporulation sparse to moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation similar to CYA.

G25N, 25 °C, 7d: Colonies 6–8 mm, raised at centre, plane; margins subsurface to low, very narrow, entire; mycelia white; sporulation absent; exudate absent, soluble pigment absent, reverse pigmentation yellowish white (2A2).

CREA, 25 °C, 7d: Colonies 20–25 mm, acid not produced.

Micromorphology — Conidiophores mostly very short monoverticillate conidiophores, although this might be considered as side branches of a very divergent conidiophore, very few true biverticillate conidiophores ever observed, coiled mycelia sometimes observed; stipes/metulae smooth walled, 9–20 × 2–3.5 [13.3±3.5 × 2.6±0.33] µm, vesicle 2.5–3.5 [2.8±0.28] µm, when definitely biverticillate stipes 50–200 µm, only 2 metulae present, often a solitary phialide borne on same level as metula; phialides 1–5, ampulliform, 5–7.5(–8) × 2–3 [6.4±0.78 × 2.8±0.28] µm; Conidia rough walled, broadly ellipsoid, 2–3 × 2–2.5 [2.6±0.17 × 2.2±0.14] µm, average width/length = 0.86±0.05, n = 77.

Notes — *Penicillium brachycaulon* typically displays weak growth on all media, with colonies that have an olive reverse pigmentation. Conidiophores can be interpreted as either monoverticillate with very short stipes, or as a conidiophore with a very divergent nature. Phialides borne directly on hyphae are not uncommon and terminal conidiophores are almost non-existent. It is closely related to *P. janthinellum* and *P. raperi,* which share many of these characters. However, restricted growth easily distinguishes the new species from *P. janthinellum*. The shorter phialides of *P. brachycaulon*, easily distinguishes it from *P. raperi* (7–9 µm).

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PLATE 22. *Penicillium brachycaulon* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–j. Conidiophores. k. Conidia (— Scale bar in d = 10 µm; — Scale bar in j = 10 µm, applies to e–k).

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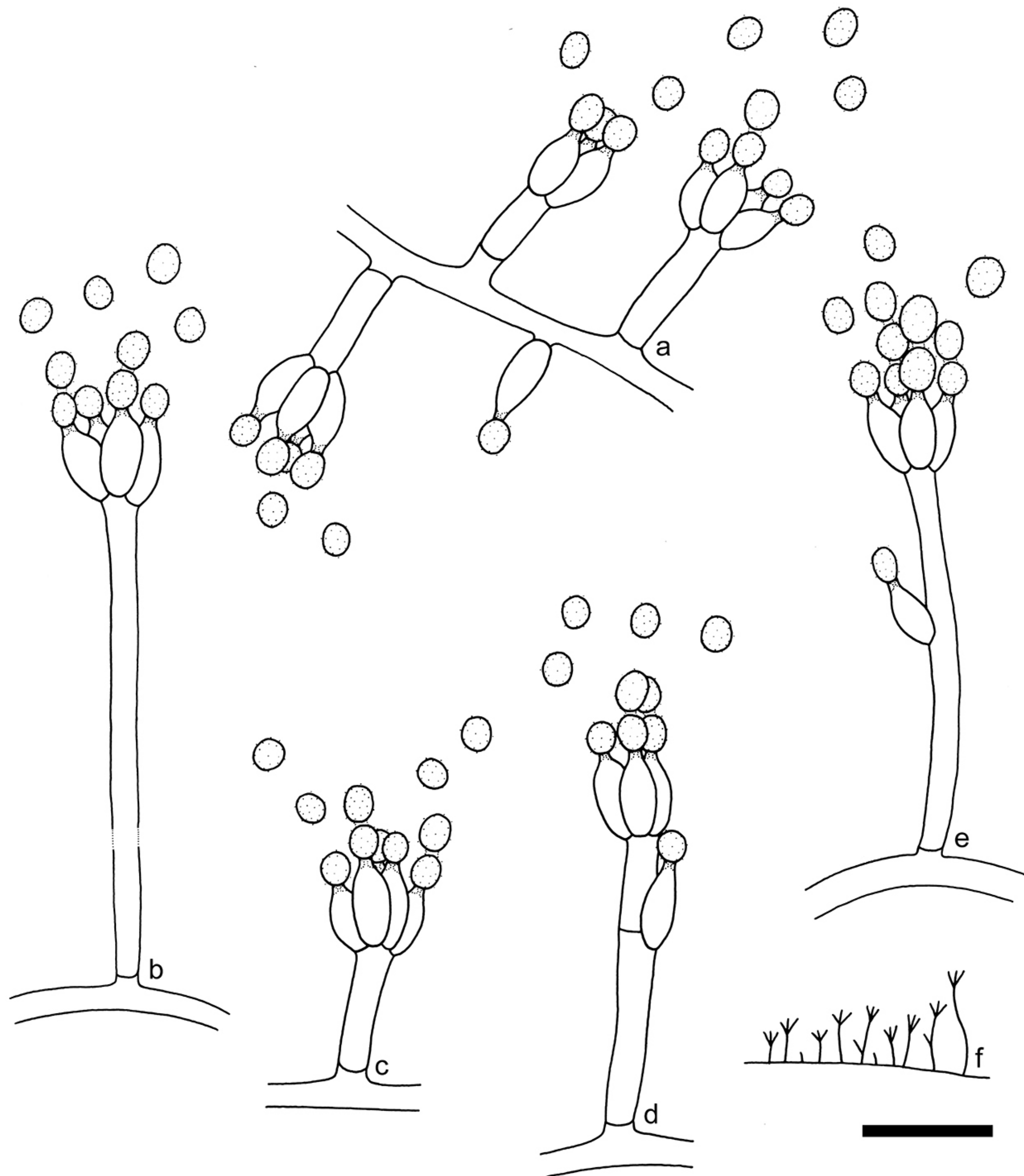

PLATE 23. Line drawing of *P. brachycaulon*. a–e. Conidiophores (— Scale bar = 10 µm). f. Conidiophore branching (— Scale bar = 50 µm).

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14. *Penicillium cremeogriseum* Chalabuda

PLATES 24, 25, 32c

Not. Syst. Crypt. Inst. Bot. Acad. Sci. USSR 6: 168. 1950.

EX-TYPE: CBS223.66 = NRRL3389 = ATCC18323
TYPE ISOLATED FROM: Soil, Kiev, Ukraine
ADDITIONAL SPECIMENS EXAMINED: CBS223.66, CV71, CV95, CV102, CV391.
ISOLATED FROM: Soil and *Protea repens* infructescence, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 40–45 mm, moderately deep, lightly radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white at margin, light yellow elsewhere; texture floccose; sporulation absent to sparse, conidia *en masse* dull green (26D3); exudate mostly absent, but sometimes clear, soluble pigment yellow, reverse pigmentation olive (3E8) becoming Lemon Yellow (3B8) near margins, brownish orange (7C6) areas also present.

At 5 °C, 7d: Germination.

At 30 °C, 7d: Colonies 53–56 mm, moderately deep, radially sulcate, having a yellow colour; margins low, narrow 2–3 mm, entire; mycelia white; sporulation absent; exudate yellow, soluble pigment absent, reverse pigmentation (5F7–5F8) at colony centre due to structures that seem to resemble that of sclerotia, although not sclerotia, elsewhere olive to dark yellow (3C6–3C8–4C6–4C8), with yellow (2A6) margin.

At 37 °C, 7d: Colonies 31–36 mm, moderately deep, radially and concentrically sulcate, having a pale yellow (1A3) colour; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation sparse, only near colony margins, conidia *en masse* greenish grey (29C2–30B2); exudate yellow, soluble pigment yellow, reverse pigmentation olive brown (4E8) at centre, fading into olive to greyish yellow (3C6–4C6).

MEA, 25 °C, 7d: Colonies 45–57 mm, moderately deep, plane; margins low, narrow, entire; mycelia white at margin, light yellow elsewhere; texture floccose; sporulation sparse to moderate, conidia *en masse* dull green (26D3); exudate absent, soluble pigment absent, reverse pigmentation olive yellow (3C8) becoming yellow (3A7) near margins.

YES, 25 °C, 7d: Colonies 40–48 mm, moderately deep, radially sulcate, also randomly furrowed; margins low, very narrow (1–2 mm), entire; mycelia white at margin, light yellow elsewhere; texture floccose; sporulation sparse to sometimes absent, conidia *en masse* dull green (26D3); exudate absent, soluble pigment absent, reverse pigmentation areas of brown to dark brown (5F8–6F8), elsewhere varying from greyish yellow to orange yellow (4B6–4B8), margins light yellow (4A4).

G25N, 25 °C, 7d: Colonies 11–14 mm, raised at centre, plane, having a yellowish color; margins low, narrow, entire; mycelia white; texture floccose; sporulation sparse, conidia *en masse* greyish green (25B3); exudate absent, soluble pigment very light yellowish pigment produced, reverse pigmentation light yellow to yellow (2A4–2A6) at centre, yellowish white (2A2) elsewhere.

CREA 25 °C, 7d: Colonies 29–33 mm, no acid produced.

Micromorphology — Conidiophores biverticillate with large number of subterminal side branches formed which can be mono- or biverticillate; stipes smooth walled, 150–550 × 2–3 µm, side branched "conidiophore" stipes 18–90 µm; metulae 2–4, mostly divergent, angle 19–50° [34±9.2°], 10–30 × 2–3 [18.1±4.91 × 2.7±0.27] µm, vesicle 2.5–4 [3.1±0.33] µm; phialides ampulliform, 3–7 per metula, 6.5–9(–10) × 2–3 [7.6±0.75 × 2.8±0.21] µm; conidia smooth walled, spheroid to subspheroid, 2.5–3.5 × 2.5–3.5 [2.9±0.2 × 2.7±0.2] µm, average width/length = 0.92±0.04, n = 76.

Notes — The species is characterized by rapidly spreading colonies, including those growing at 37 °C. Microscopically it produces irregular divergent conidiophores with smooth elements. This species used to be considered a synonym of *P. janthinellum* (Pitt 1979) and *P. simplicissimum* (Stolk & Samson 1983). However, based on phylogenetic data, Houbraken & Samson (2011) considered it a valid species. The Fynbos strains did show some sequence variation from the ex-type strain (FIGURES 6, 7), however, not enough to validate separate species. Morphologically, no distinction could be made between the ex-type culture and Fynbos strains.

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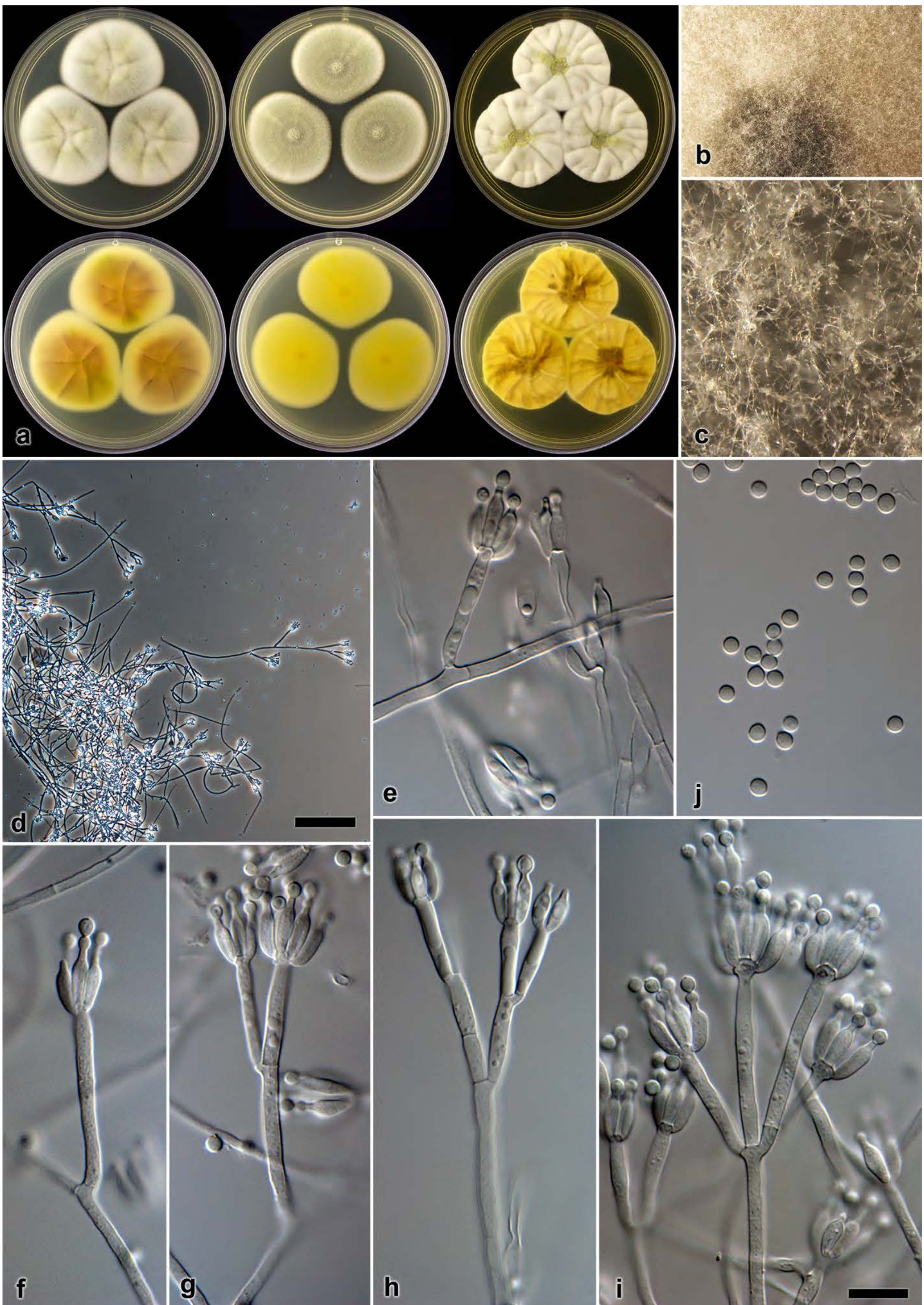

PLATE 24. *Penicillium cremeogriseum.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 10 µm; — Scale bar in i = 10 µm, applies to e–j).

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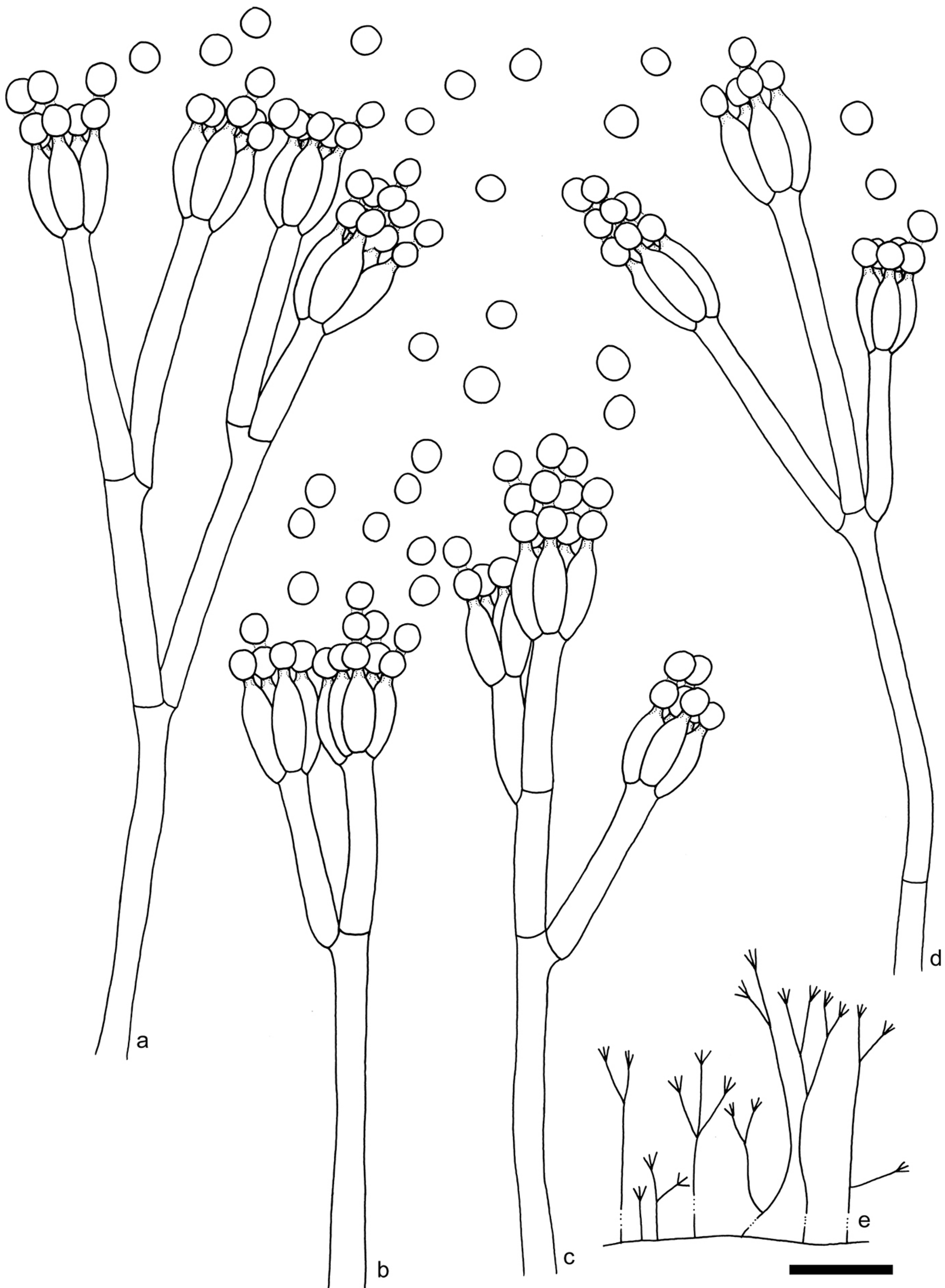

PLATE 25. Line drawing of *P. cremeogrisuem.* a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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15. *Penicillium malacosphaerula* Visagie **prov. nom.** PLATES 26, 27, 32d

ETYMOLOGY: Latin, *malacosphaerula*: *malacos* = soft, *sphaerula* = small ball; referring to the soft yellow ascocarps produced
TYPE: PREM60054
EX-TYPE: CV2855 = DTO180E6 = DAOM241161 = KAS3947
TYPE ISOLATED FROM: Soil, Malmesbury, South Africa
ADDITIONAL SPECIMENS EXAMINED: CV2848, CV2836.
ISOLATED FROM: Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 28–33 mm, low to moderately deep, radially and concentrically sulcate; margins low, wide (3 mm), entire; mycelia white and inconspicuously yellow; texture floccose; sclerotia produced especially near colony centre, giving colony a greyish yellow (4C4) color, after 4 weeks developing into cleistothecia, sporulation absent; exudate dark brown (8F8), absent in CMV271, soluble pigment bright yellow, reverse pigmentation yellowish brown (5D7–5E7) at centre, fading into greyish yellow (4B5) into a yellow (2A7–2A8) margin.

CYA, 5 °C, 7d: Mostly no germination, but some conidia do germinate.

CYA, 30 °C, 7d: Colonies 36–45 mm, all characters similar to that of colonies grown at 25 °C.

CYA, 37 °C, 7d: 34–37 mm, low to moderately deep, radially and concentrically sulcate; margins low, narrow (1 mm), somewhat irregular; mycelia white; texture floccose; sclerotia abundantly produced, giving a greyish yellow (4C4) color to colonies; sporulation moderate, conidia *en masse* dull green (26D3); exudate a few clear droplets produced, soluble pigment bright yellow, greyish green (1C5) at centre, elsewhere greenish to greyish yellow (1A7–1B7), in isolate CMV112 brownish orange (5C5) at centre.

MEA, 25 °C, 7d: Colonies 26–29 mm, low, plane; margins low, narrow (1–2 mm), irregular; mycelia white, inconspicuously yellow; texture floccose; sclerotia abundantly produced which develops into mature cleistothecia after 4 weeks, color ranging from light yellow to greyish orange (2A4–5B3); sporulation absent, conidiophores developing only after 14 days of incubation, conidia *en masse* indeterminable; exudate absent, soluble pigment absent, reverse pigmentation yellow (3A8) near centre fading into yellowish white (3A2) margin.

YES, 25 °C, 7d: Colonies 33–43 mm, low, radially and concentrically sulcate, randomly furrowed as well, sunken in at centre; margins low, narrow (1–2 mm), entire; mycelia white, inconspicuously yellow; texture floccose; sclerotia abundantly produced, giving a greyish yellow (4C4) color; sporulation absent to sparse, conidia *en masse* indeterminable; exudate absent, soluble pigment yellow, reverse pigmentation mostly light yellow (2A5–3A5) with yellow (3A6–3A7) areas present.

G25N, 25 °C, 7d: Colonies 12–16 mm, raised at centre, very lightly radialy sulcate; margins low, narrow (1 mm), entire; mycelia white; texture floccose; sporulation sparse to moderate, conidia *en masse* greyish turquoise (24B3–24C3); exudate absent, soluble pigment bright yellow, reverse pigmentation brown (5E–5E8), margin light yellow (2A5).

CREA, 25 °C, 7d: Colonies 25–30 mm, acid not produced.

Micromorphology — Conidiophores irregularly biverticillate, can almost be seen as very short monoverticillate conidiophores borne on random positions on fertile hyphae; stipes smooth walled, 100–500 × 2–3 µm, sometimes shorter, then 30–80 µm; metulae mostly 2 with occasionally 3 per stipe, verticils appressed to divergent, angle 22–41° [32±6.7°], 6.5–22 × 2–3.5 [14.1±4.4 × 2.6±0.33] µm, vesicle 2.5–4 [3.3±0.34] µm; phialides ampulliform, 3–5 per metula/stipe, (6–)7–10 × 2–3(–3.5) [7.8±0.84 ×2.77±0.27] µm; conidia smooth walled, spheroid to subspheroid, 2.5–3 × 2.5–3 [2.6±0.14 × 2.4±0.16] µm, average width/length = 0.92±0.04, n = 81; cleistothecia abundantly produced on most of the media having a brownish to dark yellowish color, 50–140 × 50–130 [83±19.61 × 73±17.6] µm; asci borne singly, 5.5–10 × 4.5–7 µm; ascospores rough walled, subspheroid to broadly ellipsoidal, 2.5–3.5 × 2–3 [3±0.18 × 2.48±0.17] µm, average width/length = 0.82±0.04, n = 83.

Notes — *Penicillium malacosphaerula* characteristically produces colonies with yellow pigmentation. It produces a sexual state with asci that is not hard walled and rough walled ascospores, rather similar in size than conidia. Sporulation commonly occurs after 10 days of incubation. Its closest relative is *P. reticulisporum*, which produces hard walled cleistothecia. *Penicillium malacosphaerula* also grows faster at 37 °C (34–37 mm) compared to *P. reticulisporum* (up to 20 mm).

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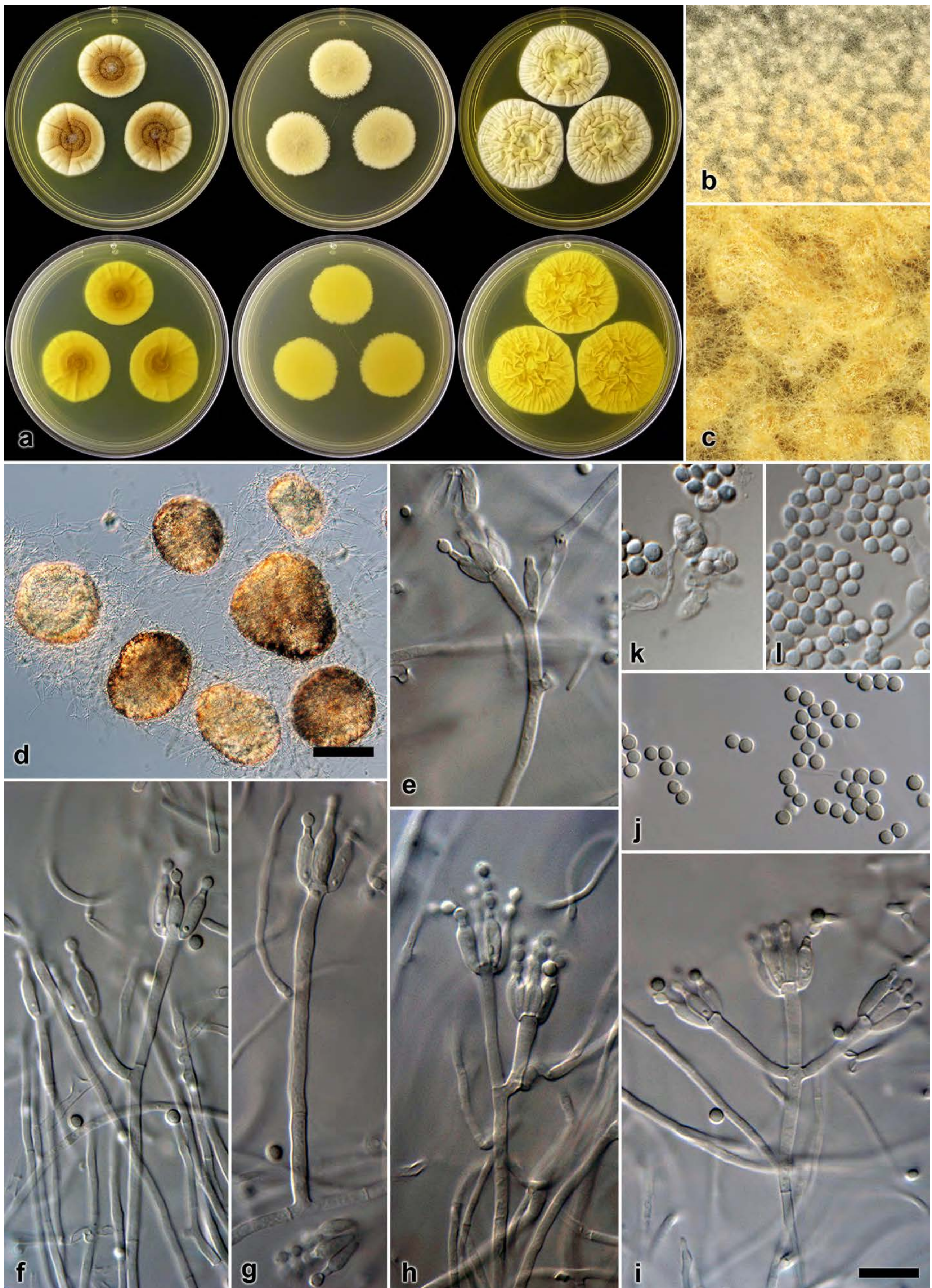

PLATE 26. *Penicillium malacosphaerula* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–d. Cleistothecia produced on MEA. e–i. Conidiophores. j. Conidia. k, l. Asci and ascospores. (— Scale bar in d = 100 µm; — Scale bar in i = 10 µm, applies to e–l).

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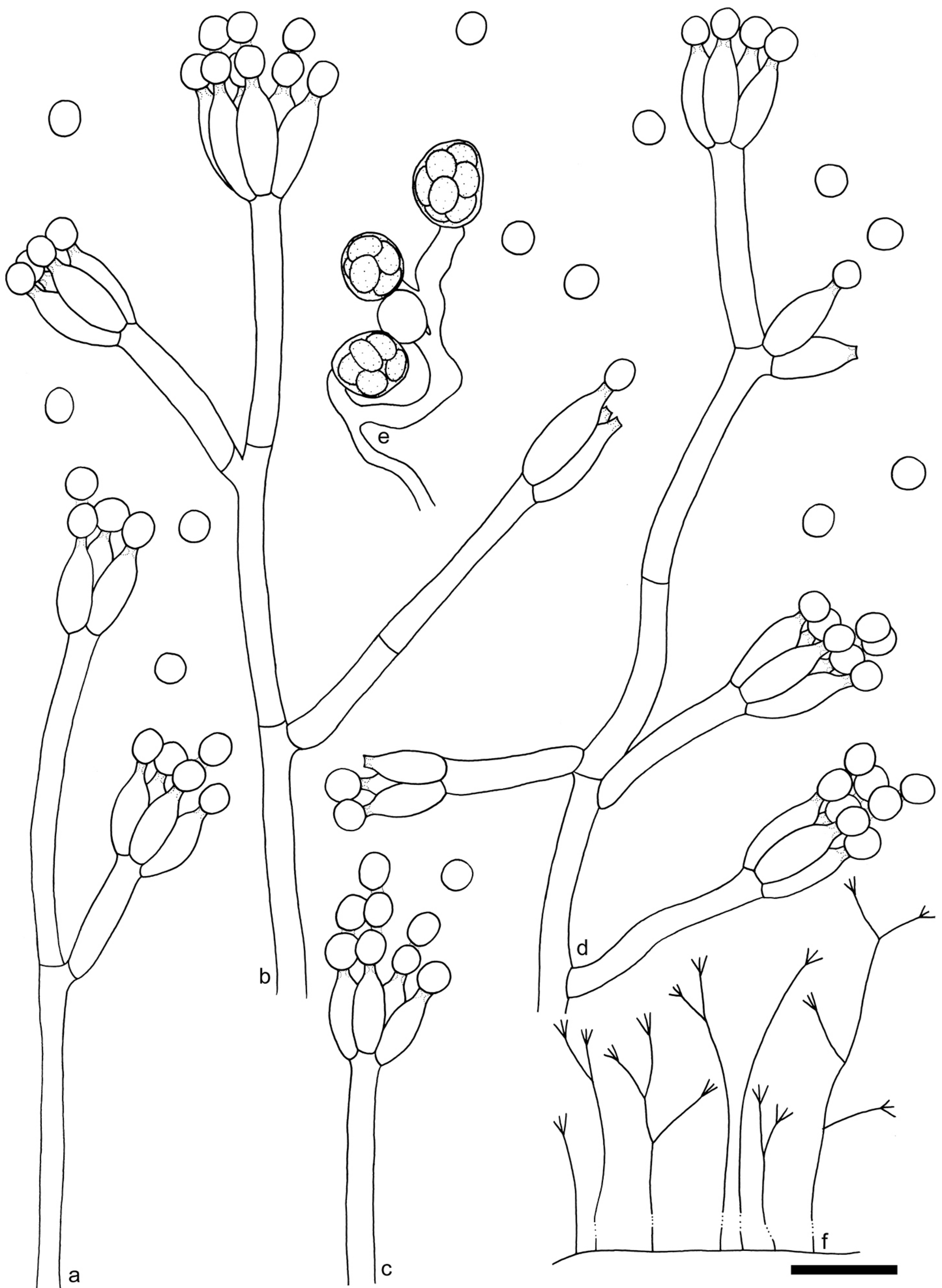

PLATE 27. Line drawing of *P. malacosphaerula*. a–e. Conidiophores and asci (— Scale bar = 10 µm). f. Conidiophore branching (— Scale bar = 50 µm).

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16. *Penicillium oxalicum* Curie & Thom PLATES 28, 29, 32e

The journal of biological chemistry 22: 289. 1915.

EX-TYPE: CBS219.30 = NRRL787
TYPE ISOLATED FROM: Soil, Connecticut, USA
SPECIMENS EXAMINED: CV822, CBS219.30.
ISOLATED FROM: Air sample, Malmesbury; commonly isolated from indoor air in the Western Cape

Macromorphology — CYA, 25 °C, 7d: Colonies 40–42 mm, rather deep due to long chains of conidia tightly packed together, plane to slightly raised at centre; margins low, narrow to sometimes wide (1–3 mm), entire; mycelia white; texture velutinous with crusts of conidia formed; sporulation very dense, conidia *en masse* dull green (26F4), greyish green (25D5) in less dense conidial areas; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B4) at centre, elsewhere areas of pale yellow (2A3) and greyish yellow (2B3–2C3).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 37–40 mm, same characters as colonies at 25 °C except for reverse pigmentation dull to greyish yellow (3B4–3B5) and light yellow (3A4–3A5) areas.

CYA, 37 °C, 7d: 22–23 mm, low, random sulcation; margins low, narrow (2–3 mm), irregular; mycelia white; texture velutinous; sporulation moderate, conidia *en masse* (27E7–28E8); exudate absent, soluble pigment absent, reverse pigmentation greyish green (1D4–1D5) at centre, margin greenish grey to greyish yellow (1B2–1B3).

MEA, 25 °C, 7d: Colonies 18–21 mm, low, plane; margins low to subsurface, narrow (1–2 mm), entire; mycelia white; texture velutinous with crusts of conidia formed; sporulation dense, conidia *en masse* dark green (27F6–27F8); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A4) at centre, elsewhere greyish to olive yellow (2B4–2C6–3C6).

YES, 25 °C, 7d: Colonies 34–37 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation light to greyish yellow (4A5–4B5–4B6).

G25N, 25 °C, 7d: Colonies 16–18 mm, low, plane; margins low, narrow (1–2 mm), entire; mycelia white; sporulation moderate, conidia *en masse* greyish green (25E6–25F6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B3) at centre, dull to greyish green (27D4–27D5) elsewhere.

CREA, 25 °C, 7d: Colonies 27–35 mm, strong acid production.

Micromorphology — Conidiophores almost exclusively biverticillate, with very few monoverticillate, and very few having subterminal verticils; Stipes smooth walled, 100–320 × 2.5–4 µm; Metulae 2–4 (–5), closely appressed, angle 7–23° [13.13±5.18°], 12–25 × 3–4.5 [18.6±2.4 × 3.6±0.33] µm, vesicle 3–4.5 [3.79±0.36] µm; Phialides ampulliform–acerose with very short neck, 4–5 per metula, 9–13 × 2.5–4 [11.1±0.92 × 3.3±0.29] µm; Conidia finely rough walled pattern forming ridges, ellipsoidal, 4–5(–6) × 2–4 [4.4±0.31 × 3±0.26] µm, average width/length = 0.67±0.06, n = 126.

Notes — *Penicillium oxalicum* in general produces fast growing colonies and abundant sporulation. These conidia easily break off in crusts when disturbed. Conidiophores are also distinct from closely related species. It has almost ellipsoidal phialides, although Pitt (1979) considered them to be acerose. Furthermore, it produces very big ellipsoidal conidia that are commonly smooth to rough-walled in ridges. Strong acid is produced on CREA.

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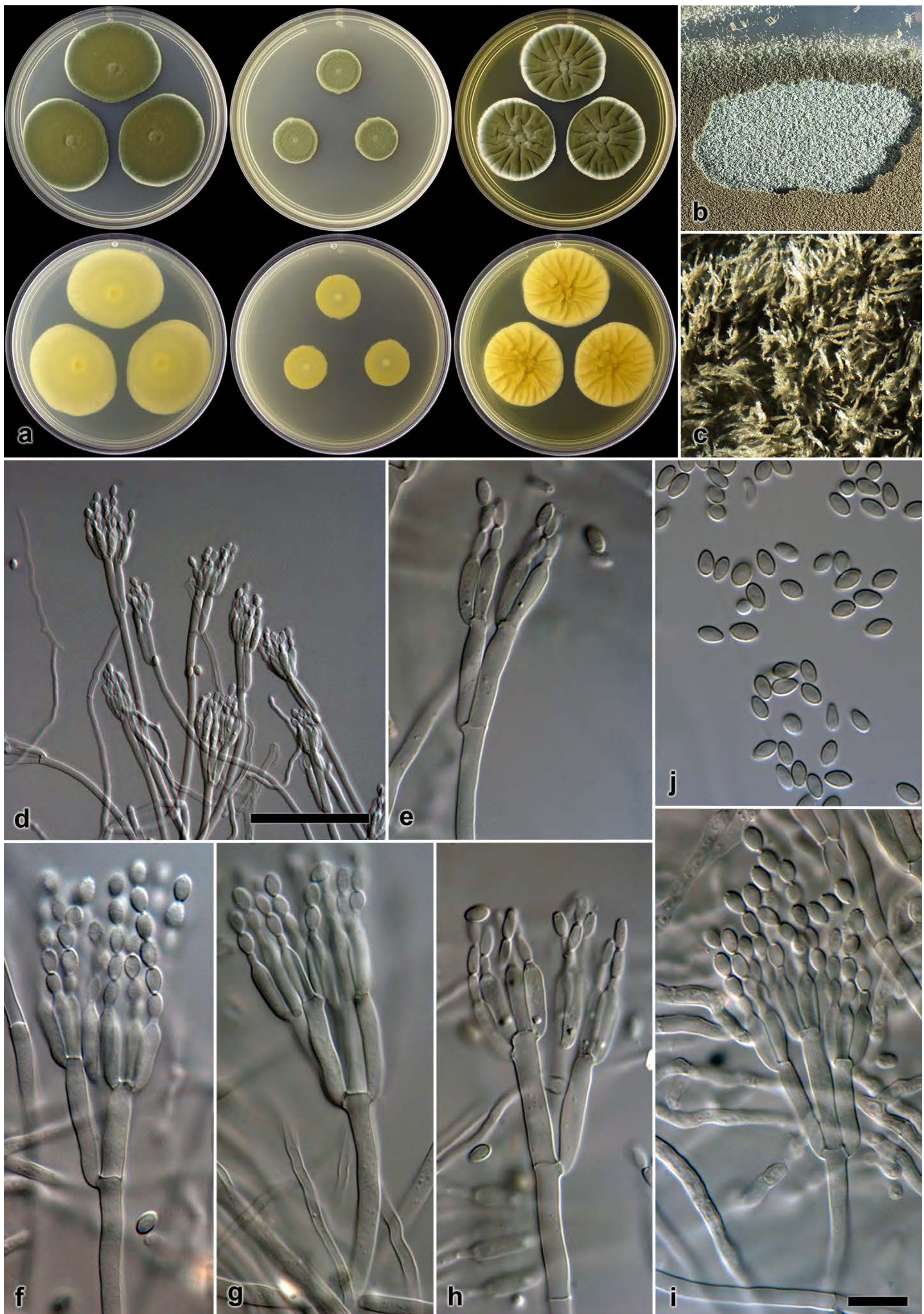

PLATE 28. *Penicillium oxalicum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 50 µm; — Scale bar in i = 10 µm, applies to e–j).

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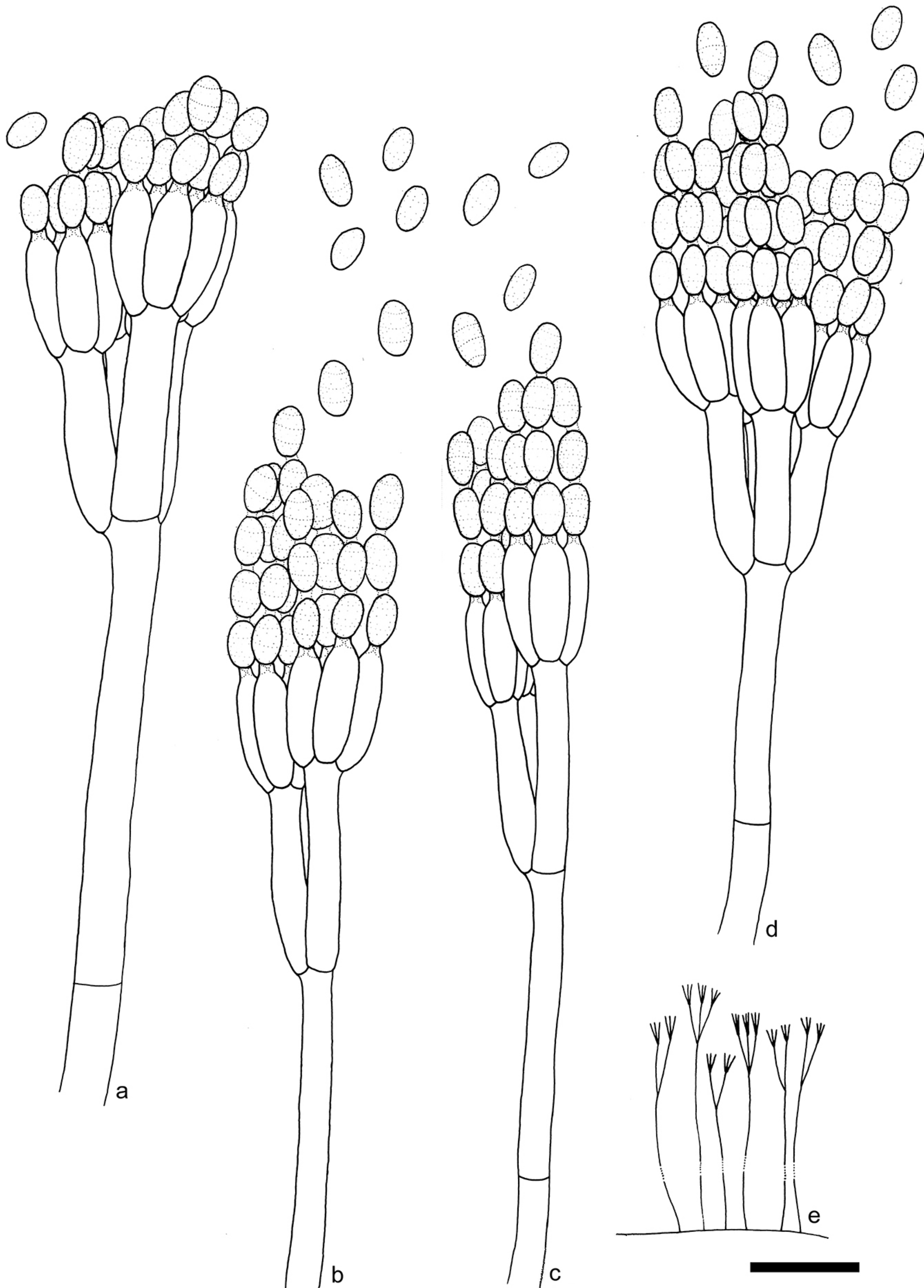

PLATE 29. Line drawing of *P. oxalicum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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17. *Penicillium skrjabinii* Schmotina & Golovleva PLATES 30, 31, 32f

Mikol. Fitopatol. 8: 530. 1974.

EX-TYPE: CBS439.75 = IMI196528
TYPE ISOLATED FROM: Soil, Russia
SPECIMENS EXAMINED: CV85.
ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 39–42 mm, low to moderately deep, plane; margins low, wide (3–4 mm); mycelia white; texture floccose; sporulation moderate, conidia *en masse* greyish to dull green (25D5–25D4–25E4–25E6); exudate absent, soluble pigment absent, reverse pigmentation areas of yellow (3A6–3B8) and pale yellow (1A3).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 44–48 mm, characters similar to colonies at 25 °C, except for sporulation that are less dense as well as reverse pigmentation greyish yellow (4B5–4B6), yellowish grey to greyish yellow (4B2–4B3) and yellowish white (1A2) areas.

CYA, 37 °C, 7d: Sometimes microcolonies, sometimes up to 9 mm, moderately deep, sunken in at centre; margins low, very narrow (< 1 mm), entire; mycelia white; sporulation absent; exudate absent, soluble pigment very light reddish brown, reverse pigmentation brownish orange (6C7) at centre, margin brown (6E7).

MEA, 25 °C, 7d: Colonies 43–47 mm, low, plane; margins subsurface to low, wide (4 mm); mycelia white; texture velutinous and floccose; sporulation moderate, conidia *en masse* greyish green (25E4–25E6); exudate absent, soluble pigment absent, reverse pigmentation (3C8) at point of inoculation, light yellow (2A5) fading into yellowish grey (2C2–3C2).

YES, 25 °C, 7d: Colonies 45–50 mm, low to moderately deep, radially and sometimes lightly concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture floccose; sporulation moderate, conidia *en masse* greyish green (25C3–25E6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B5–4B6) and (3B5–3B6) areas.

G25N, 25 °C, 7d: Colonies 9–16 mm, rising towards centre, concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; sporulation moderate, conidia *en masse* greyish green (25C5–25E5); exudate absent, soluble pigment absent, reverse pigmentation olive (2E5) at centre, fading into greyish yellow (2C3) elsewhere.

CREA, 25 °C, 7d: Colonies 25–30 mm, acid not produced.

Micromorphology — Conidiophores mostly biverticillate, often irregular with subterminal verticils present as well as some terverticillate conidiophores; stipes rough walled, 90–450 × 2.5–3.5 µm; rami/branches 15–40 [21.8±7.3] µm; metulae mostly 3 with some up to 5 per stipe/branch, mostly appressed, sometimes divergent, angle 12–50° [28.1±8.33°], 9–17 × 2.5–4 [13.5±2.01 × 3.1±0.33] µm, vesicle 3–4 [3.5±0.29] µm; phialides rough walled, ampulliform, 4–5 per metula, 7–10 × 2.5–3.5 [8.7±0.63 × 2.9±0.22] µm; conidia rough walled, subspheroidal, 3–3.5 × 2.5–3 [3.1±0.16 × 2.6±0.14] µm, average width/length = 0.83±0.14, n = 107.

Notes — *Penicillium skrjabinii* characteristically produces fast growing colonies on all media, with variable growth at 37 °C. Its most striking feature is the heavy rough walled conidiophores and conidia. Phialides were also consistently rough walled, a feature not reported in the original description or subsequent studies (Pitt 1979, Ramirez 1982).

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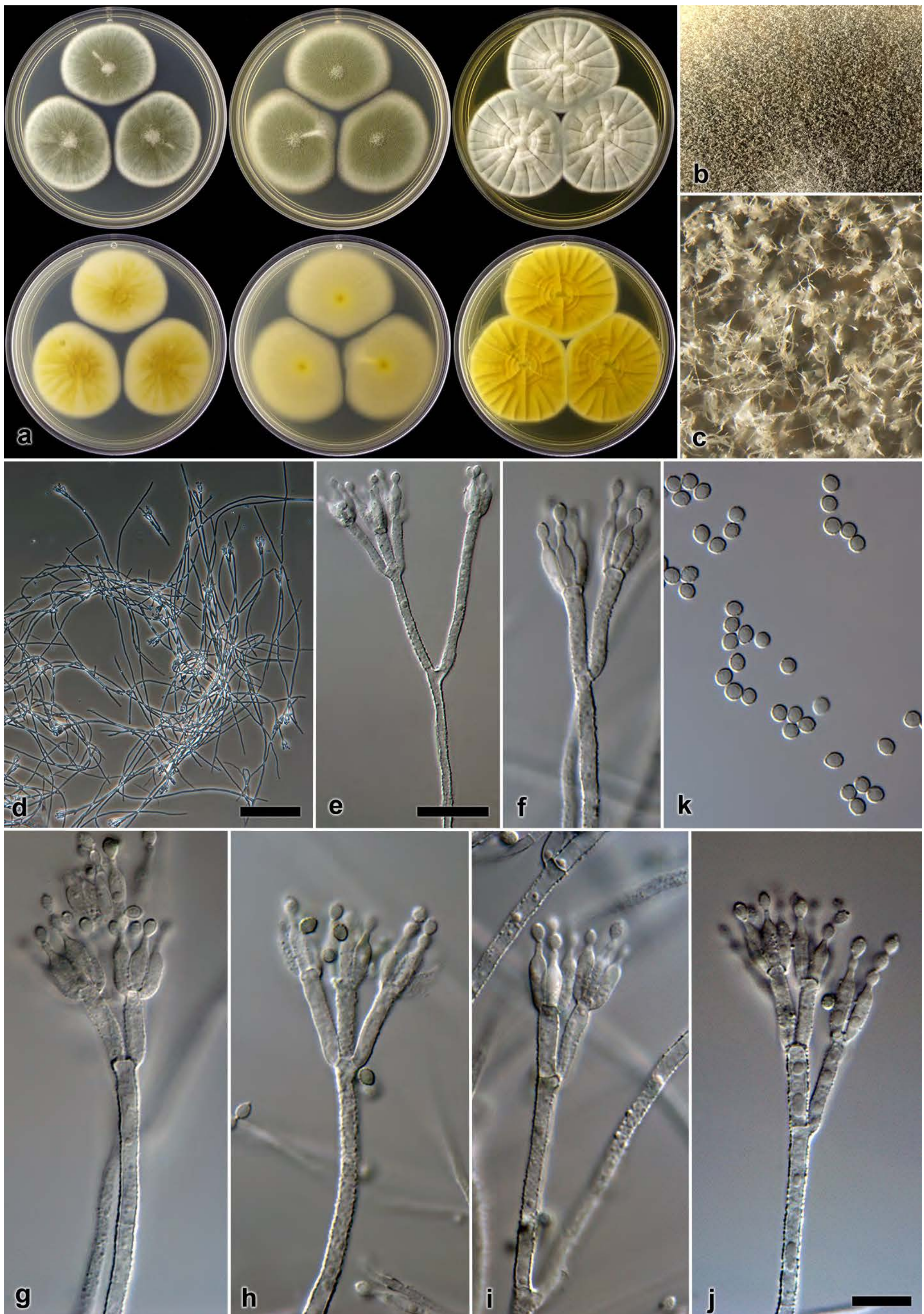

PLATE 30. *Penicillium skrjabinii* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–j. Conidiophores. k. Conidia (— Scale bar in d = 100 µm; — Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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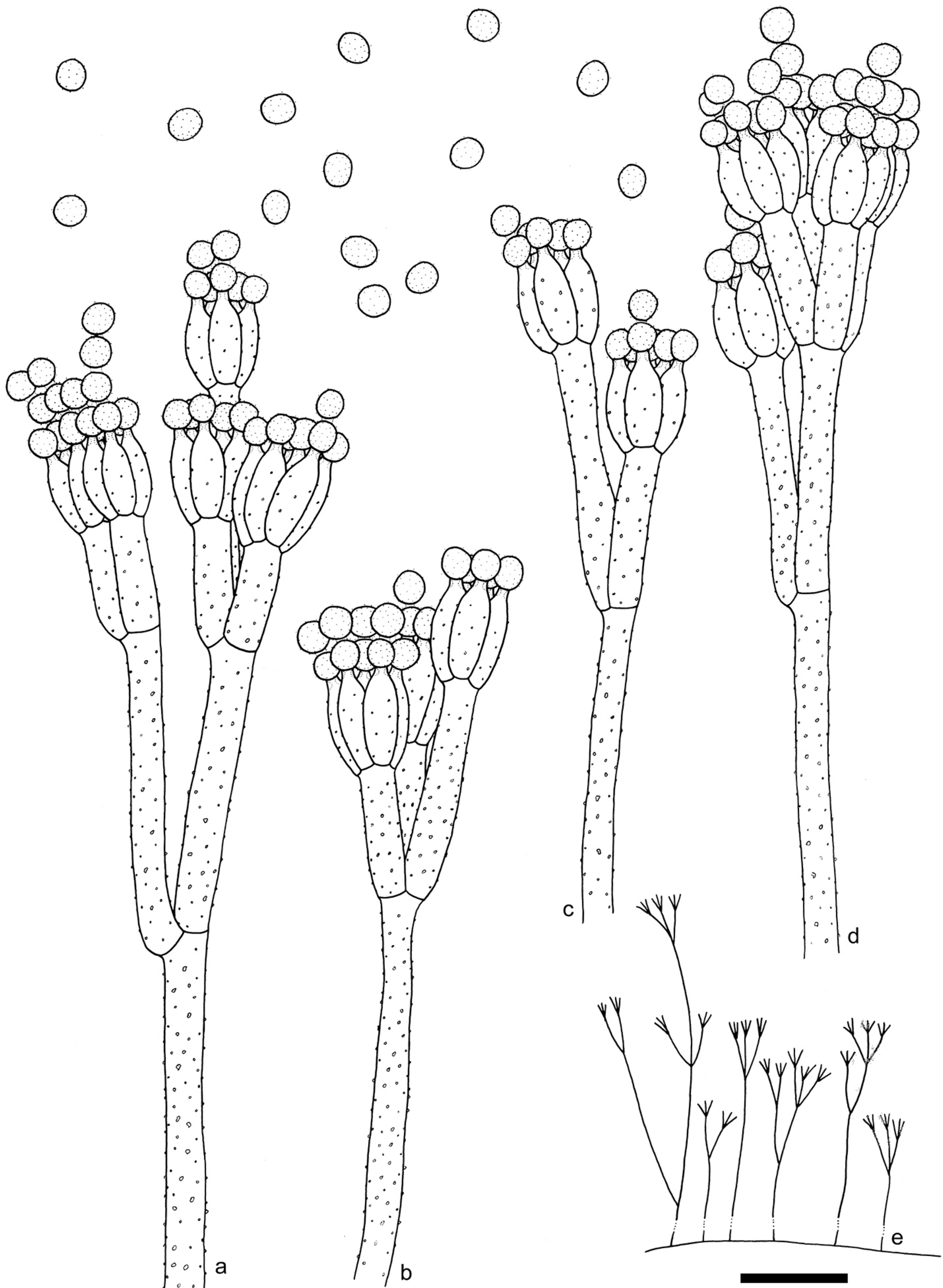

PLATE 31. Line drawing of *P. skrjabinii*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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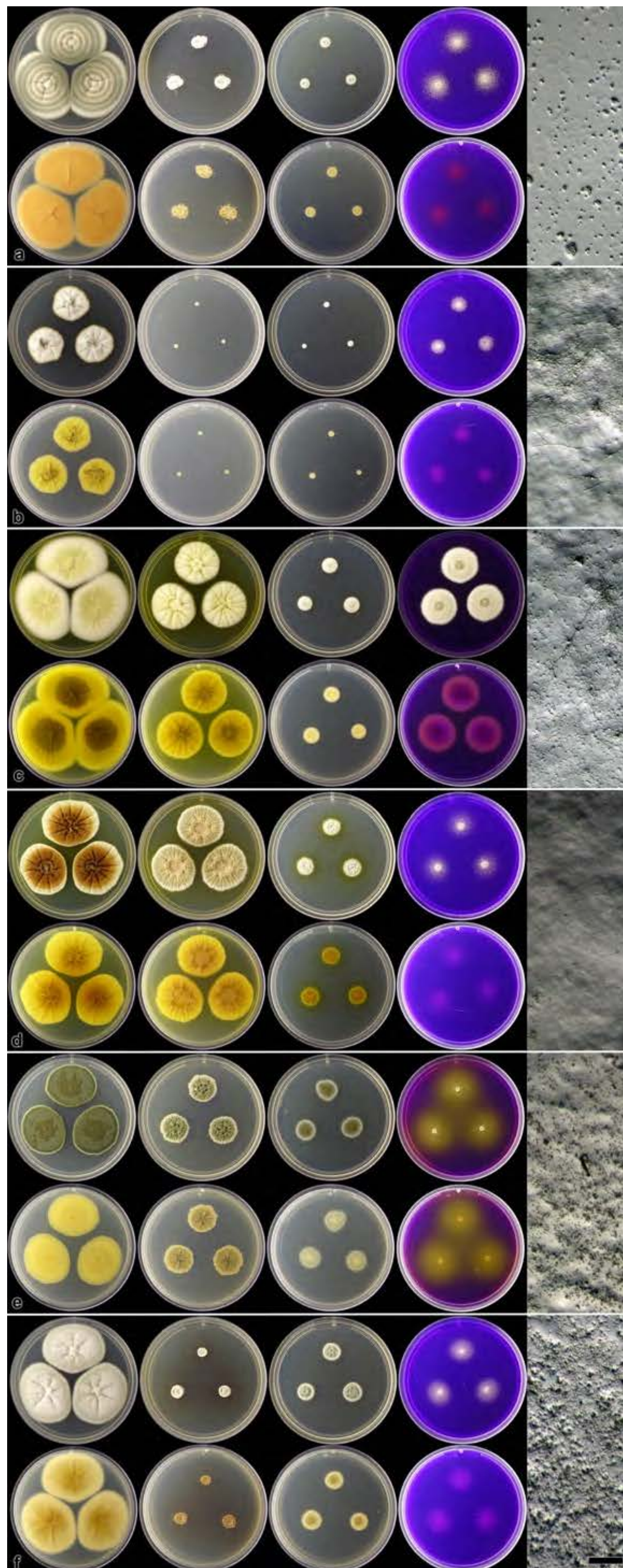

PLATE 32. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium annulatum.* b. *P. brachycaulon.* c. *P. cremeogriseum.* d. *P. malacosphaerula*. e. *P. oxalicum*. f. *P. skrjabinii.*

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The section *Sclerotiora* Houbraken & Samson

Studies in Mycology 70: 32. 2011.

TAXONOMIC NOVELTIES: *Penicillium compactum* prov. nom.

SPECIES TREATED: *Penicillium bilaiae*, *P. hirayamae* & *P. sclerotiorum*

Penicillium section *Sclerotiorum* contains 17 species, and typically produces monoverticillate conidiophores. Some exceptions include *P. herquei, P. malachiteum* and *P. nodositatum* that have biverticillate conidiophores. In general colonies produce bright yellow or orange pigments, whether it may be mycelia, sclerotia, ascocarps, soluble pigments or colony reverses (Pitt 1979, Houbraken & Samson 2011, Rivera & Seifert 2011). Also, these species often produce loosely funiculose colony textures and have conidiophores with short stipes, as seen in *P. bilaiae, P. hirayamae* and *P. viticola* (Nonaka *et al*. 2009). Section *Sclerotiora* is phylogenetically a well-studied group and a number of recent studies have introduced new species in this section (Peterson 2000, Peterson *et al*. 2003, Peterson *et al*. 2004, Nonaka *et al*. 2009, Rivera & Seifert 2011). Rivera & Seifert (2011) reviewed the *P. sclerotiorum* complex using a five-gene phylogeny, while Houbraken & Samson (2011) added the RPB2 gene for this group.

The Fynbos strains from this clade were found to represent four species. They were identified as *P. bilaiae, P. hirayamae* and *P. sclerotiorum,* as well as one previously undescribed species, *P. compactum*. The undescribed species is closely related to *P. adametzii*. *Penicillium adametzii* typically produces strongly funiculose colonies, non-vesiculate monoverticillate conidiophores with five to six phialides per stipe. However, *Penicillium compactum* produces a loosely funiculose colony texture and have conidiophores with vesiculate stipes and sometimes up to 24 phialides per stipe. Microscopically it shares similar characters to *P. bilaiae*. The bright yellow soluble pigments produced on CYA and MEA, as well as strong acid production on CREA makes *P. bilaiae* easily distinguishable from the new species.

Strains identified as *P. hirayamae* consistently resolved in a clade distinct from the ex-type sequence (FIGURE 8). However, based on morphology these strains did not display any differences from the ex-type strain. The species was originally described from rice in Thailand (Udagawa 1959), but has also been reported from cereals in the USA, India and South Africa, as well as from soil from various parts of the world (Pitt 1979). In this study *P. hirayamae* was isolated on a number of occasions from soil collected at the Malmesbury site. Pitt (1979) mentions that it has been isolated from widely separated parts of the world. Unfortunately, these strains were not obtained in this study. Since no morphological differences was observed between the Fynbos strains and the ex-type strain studied, additional data from strains are necessary for species delineation. Therefore, at present the Fynbos strains are considered to represent *P. hirayamae*.

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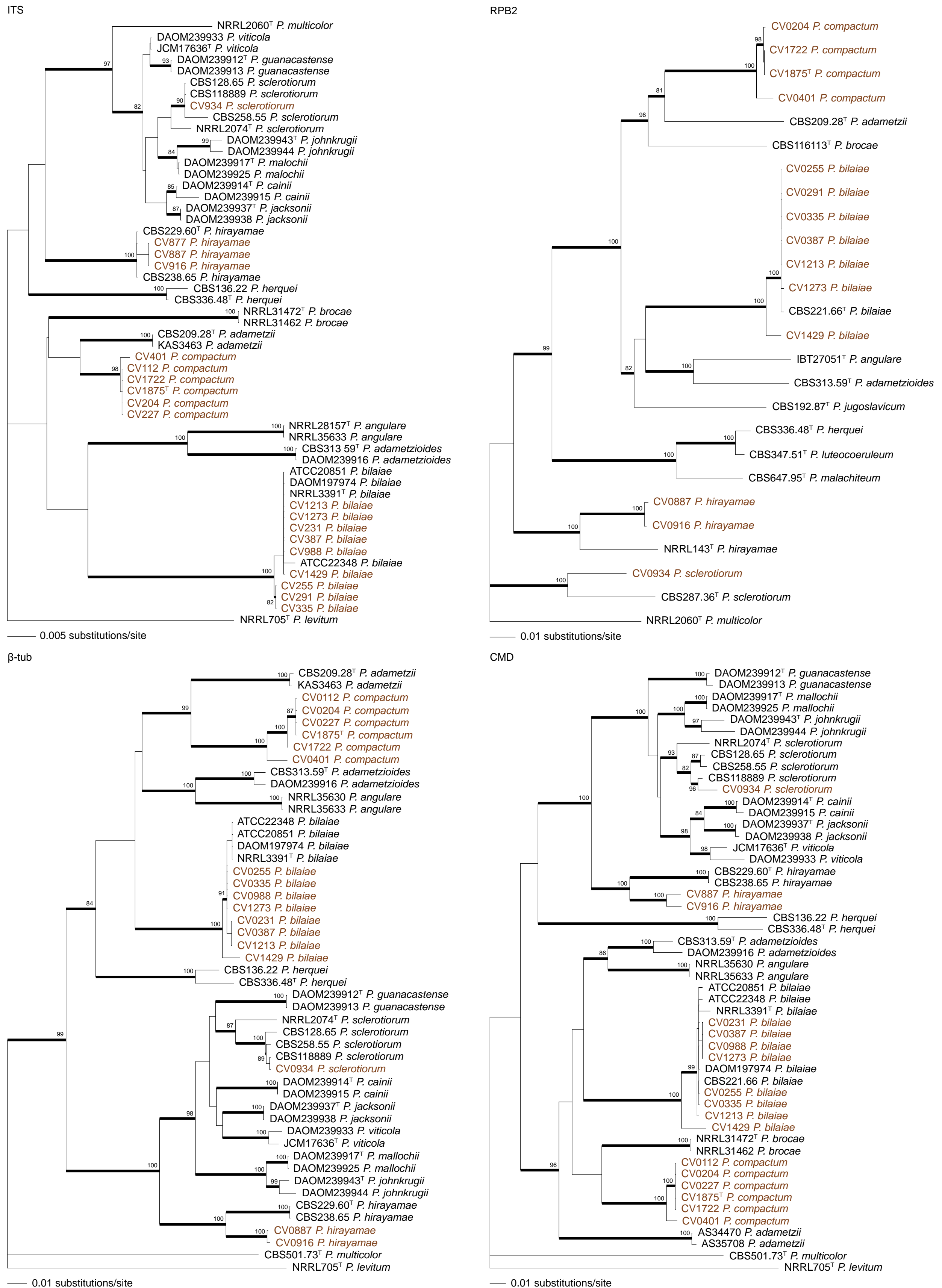

FIGURE 8: Phylogenetic trees based on ITS, RPB2, β-tubulin and Calmodulin, showing relationship of species in the section *Sclerotiora*. *Penicillium levitum* was chosen as outgroup for ITS, β-tubulin and Calmodulin. *Penicillium multicolor* was chosen as outgroup for RPB2. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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18. ***Penicillium bilaiae*** Chalabuda PLATES 33, 34, 41a

Not. Syst. Crypt. Inst. bot. Acad. Sci. USSR 6:165. 1950.

EX-TYPE: CBS221.66 = ATCC22348 = ATCC48731 = FRR3391 = IMI113677 = MUCL31187

TYPE ISOLATED FROM: Soil, Kiev, Ukraine

SPECIMENS EXAMINED: CV231, CV255, CV335, CV387, CV988, CV1213, CV1273, CV1429, CBS221.66.

ISOLATED FROM: Air, soil, mites and bracts from *Protea repens* infructescences, Stellenbosch, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 31–33 mm, low, lightly radially sulcate and strongly concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderate to dense, conidia *en masse* greyish turquoise to greyish green (24E3–24E4–25E4), to greyish turquoise (24D7) near margin; exudate clear to yellow droplets present, soluble pigment bright yellow to somewhat darker in some isolates, reverse pigmentation yellow (3A6) at centre fading into near light yellow (2A4–2A5) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 33–35 mm, low, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderate to dense, conidia *en masse* dark green (25F4–25F5), fading to greyish turquoise (24E4) near margin; exudate clear to yellowish, soluble pigment bright yellow to somewhat darker yellow in some isolates, reverse pigmentation greyish yellow (4B5–4B6) at centre, to a lighter greyish yellow to yellow (2B7–2B8) elsewhere.

CYA, 37 °C, 7d: Colonies 8–9 mm, moderately deep, sunken in at centre; margins deep, narrow, entire; mycelia white; sporulation absent; exudate absent, soluble pigment very light yellow halo surrounding colonies, reverse pigmentation light yellow (2A4) at point of inoculation, greyish yellow (3B5) at margin.

MEA, 25 °C, 7d: Colonies 26–29 mm, low, plane, sometime somewhat raised at centre; margins very low to subsurface, narrow (2–3 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise (24E3) at centre fading into an almost deep turquoise (24E8) near margin; exudate absent, soluble pigment yellow, although absent in some isolates, reverse pigmentation greenish yellow (1A6) at centre fading to greyish green (1C6) at margin.

YES, 25 °C, 7d: Colonies 31–35 mm, low, randomly sulcate; margins low, very narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense to dense, conidia *en masse* dull to greyish green (26E4–26E6) at centre, fading into greyish green (25D6) at margin; exudate absent, soluble pigment absent, reverse pigmentation (3C5) in most of the colony, orange (6B8) at colony centre, although not present in all isolates.

G25N, 25 °C, 7d: Colonies 11–13 mm, low, concentrically sulcate, rising with each ring; margins low, very narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation dull to greyish yellow (3B3–3C3–3C5).

CREA, 25 °C, 7d: Colonies 20–27 mm; moderate to good acid production.

Micromorphology — Conidiophores strictly monoverticillate; stipes smooth walled, 20–160 × 2–3 µm, vesicle 4–6 [5.04±0.47] µm, vesicle sometimes elongated vertically; phialides ampulliform, 12–24 per stipe, 6.5–9.5 × 2.5–3 [8.05±0.61 × 2.73±0.17] µm; conidia rough walled, connectives visible, spheroidal to subspheroidal, 2.5–3 × 2–3 [2.50±0.12 × 2.44±0.15] µm, average width/length = 0.95±0.03, n = 88.

Notes — *Penicillium bilaiae* characteristically produce conidiophores borne on funicles and have short vesiculate stipes, similar to *P. compactum*. One striking feature is the consistent bright yellow soluble pigments produced on most media, as well as strong acid production on CREA, which is absent in *P. compactum.* Pitt (1979) mentions that this species is widely distributed in soil, although not often isolated during surveys. This study isolated the species from only one soil sample collected at Malmesbury, but found it to be common in *Protea repens* infructescences, from where it was isolated numerous times. This might suggest that this species is more common on plants and explains why it is not more commonly found in soil, although it has a wide occurrence around the world.

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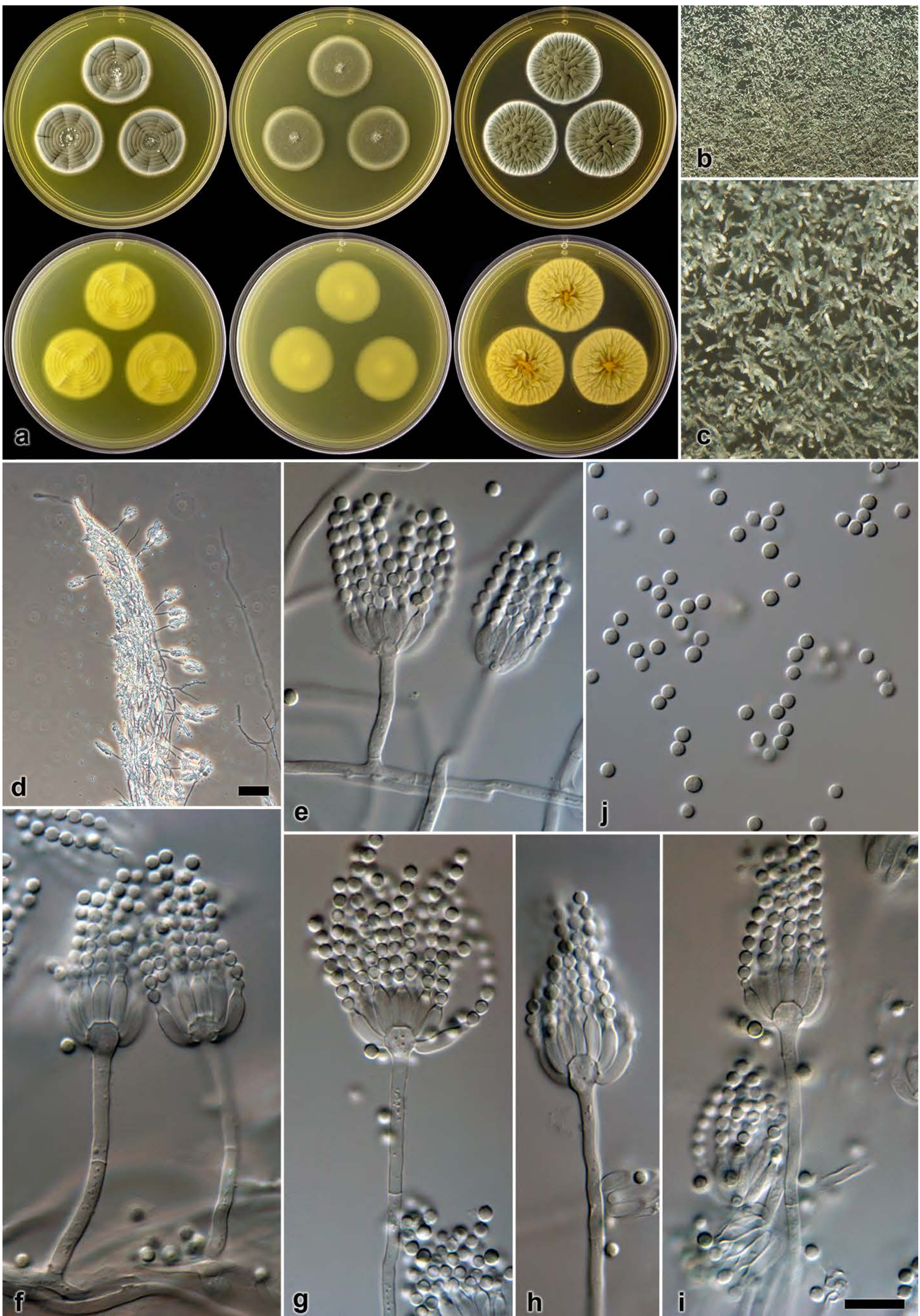

PLATE 33. *Penicillium bilaiae* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 50 µm; — Scale bar in i = 10 µm, applies to e–j).

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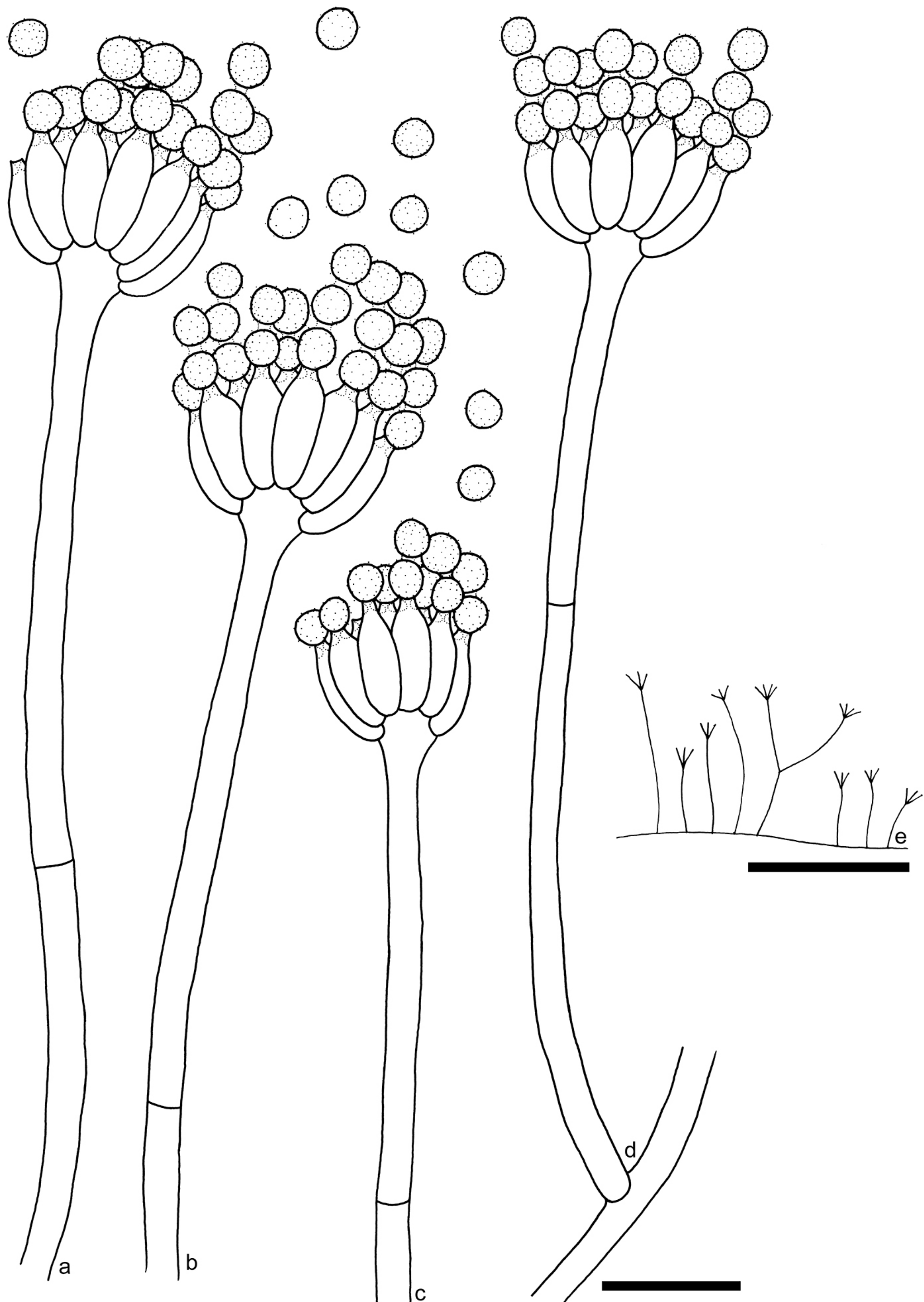

PLATE 34. Line drawing of *P. bilaiae*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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19. *Penicillium compactum* Visagie **prov. nom.** PLATES 35, 36, 41b

ETYMOLOGY: Latin, *compactum* = meaning compact; named after its short and compact conidiophores
EX-TYPE: CV1875 = DTO183F3 = KAS4053 = DAOM241034
TYPE ISOLATED FROM: *Protea repens* infructescence, Struisbaai, South Africa
SPECIMENS EXAMINED: CV112, CV1722, CV1875, CV204, CV227, CV401.
ISOLATED FROM: Air, soil, mites and bracts from *Protea repens* infructescence, Stellenbosch and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 20–28 mm, moderately deep, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull green to greyish green (27D4–27D5), areas greenish grey to greyish green (27B2–27B4) especially near margin; exudate clear to almost yellowish brown, soluble pigment absent, reverse pigmentation pale to light yellow (2A3–2A4), in some isolates a darker dull yellow (3B4).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies showing no differences from those grown on CYA at 25 °C.

CYA, 37 °C, 7d: Sometimes only germination, mostly microcolonies formed.

MEA, 25 °C, 7d: Colonies 29–32 mm, low, plane; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (27D5–27E5–27E6–27D6); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (2A3) at centre, greyish yellow to greyish green (1B3–1C3) elsewhere.

YES, 25 °C, 7d: Colonies 30–35 mm, moderately deep, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* green to greyish green (27D4–27D5), when sporulation more dense, dull to greyish green (27E4–27D7); exudate absent, soluble pigment absent, reverse pigmentation greyish to olive yellow (3B6–3C6) at centre, pale to pastel yellow (2A3–2A4) near margin.

G25N, 25 °C, 7d: Colonies 9–14 mm, low to moderately deep, lightly radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25C4–25C6); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (2A3–3A3).

CREA, 25 °C, 7d: Colonies 10–17 mm, moderate acid production within colony diameter.

Micromorphology — Conidiophores strictly monoverticillate; stipes smooth walled, 20–95 × 2–3 µm, vesicle 4.5–7(–9) [6.27±0.78] µm; phialides ampulliform, 12–24 per stipe, 6.5–9 × 2.5–3.5 [7.7±0.5 × 2.9±0.23] µm; conidia rough walled, connectives visible, spheroidal to subspheroidal, 2–3 × 2–2.5 [2.52±0.14 × 2.41±0.13] µm, average width/length = 0.93±0.04, n = 127.

Notes — *Penicillium compactum* is characterized by monoverticillate conidiophores that have a vesiculate stipe and produce spheroid rough-walled conidia. It grew relatively well on all media. The yellow pigmentations often seen in colonies of section *Sclerotiora* species, were not observed in the new species. Conidia *en masse* were also observed to be a very light green, something not observed for other section *Sclerotia* species isolated from Fynbos. Phylogenetically it forms a distinct clade, separate from any other species (FIGURE 8). It is closely related to both *P. adametzii* and *P. bilaiae*. However, *P. compactum* has short vesiculated stipes and numerous phialides, similar to *P. bilaiae* conidiophores, but distinguishes it from *P. adametzii*. *Penicillium compactum* can, however, be separated from *P. bilaiae* based on the absence of yellow pigmentation on CYA and MEA, as well as the lack of acid production on CREA.

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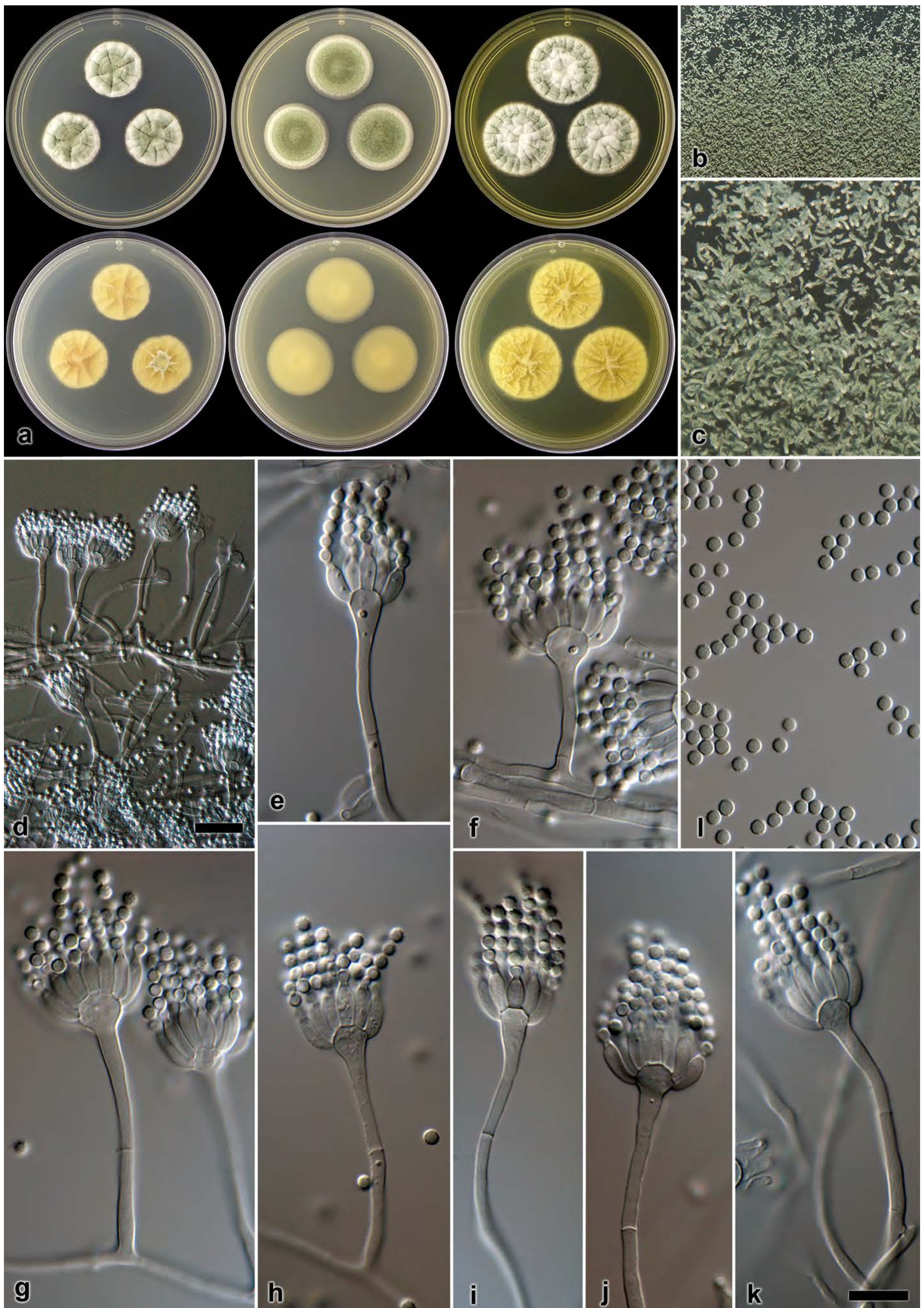

PLATE 35. *Penicillium compactum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–k. Conidiophores. l. Conidia (— Scale bar in d = 50 µm; — Scale bar in k = 10 µm, applies to e–l).

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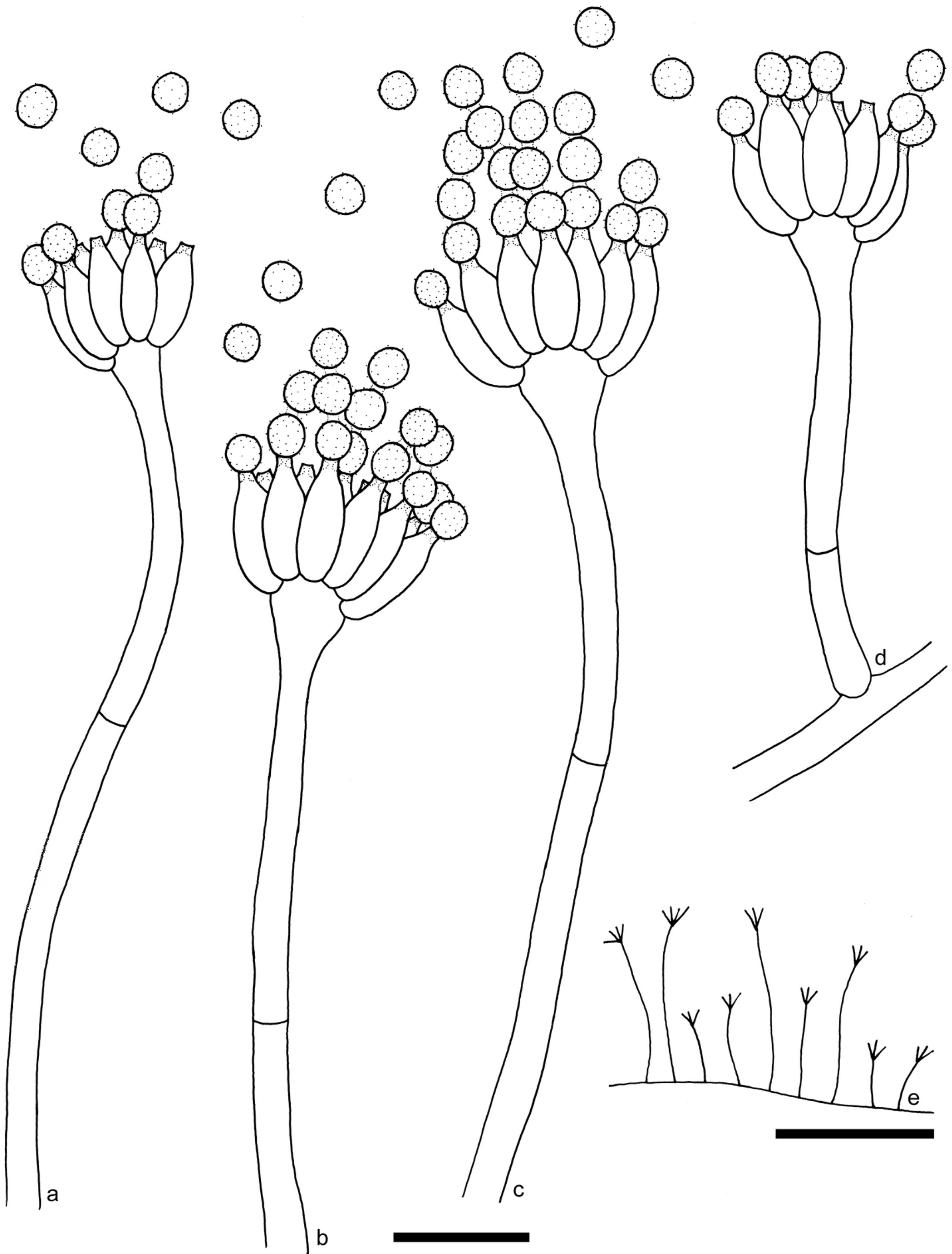

PLATE 36. Line drawing of *P. compactum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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20. ***Penicillium hirayamae*** Udagawa

PLATES 37, 38, 41c

Journal of Agricultural Sciences Tokyo 5: 6. 1959.

SYNONYM: *Eupenicillium hirayamae* Scott & Stolk
EX-TYPE: NRRL143 = CBS229.60 = ATCC18312 = IMI078255
TYPE ISOLATED FROM: Milled rice, Thailand
SPECIMENS EXAMINED: CV877, CV878, CV887, CV916, NRRL143.
ISOLATED FROM: Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 24–31 mm, low, raised centrally, radially sulcate, orange cleistothecia present; margins low, narrow (1–2 mm), entire; mycelia white at margins, yellow to orange near centre; texture typically loosely funiculose, velutinous areas at margin; sporulation sparse to moderate, conidia *en masse* greyish turquoise to dull green (24D3–26D3), when sparse greyish green (25B3–26B3); exudate yellowish orange, soluble pigment absent, reverse pigmentation orange (5A6–5A7), pale green (30A3) at margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: All features similar to CYA at 25°C, except for colonies 26–30 mm, and sclerotia more yellow than orange.

CYA, 37 °C, 7d: Colonies 7–18 mm, low, craterform, radially and concentrically sulcate, orange to yellow sclerotia present in "crater"; margins low, very narrow (1 mm), entire; mycelia white, with some inconspicuously yellow areas; texture velutinous; sporulation sparse to absent, conidia *en masse* pastel green (25A4); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (1B3–1B4).

MEA, 25 °C, 7d: Colonies 20–32 mm, low, plane, yellow and orange sclerotia present; margins low, narrow to wide (1–3 mm), entire; mycelia white at margins, yellow and orange elsewhere; texture velutinous at margins, loosely funiculose elsewhere; sporulation moderately dense, conidia *en masse* greyish green (25C4–26C4); exudate yellowish orange, sometimes absent, soluble pigment absent, reverse pigmentation deep orange to orange (6A8–6B8), in some isolates light yellow (4A5).

YES, 25 °C, 7d: Colonies 29–34 mm, low, rising steeply at colony centre, radially sulcate, yellow and orange sclerotia present at centre; margins low, narrow (1–2 mm), entire; mycelia white at margin, yellow to orange; texture velutinous and loosely funiculose; sporulation moderately dense, conidia *en masse* greyish turquoise (24C3–24C4); exudate absent, sometimes a few yellowish orange droplets present, soluble pigment absent, reverse pigmentation orange (6B8) at centre, fading into a greyish yellow (2B4) at margin.

G25N, 25 °C, 7d: Colonies 10–14 mm, low, radially and concentrically sulcate, orange sclerotia sparsely produced at colony centre; margins low, very narrow (1 mm), entire; mycelia white at margin, yellowish orange present at centre; texture velutinous and loosely funiculose; sporulation sparse to almost moderately dense, absent in some isolates, conidia *en masse* greyish green (25B4–25C4); exudate yellow to orange, soluble pigment absent, reverse pigmentation greyish yellow (4B5) to orange (6B8) at centre, dull green (28D3–28D4).

CREA, 25 °C, 7d: Colonies 6–8 mm, no acid production.

Micromorphology — Conidiophores borne from funicles, largest proportion monoverticillate although some form of biverticillate conidiophores present; stipes/branches smooth walled, 17–65 × 2–3 µm, vesicle 3–5 [3.8±0.4] µm; phialides ampulliform, 7–12 per metula, 5.5–9 × 2–3 [6.6±0.48 × 2.5±0.15] µm; conidia smooth, spheroidal with some subspheroidal, 2–2.5 × 2–2.5 [2.3±0.14 × 2.3±0.15] µm, average width/length = 0.97±0.02, n = 77; sclerotia/cleistothecia 90–180 × 65–150 µm.

Notes — *Penicillium hirayamae* typically produces bright orange and yellow pigments in mycelia, sclerotia/ascocarps and reverse pigmentation. Monoverticillate conidiophores are borne from loosely funiculose mycelia. A sexual state has been reported (Scott & Stolk 1967, Pitt 1979), but this was not seen for the Fynbos isolates even after months of incubation on Oatmeal agar. This species is phylogenetically not coherent, with Fynbos strains consistently resolving in a clade separate from the ex-type sequence (FIGURE 8). Morphologically the strains showed no variation. Therefore, this species possibly represent a species complex and needs further investigation.

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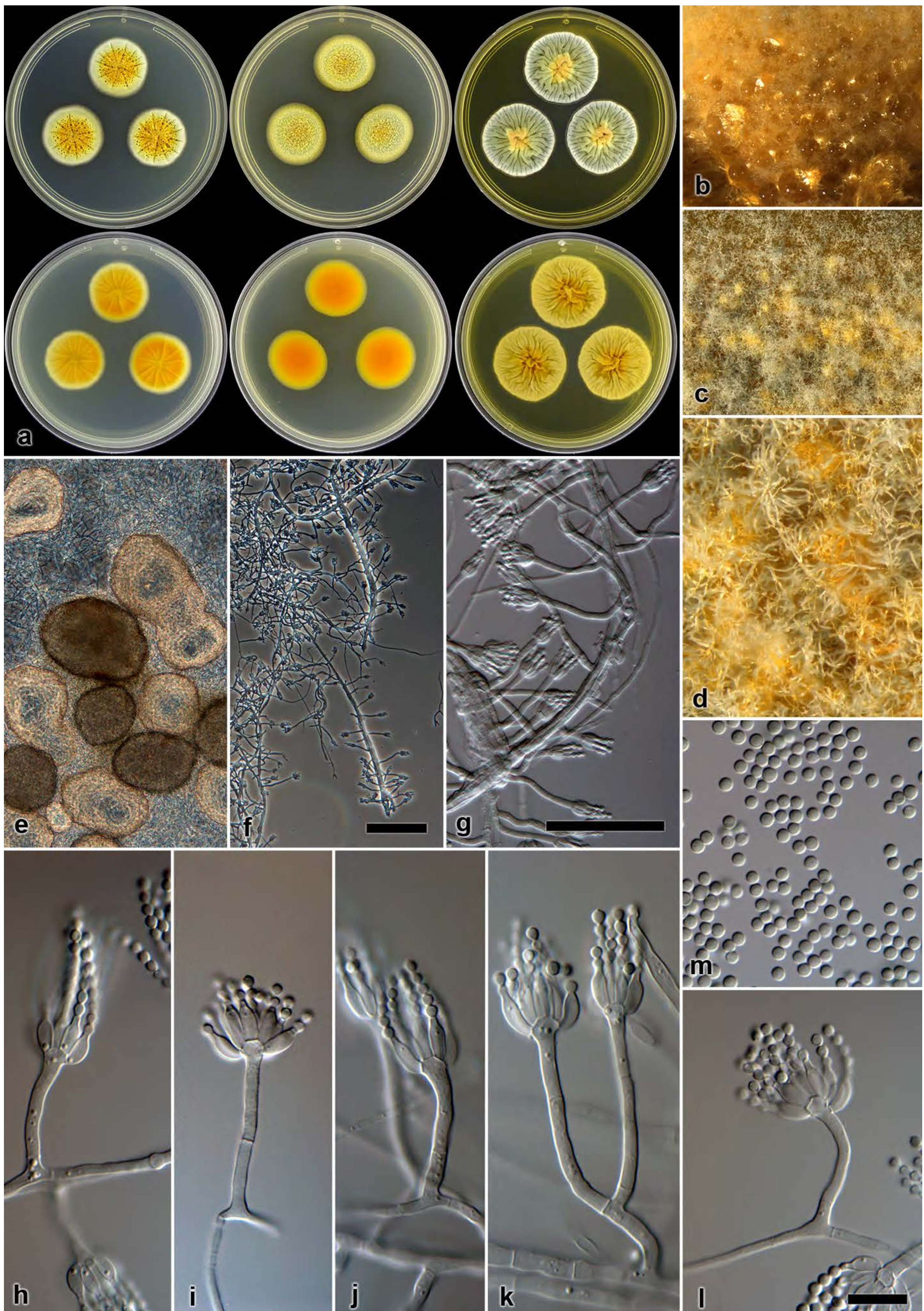

PLATE 37. *Penicillium hirayamae* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia from MEA. f–l. Conidiophores. m. Conidia (— Scale bar in f = 100 µm, applies to e, f; — Scale bar in g = 50 µm; — Scale bar in l = 10 µm, applies to h–m).

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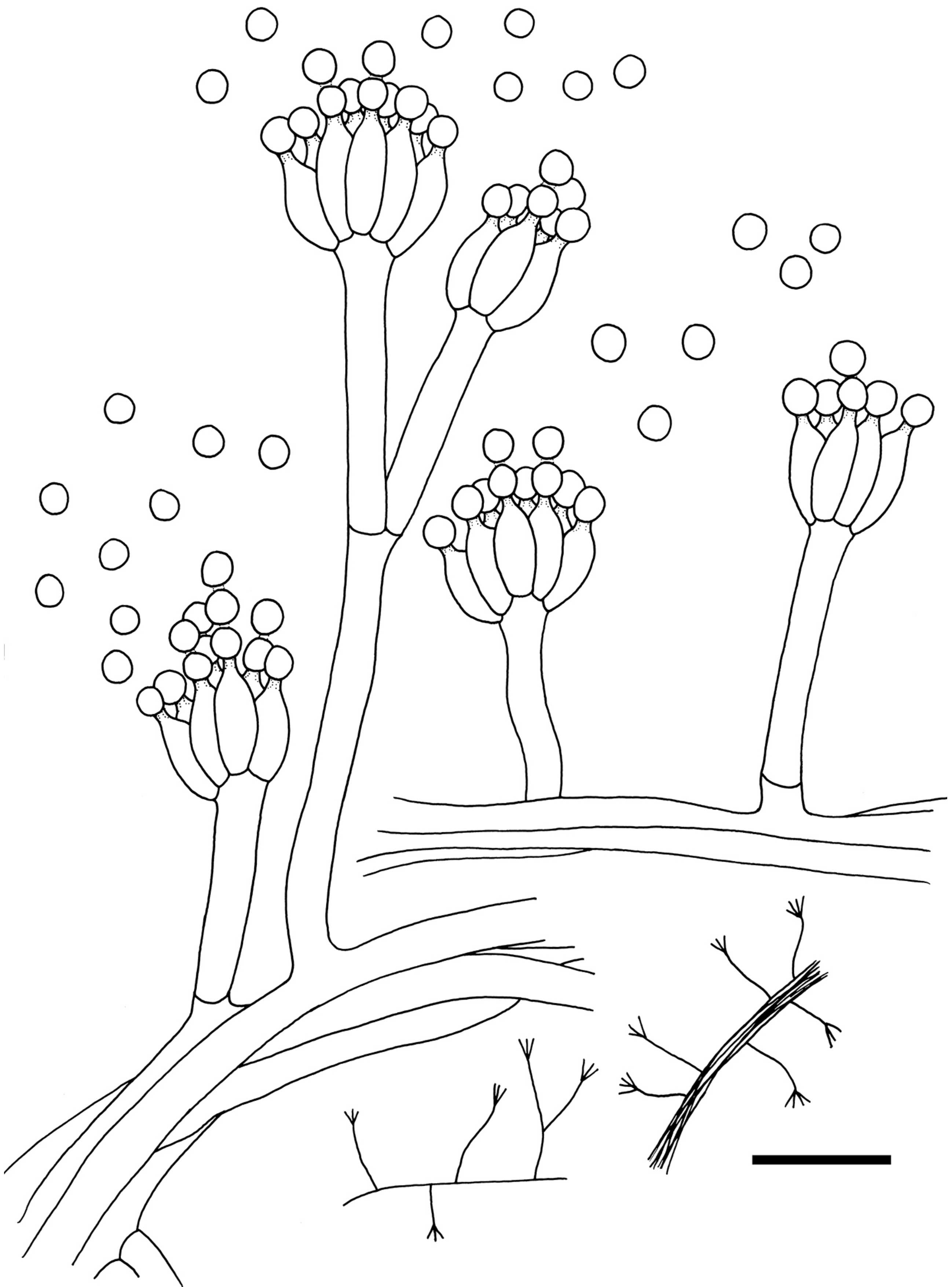

PLATE 38. Line drawing of *P. hirayamae*. a, b. Conidiophores (— Scale bar = 10 μm). c, d. Conidiophore branching (— Scale bar = 50 μm).

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21. *Penicillium sclerotiorum* van Beyma PLATES 39, 40, 41d

Zentralblatt für Bakteriologie und Parasitenkunde, Abteilung II 96: 418. 1937.

EX-TYPE: CBS287.36 = ATCC10494 = IMI040569 = NRRL2074
TYPE ISOLATED FROM: Air, Buitenzorg, Java, Indonesia
SPECIMENS EXAMINED: CV934.
ISOLATED FROM: Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 43–45 mm, low, very lightly radially and concentrically sulcate, bright florescent orange sclerotia present, especially at colony margins facing each other; margins low, wide (3–4 mm), entire; mycelia white and bright orange; texture velutinous and floccose; sporulation dense, conidia *en masse* dull to greyish green (26E4–26E5); exudate mostly absent, when present clear to inconspicuously orange, soluble pigment absent, reverse pigmentation patchy, reddish orange (7B8), golden yellow (5B7), yellowish orange (4A6) areas.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 32–34 mm, low, radially sulcate, bright orange sclerotia produced; margins low, narrow (1–2 mm), entire; mycelia white and orange present; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (26E4–26E6); exudate absent, soluble pigment absent, reverse pigmentation reddish orange (7B8) and yellow orange (4A6) areas near centre, greenish white at margin (29A2–30A2).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 38–44 mm, low, plane, bright florescent orange sclerotia produced sporadically; margins subsurface, wide (3–5 mm), entire; mycelia underneath conidial areas orange, elsewhere; texture velutinous; sporulation dense, conidia *en masse* dull to greyish green (25E4–25E6); exudate absent, soluble pigment absent, reverse pigmentation mostly reddish orange (7B8) to brighter reddish orange (7A8) underneath sclerotial areas.

YES, 25 °C, 7d: Colonies 42–45 mm, low to moderately deep, radially and concentrically sulcate, light orange sclerotia present; margins low, narrow (1–2 mm), entire; mycelia white, light orange in regions; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25D5–25E4); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (7C8) at centre, with pale yellow (2A3–3A3) at margin.

G25N, 25 °C, 7d: Colonies 16–18 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25°C; exudate absent, soluble pigment absent, reverse pigmentation yellowish orange (4B8) at centre, light yellow (4A5) at margin.

CREA, 25 °C, 7d: Colonies 27–30 mm, strong acid production.

Micromorphology — Conidiophores strictly monoverticillate; stipes smooth walled, 100–380 × 2–3 µm, vesicle 4.5–7 [5.7±0.55] µm; phialides ampulliform, 10–16 per stipe, 8–11 × 2.5–4 [9.6±0.6 × 3.2±0.25] µm; conidia smooth walled, ellipsoidal, 2.5–3.5 × 2–2.5 [2.9±0.15 × 2.1±0.14] µm, average width/length = 0.75±0.04, n = 78; sclerotia 180–250 × 130–200 µm.

Notes — *Penicillium sclerotiorum* belongs to a clade of species that produce bright orange mycelia and sclerotia. Rivera & Seifert (2011) reviewed this clade in a recent study and described multiple new species. The smooth walled stipes and ellipsoidal conidia produced by *P. sclerotiorum* is considered diagnostic for the species. Also, it displays a strong acid production on CREA.

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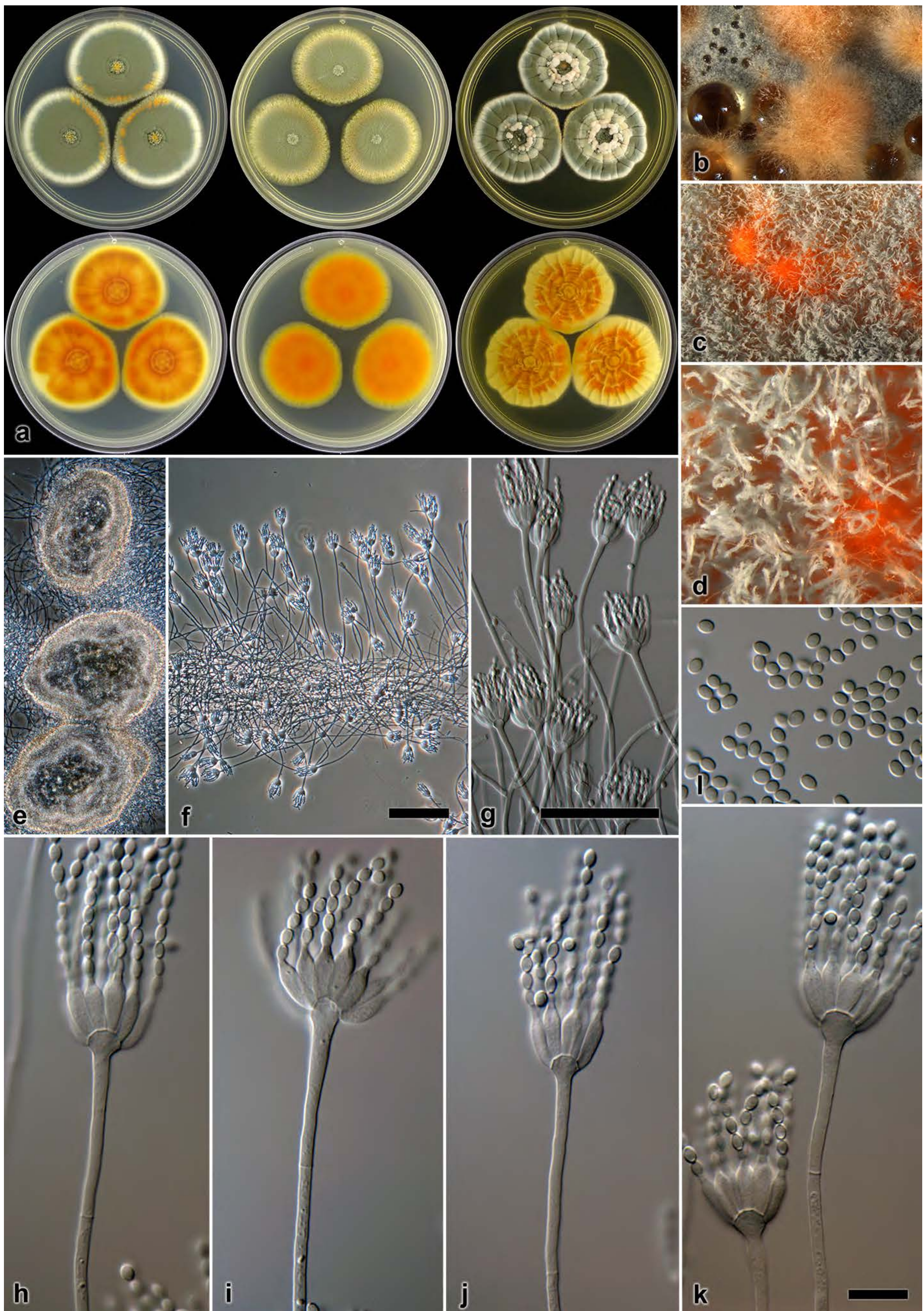

PLATE 39. *Penicillium sclerotiorum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia. f–k. Conidiophores. l. Conidia (— Scale bar in f = 100 μm, applies to e, f; — Scale bar in g = 50 μm; — Scale bar in k = 10 μm, applies to h–l).

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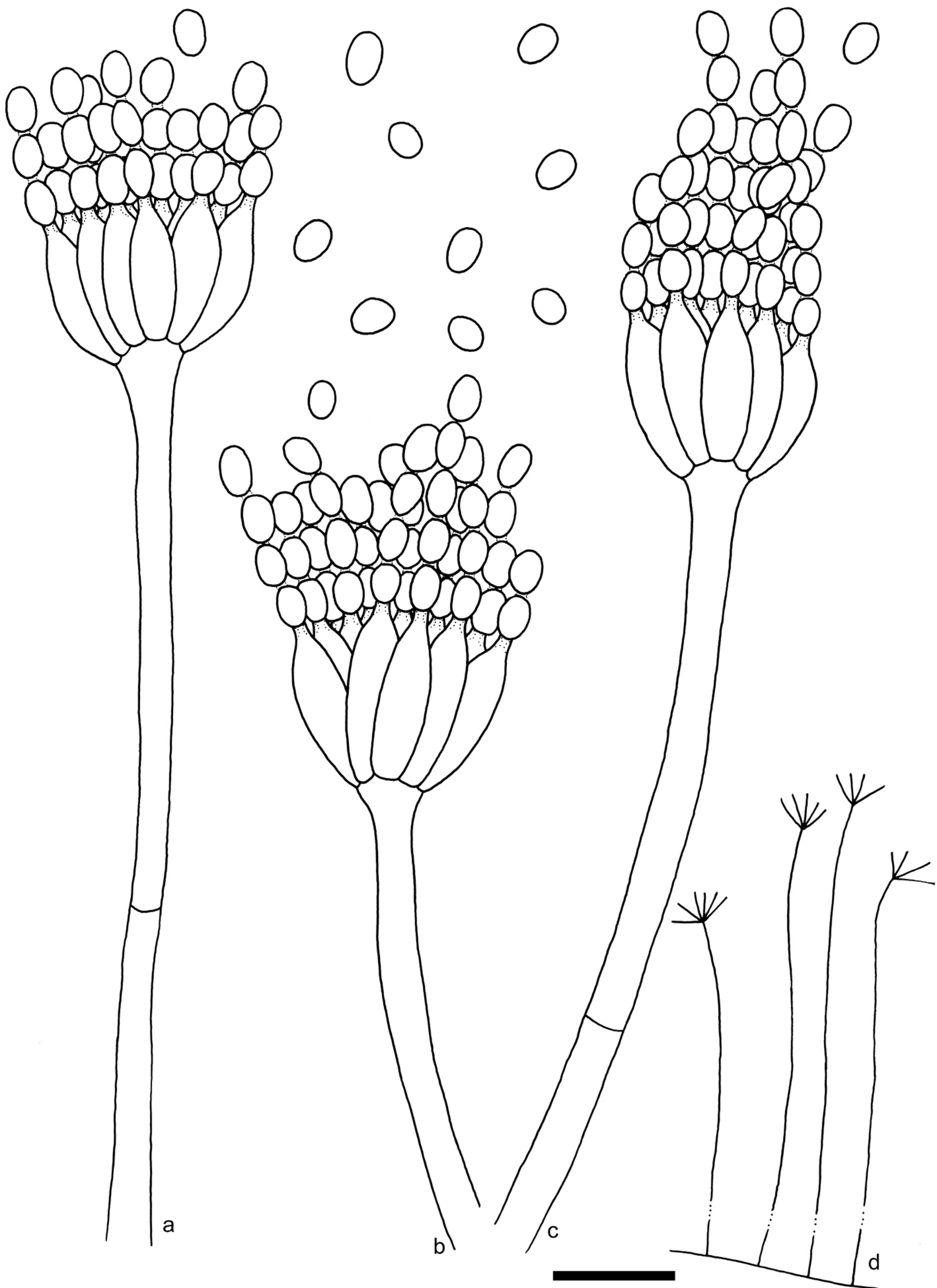

PLATE 40. Line drawing of *P. sclerotiorum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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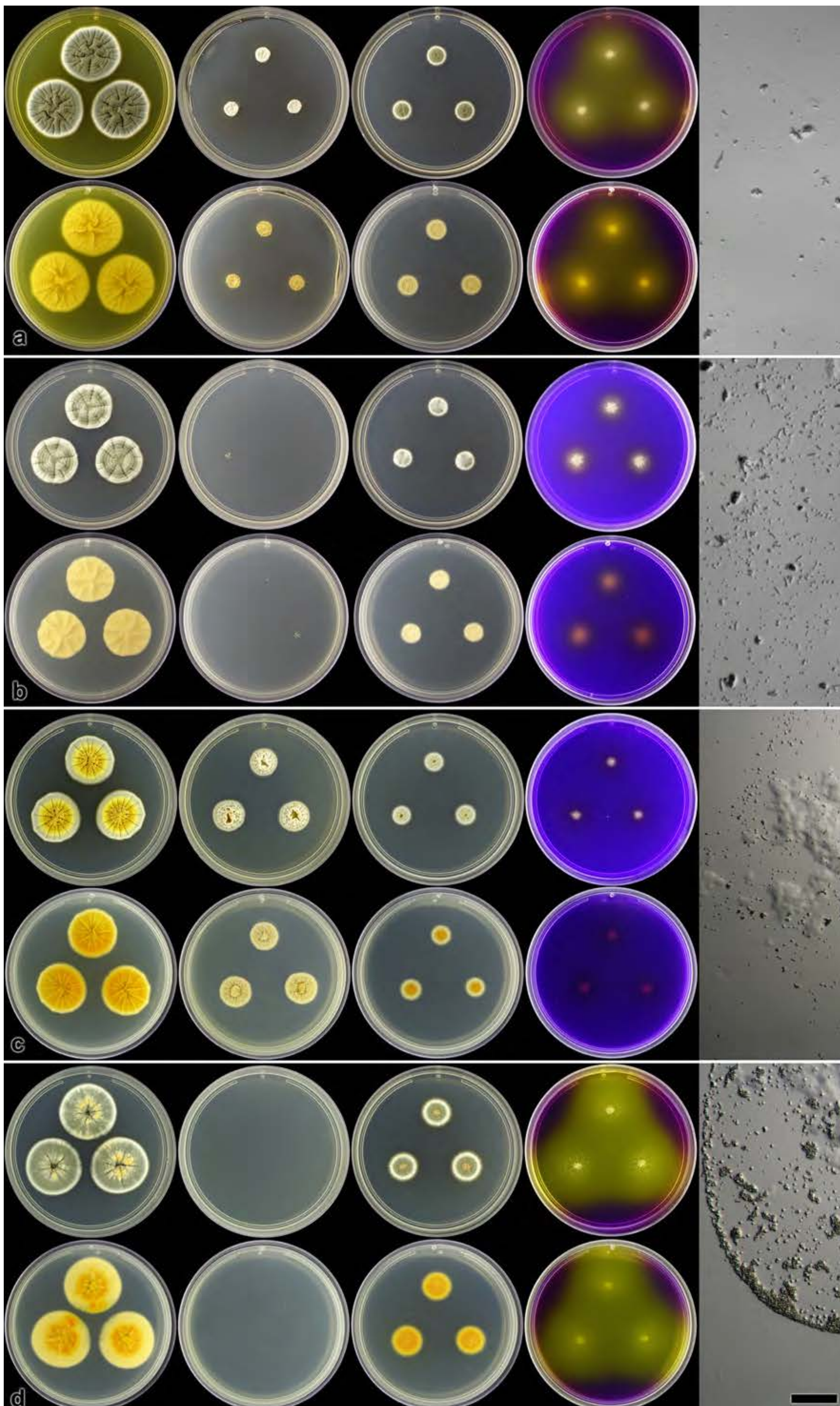

PLATE 41. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium bilaiae*. b. *P. compactum*. c. *P. hirayamae*. d. *P. sclerotiorium*.

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The section *Aspergilloides* Pitt

The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*: 169. 1979.

TAXONOMIC NOVELTIES: *Penicillium brunneoconidia* prov. nom., *P. caseidecus* prov. nom., *P. clavistipa* prov. nom., *P. cumulacinatum* prov. nom., *P. flavosclerotia* prov. nom., *P. infra-aurantiacum* prov. nom., *P. malmesburiensis* prov. nom., *P purpuroides* prov. nom., *P. vagum* prov. nom.

SPECIES TREATED: *Penicillium fuscum, P. glabrum, P. thomii* (morphogroup 1), *P. thomii* (morphogroup 2)

Pitt (1979) introduced the section *Aspergilloides* for monoverticillate species that have vesiculated stipes. This understanding of the section was expanded by Houbraken & Samson (2011) based on multigene phylogenies, to include non-vesiculate species such as *P. montanense, P. quercetorum, P. lividum* and *P. fuscum* that form well supported clades within this group. Members in the section show fast growth on all media (Barreto *et al*. 2011). However, four newly described Fynbos species displayed restricted growth, a character not observed for other species in the section.

Differentiation between closely related species in this section is often problematic. This was especially true in the case of *P. glabrum, P. spinulosum, P. purpurescens* and *P. montanense* (Raper & Thom 1949, Pitt 1979, Pitt *et al*. 1990, Barreto *et al.* 2011). These species were, for a long time, differentiated based on characters such as heavy rough walled conidia in the case of *Penicillium purpurescens* (Raper & Thom 1949, Pitt 1979, Pitt *et al*. 1990) or more restricted growth rate on CYA as in the case of *Penicillium montanense* (Pitt 1979, Pitt *et al*. 1990). Also, the looser colony texture and roughened conidia of *P. spinulosum* was used to distinguish it from *P. glabrum* (Raper & Thom 1949, Pitt 1979). These characters were always difficult to apply (Pitt *et al*. 1990). As such, Pitt *et al*. (1990) evaluated the species boundaries in this group. After careful consideration of a broad number of characters, they accepted the four species as distinct. Conidial wall texture, growth rates on CYA and G25N, as well as width of stipe vesicles and phialides was considered diagnostic (Pitt *et al*. 1990). However, the practical use of these criteria for identifications to a large extent remained problematic, especially for non-taxonomists (Barreto *et al*. 2011). Barreto *et al*. (2011) reviewed this group and used morphology, extrolite analysis and multigene phylogenies for comparisons of a large number of *P. glabrum* and *P. spinulosum* strains. They reported considerable intraspecies variation for both species, but could distinguish between them based on growth rates, especially on CREA where *P. spinulosum* grows faster than *P. glabrum* and reaching colony diameters bigger than 25 mm. Furthermore, extrolite analysis and multigene sequences resulted in consistent clades that separate these species. Although these species are still difficult to distinguish based on morphology, it is phylogenetically and physiologically well defined. Based on this polyphasic approach, Barreto *et al*. (2011) reduced a large number of species to synonymy with either *P. glabrum* or *P. spinulosum*.

Similar difficulties to Barreto *et al*. (2011) were also experienced during the Fynbos study where a large number of *P. glabrum* strains were isolated that showed variations in both morphology and gene sequences. Phylogenetically the strains resolved in the *P. glabrum* clade and are, therefore, identified as such. However, a number of strains (CV6, CV7, CV15, CV728), which produce red colony reverse pigmentation clustered in a separate clade (FIGURES 9, 10). Although Barreto *et al*. (2011) did not observe red-pigmented strains in *P. glabrum*, Pitt (1979) did include this character in his descriptions. It thus seems that this group might warrant further studies that include extrolite analysis, which will add to knowledge on the diverse nature of *P. glabrum*.

Penicillium purpuroides and *P. vagum* represent two novel Fynbos species that resolved in the *P. glabrum – P. spinulosum – P. purpurescens* clade. It should be noted that even though RPB2 resolved *P. vagum* as a close relative to the *P. montanense* clade, all the other genes place it as a close relative to the *P. spinulosum* clade. Both the new species produce heavy rough walled spheroid conidia, similar to *P. purpurescens*. However, the larger conidia of *P. purpurescens* distinguish it from both the novel species. The two Fynbos species are easily distinguished from each other based on growth rates on CYA and MEA at 25 °C.

The two species described here, as *P. infra-aurantiacum* and *P. malmesburiensis* are phylogenetically distinct from all previously described species and form a distinct clade, basal to the rest of the section (FIGURE 9). *Penicillium malmesburiensis* is distinguished from *P. infra-aurantiacum* based on the orange exudate and reverse pigmentation typical of the latter species. *Penicillium infra-aurantiacum*, also have longer stipes compared to *P. malmesburiensis*.

Although the largest proportion of species from section *Aspergilloides* display fast growth, five species isolated from Fynbos showed restricted growth, especially on CYA. Multigene phylogenies resolved these strains in a clade together with *P. fuscum* (= *E. pinetorum*), *P. montanense*, *P. abeanum* and *P. ardesiacum* (FIGURES 9, 11). These species, excluding *P. fuscum*, also display the characteristic fast growth seen for other section *Aspergilloides* species. Strain CV531 was identified as *P. fuscum* based on the similarities of its morphology and sequence data to other reference strains. However, four species had unique morphologies and are

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described here as new, with their novelty confirmed by multigene phylogenies. The restricted growth on CYA was found diagnostic for separation of Fynbos strains from the previously described species in this clade. Conidiophore morphology was found diagnostic for differentiation between the four newly described Fynbos species. For instance, *P. clavistipa* are easily distinguished from the other species by its rough walled stipes and rather broad vesicles. *Penicillium brunneoconidia* and *P. caseidecus* produces very short stipes. However, *P. brunneoconidia* characteristically produce dark green conidia compared to the greyish turquoise conidia of *P. caseidecus*. *Penicillium flavosclerotia* are distinguished by its typical yellow sclerotia produced on most media. In this regard, it is similar to *P. fuscum*. However, growth rates on CYA at 30 °C and YES makes *P. flavosclerotia* distinct from *P. fuscum*.

A large number of strains from this study resembled *Penicillium thomii.* However, in keeping with the rest of this section, this group appears to represent a species complex within section *Aspergilloides*. Strains in this species produce monoverticillate conidiophores with vesiculate stipes and ellipsoid rough walled conidia. Colonies produce sclerotia that range in color from apricot to pale on MEA and pinkish to pale brown on CYA (Pitt 1979). Pitt (1974, 1979) reduced a number of species to synonymy with *P. thomii*, including *P. aurantioviolaceum, P. yezoense, P. parallelosporum, P. crocicola* and *P. thomii* var. *flavescens*. Material for these species were not examined, however, ex-type sequences for *P. thomii, P. thomii* var. *flavescens* *P. crocicola* and *P. yezoense* show that these species, although closely related, do show variation and most probably represents distinct species. A revision of this species complex is needed in order to delineate and determine species boundaries for *P. thomii* and its previously considered synonyms. Strains isolated from Fynbos formed two morphogroups that clustered into three phylogroups (FIGURES 9, 10). However, in the absence of clearly delineated species, the Fynbos strains were identified as *P. thomii* morphogroup 1 and morphogroup 2. Differences between these two morphogroups most notably include conidial color and reverse pigmentation, as well as the pale sclerotia formed in morphogroup 2, compared to the apricot colored sclerotia in morphogroup 1. Strains in morphogroup 2, also consistently produced acid on CREA, which were absent in morphogroup 1. One of the new Fynbos species is morphologically similar to species from this complex, also producing brownish sclerotia. However, strains described here as *P. cumulacinatum* produce spheroid conidia and is consistently resolved in a clade separate of the *P. thomii* complex (FIGURES 9, 10).

Sequence data were shown to be a very useful character for species delineation in a section where morphological differences are often difficult to apply. Although a number of taxonomic difficulties remain in the section, most notably the *P. thomii* species complex, the Fynbos strains resolved in consistent clades and are well defined morphologically and phylogenetically.

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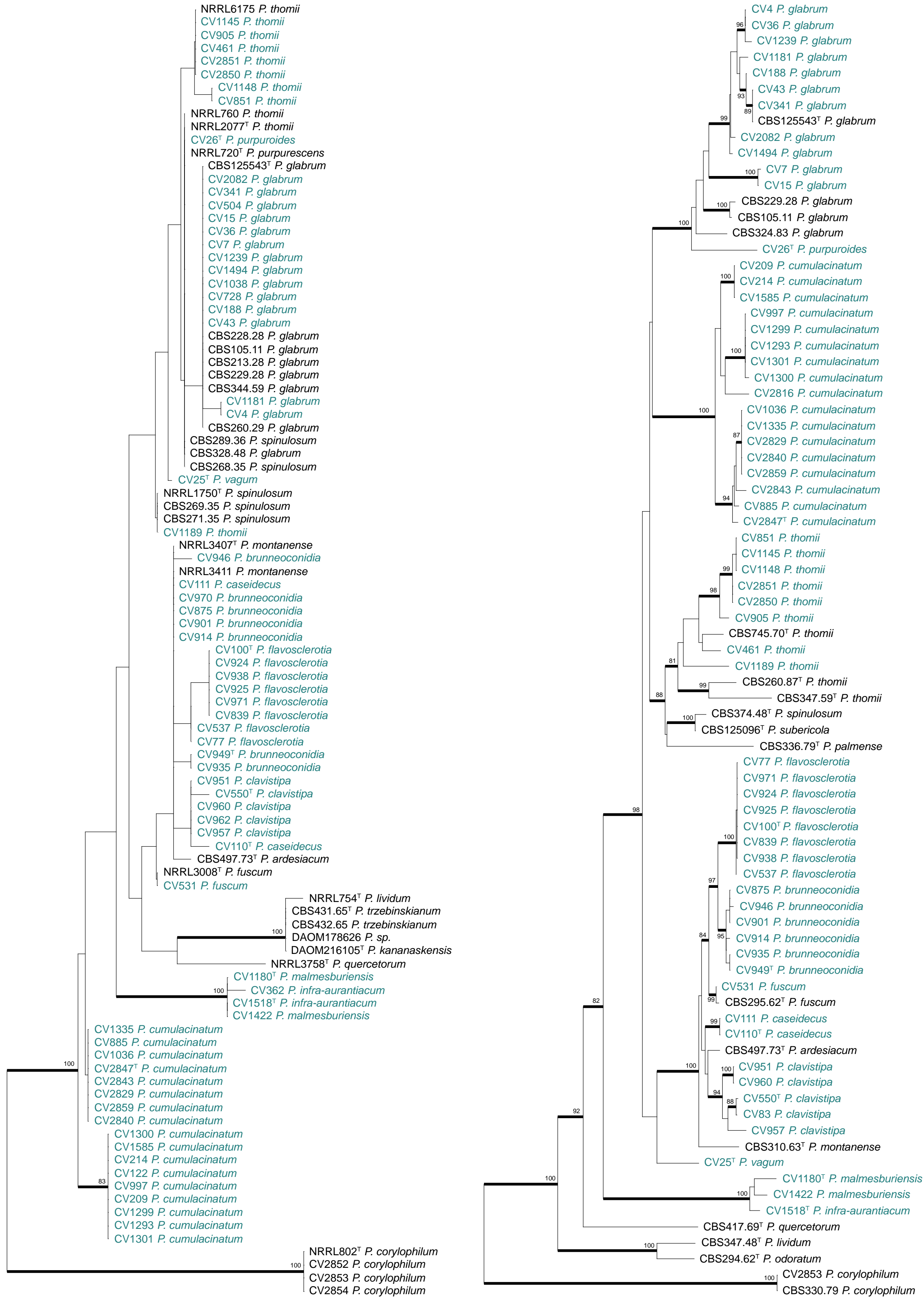

FIGURE 9: Phylogenetic trees based on ITS and RPB2, showing relationship of species in the section *Aspergilloides*. *Penicillium corylophilum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type). Colored names indicate strains isolated from Fynbos.

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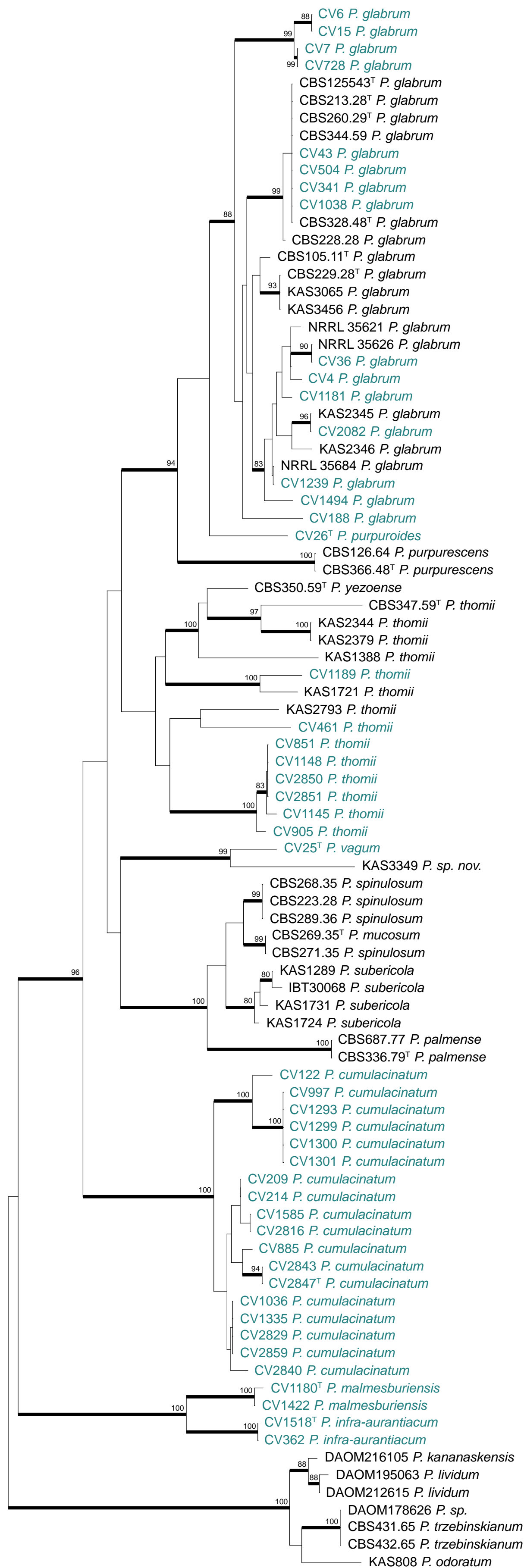

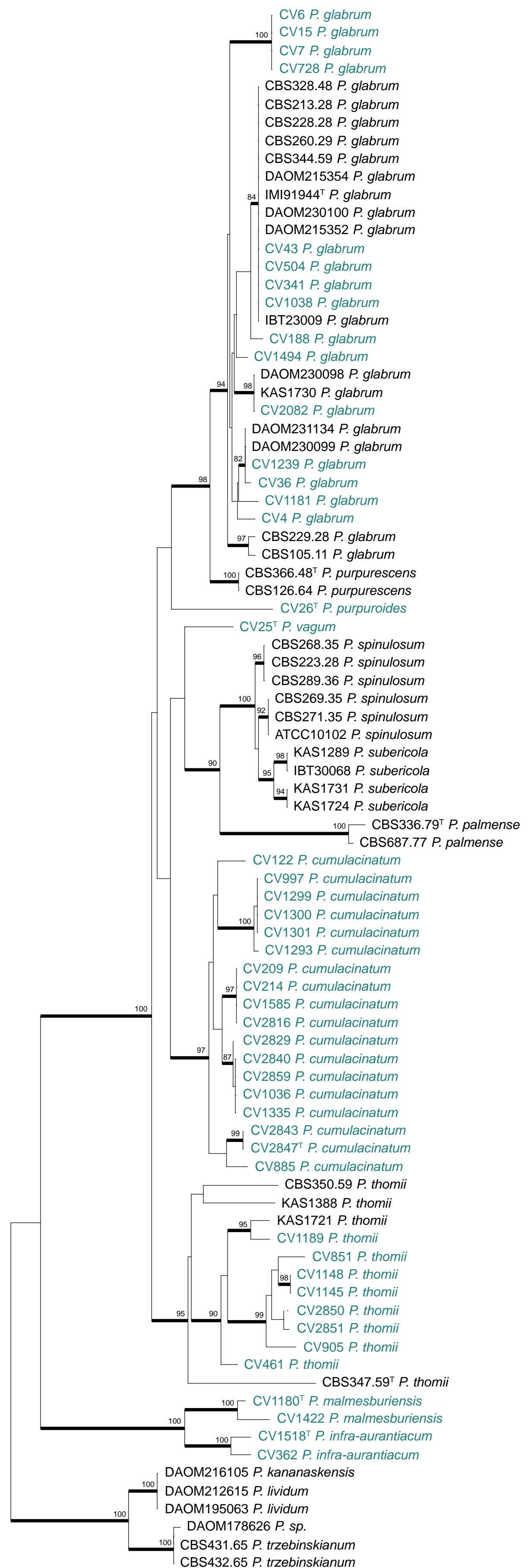

FIGURE 10: Phylogenetic trees based on β-tubulin and Calmodulin, showing relationship of species of morphological similar species in the section *Aspergilloides*. The *P. lividum* clade was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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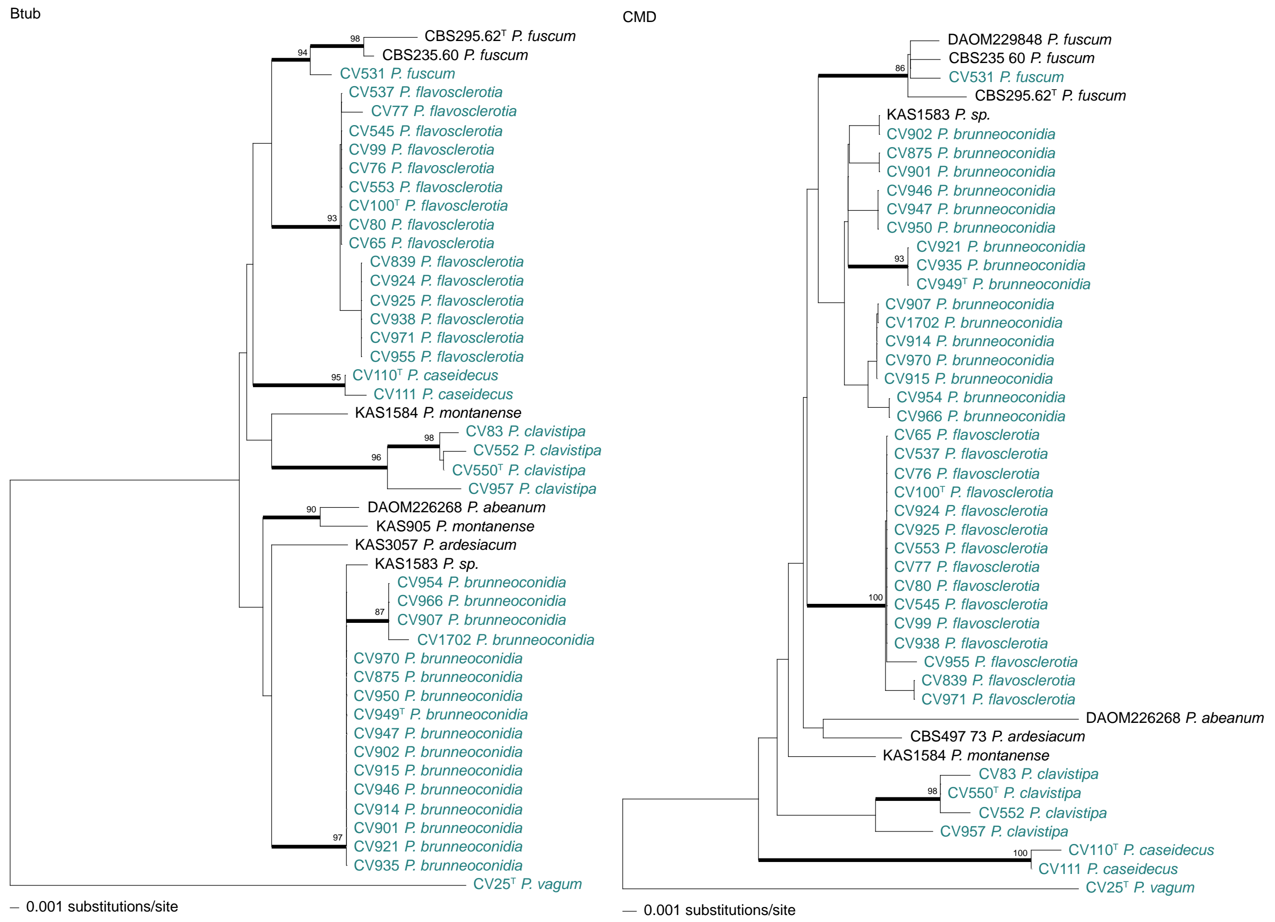

FIGURE 11: Phylogenetic trees based on β-tubulin and Calmodulin, showing relationship of species of morphological similar species in the section *Aspergilloides*. *Penicillium VAGUM* was chosen as outgroup, based on its placement as close relative based on RPB2. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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22. *Penicillium brunneoconidia* Visagie **prov. nom.** PLATES 42, 55a

ETYMOLOGY: Latin, *brunneoconidia* = meaning brown conidia; named after the species conidia that becomes brown with age
EX-TYPE: CV949 = DTO182E4 = KAS4214 = DAOM241359
TYPE ISOLATED FROM: Soil, Malmesbury
SPECIMENS EXAMINED: CV914, CV915, CV921, CV935, CV954, CV970, CV946, CV875, CV901, CV902, CV907, CV947, CV950, CV966, CV1702.
ISOLATED FROM: Soil, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 12–20 mm, low, radially and concentrically sulcate, sometimes plane; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous to floccose; sporulation moderately dense, conidia *en masse* greenish grey to dark green (30F2–30F6), becoming brown (5F8); exudate absent, soluble pigment absent, reverse pigmentation greenish grey to dull green (30D2–30D5) at centre, fading into greyish green (30C3), brown (5F8) in some strains.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: No growth to colonies 9–11 mm, consisting of white mycelia.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 20–32 mm, low, plane; margins low, wide (2–4 mm), entire; mycelia white; texture velutinous near margin, loosely funiculose at centre; sporulation moderately dense, conidia *en masse* greenish grey to dark green (30F2–30F6); exudate absent, soluble pigment absent, reverse pigmentation dull green (27D3) to dark green (27F5) in some isolates near centre, fading into greenish white (27A2) at margin.

YES, 25 °C, 7d: Colonies 17–26 mm, craterform, radially sulcate; margins low, wide (2 mm), entire; mycelia white; texture velutinous, but dominated by funiculose mycelia; sporulation moderately dense centrally, absent in some isolates, conidia *en masse* dull to dark green (25E4–25E3–25F3); exudate absent, soluble pigment absent, reverse pigmentation in sporulating isolates (28F4) at centre, fading into greyish green (1D4) near yellowish white (1A2) margin, in other isolates dull yellow (3B4) to reddish yellow (4A6) near centre, pale yellow (3A3) margin.

G25N, 25 °C, 7d: Colonies 1–5 mm, consisting of white mycelia.

CREA, 25 °C, 7d: Colonies 2–3 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 7.5–30 × 1.5–2.5 µm, minor proportion up to 120 µm, vesicle 2.5–5 [3.4±0.4] µm, average vesicle/stipe width 1.5; phialides ampulliform, 3–6 per stipe, 5–7.5 × 2.5-3.5 [6.1±0.6 × 3.1±0.2] µm; conidia very heavy, thick and rough walled, spheroid, 3.5–4.5 × 3.5–4.5 [3.9±0.3 × 3.9±0.2] µm, average width/length = 0.98±0.02, n = 54.

Notes — *Penicillium brunneoconidia* is characterized by its dark green to olive green conidia becomes dark brown with age. This character was not observed in other species in the clade. It resolved in a clade together with *P. fuscum, P. montanense, P. ardesiacum, P. abeanum* and three new Fynbos species (FIGURES 9, 11). However, *P. brunneoconidia* colonies show restricted growth, which distinguishes it from *P. fuscum*, *P. montanense*, *P. abeanum* and *P. ardesiacum*. The new species produces conidiophores characterized by short stipes (7.5–30 µm) and produce big conidia (3.5–4.5 µm) with thick walls and protuberances. This easily distinguishes it from other novel Fynbos species in the clade (*P. flavosclerotia, P. caseidecus, P. clavistipa*). Compared to *P. brunneoconidia*, *P. flavosclerotia* (23–80 µm) and *P. clavistipa* (20–120 µm) produces longer stipes. *Penicillium caseidecus* produces turquoise conidia, which distinguishes it from the green conidia of *P. brunneoconidia*.

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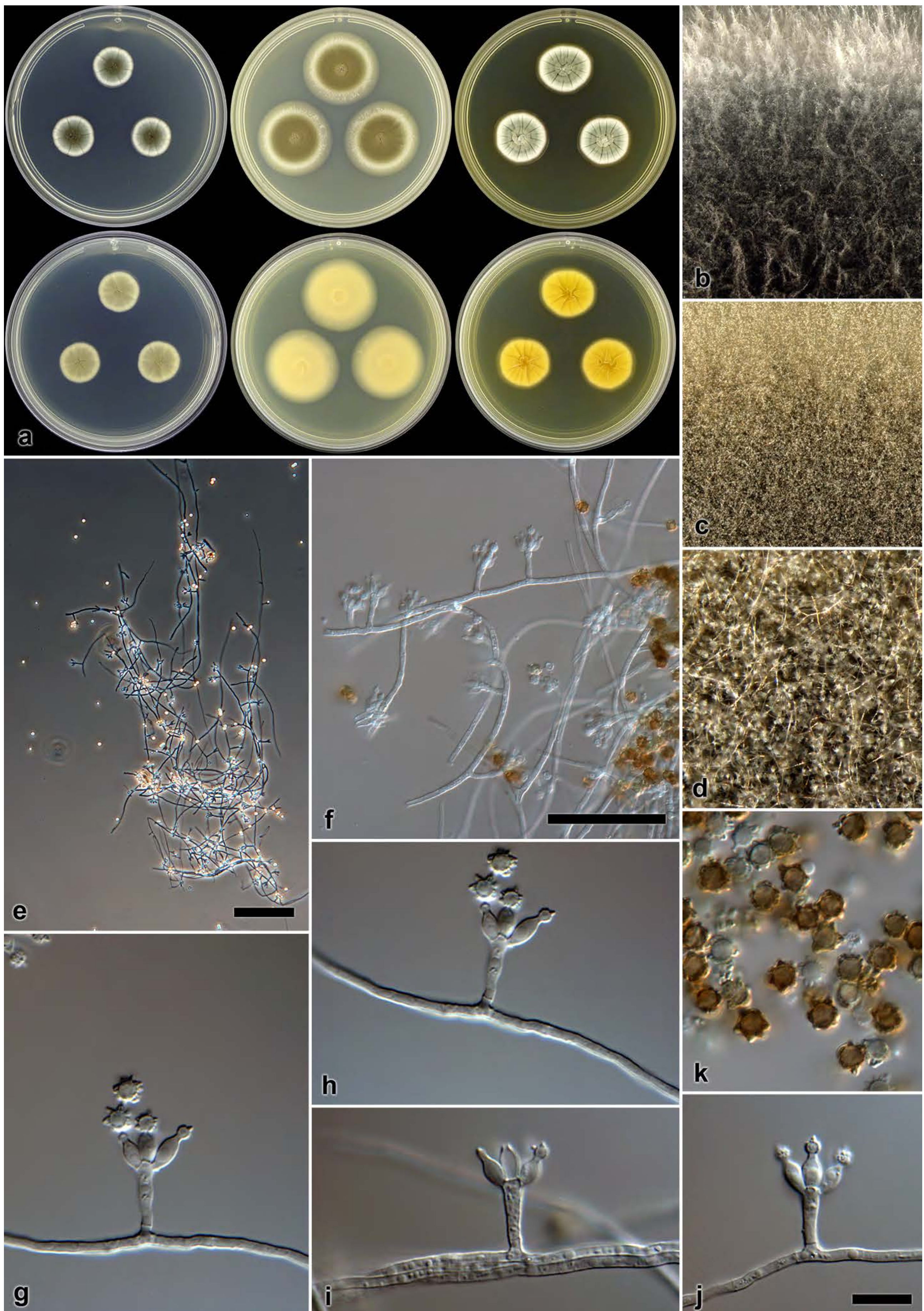

PLATE 42. *Penicillium brunneoconidia* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in j = 10 µm, applies to g–k).

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23. ***Penicillium caseidecus*** Visagie **prov. nom.** PLATES 43, 55b

ETYMOLOGY: Latin, *caseidecus* = meaning blue cheese; named after the light blue turquoise color of conidia on MEA
EX-TYPE: CV110 = DTO181A3 = KAS3970 = DAOM241130
TYPE ISOLATED FROM: Soil, Stellenbosch
SPECIMENS EXAMINED: CV111.
ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 15–20 mm, low, sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25C5–25D5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B6) at centre, fading into a lighter greyish yellow (4B3) near margin

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 5–8 mm, deep, raised at centre, random bulges; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous; sporulation sparse, conidia *en masse* turquoise white to pale turquoise (24A2–24A3); exudate absent, soluble pigment absent, reverse pigmentation dull yellow (3B4).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 28–30 mm, low, plane; margins subsurface, wide (5 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish turquoise (24C4–24C5); exudate absent, soluble pigment absent, reverse pigmentation (4B6) at centre.

YES, 25 °C, 7d: Colonies 22–25 mm, moderately deep, sulcate, having almost beige color; margins low, narrow (1 mm), entire; mycelia white; sporulation absent; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B2–4B6).

G25N, 25 °C, 7d: Colonies 5–6 mm, consisting of white mycelial mass.

CREA, 25 °C, 7d: Colonies 4–5 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate, mycelia on which conidiophores are borne often rough walled; stipes smooth walled, 6–30 × 1.5–3 µm, vesicle 3–6 [4.3±0.7] µm, average vesicle/stipe width 1.9; phialides ampulliform, 12–18 per stipe, 5–6.5 × 2.5–3.5 [5.5±0.5 × 2.7±0.25] µm; conidia rough walled, spheroid, 2–2.5 × 2–2.5 [2.5±0.09 × 2.5±0.1] µm, average width/length = 0.98±0.02, n = 40.

Notes — *Penicillium caseidecus* resolved in a clade together with other Fynbos species that displays restricted growth, as well as *P. fuscum, P. montanense, P. ardesiacum* and *P. abeanum* (FIGURES 9, 11). Its restricted growth separates it from the faster growing *P. fuscum, P. montanense, P. abeanum* and *P. ardesiacum*. *Penicillium caseidecus* produces very short and rather broad conidiophore stipes (6–30 µm), which distinguishes it from the closely related *P. clavistipa* and *P. flavosclerotia*. *Penicillium brunneoconidia* has similar stipe lengths (7.5–30 µm), however, it produces dark green conidia compared to the turquoise conidia in *P. caseidecus*.

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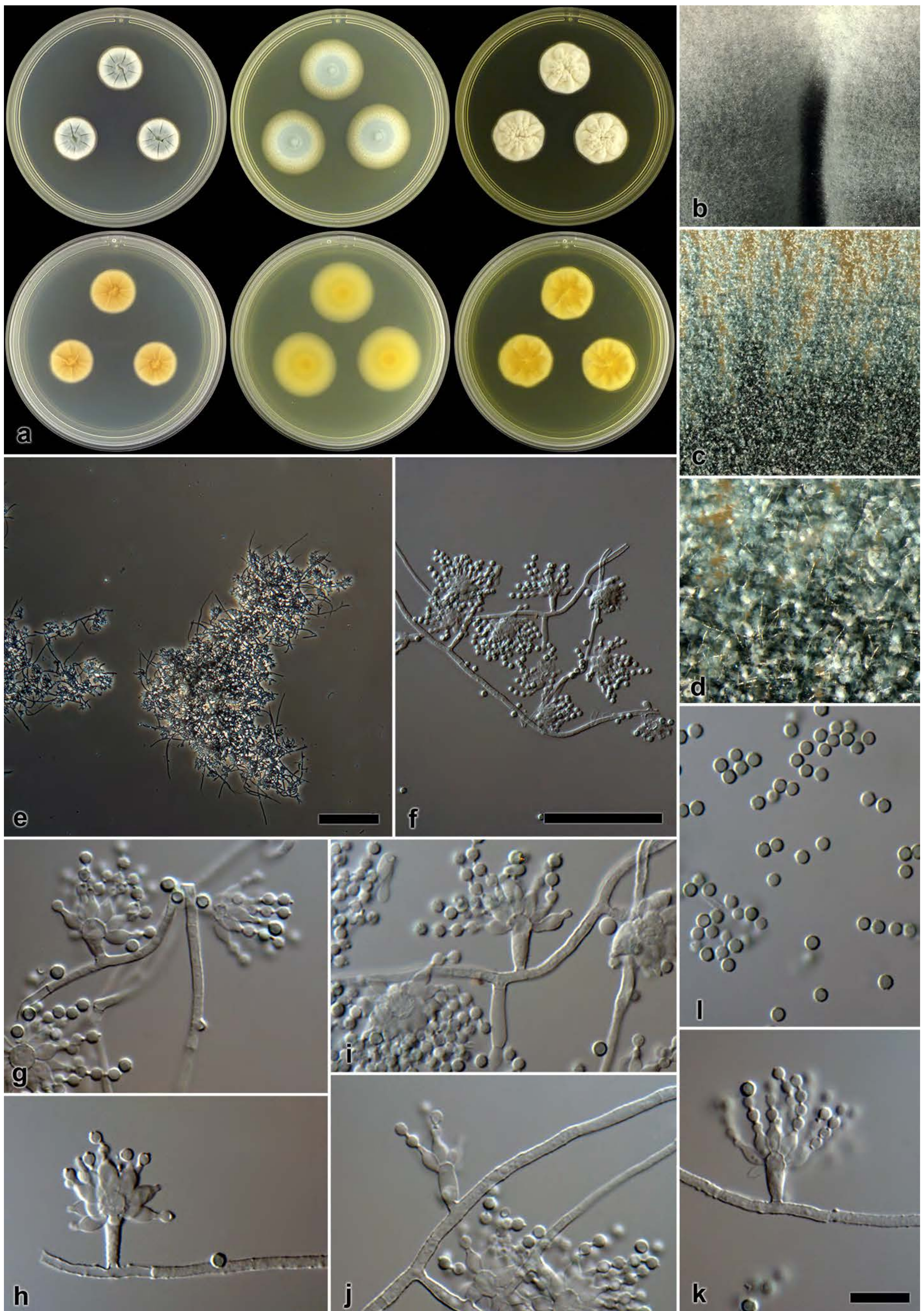

PLATE 43. *Penicillium caseidecus* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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24. *Penicillium clavistipa* Visagie **prov. nom.** PLATES 44, 55c

ETYMOLOGY: Latin, *clavistipa* = meaning club-shaped stipe; named after its vesiculated stipes
EX-TYPE: CV550 = DTO181I3 = KAS4156 = DAOM241129
TYPE ISOLATED FROM: Soil, Stellenbosch
SPECIMENS EXAMINED: CV83, CV552, CV957.
ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 10–15 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish yellow (2C3–3C3); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2C3) at centre, yellowish white (2A2) near margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 1–12 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish yellow (2C3); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2C3) at centre, yellowish white (2A2) near margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 23–32 mm, low, plane; margins low, wide (2–4 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* olive (1F5–2F5); exudate absent, soluble pigment absent, reverse pigmentation greenish grey (30B2) at centre and margin, greyish green (1C3) elsewhere.

YES, 25 °C, 7d: Colonies 12–20 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (29E5) near centre, greyish turquoise (24B3) near margin; exudate absent, soluble pigment absent, reverse pigmentation reddish yellow (4A7) at centre, greyish yellow (4B4) elsewhere.

G25N, 25 °C, 7d: Colonies 5–9 mm, consisting of white mycelial mass.

CREA, 25 °C, 7d: Colonies 8–13 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate; stipes rough walled, 20–120 × 2.5–3.5 µm, vesicle 5.5–10 [7.1±0.1] µm, average vesicle/stipe width 2.56; phialides ampulliform, 22–35 per stipe, 7–9 × 2.5–3.5 [8±0.6 × 3.1±0.27] µm; conidia spheroid, heavy rough walls, 2.5–3 × 2.5–3 [2.53±0.09 × 2.52±0.1] µm, average width/length = 0.98±0.02, n = 45.

Notes — *Penicillium clavistipa* resolved in a clade with *P. fuscum, P. montanense, P. ardesiacum, P. abeanum* and three new Fynbos species (FIGURES 9, 11). It characteristically displays restricted growth on CYA and MEA, which distinguishes it from *P. fuscum, P. montanense, P. ardesiacum* and *P. abeanum*. Conidiophore stipes are rough walled and end in vesicles up to 10 µm, a character not observed in any other species in the clade. Colonies resemble those of *P. brunneoconidia*. However, conidia of *P. clavistipa* on CYA are greyish yellow compared to the greyish to olive-green conidia in *P. brunneoconidia*.

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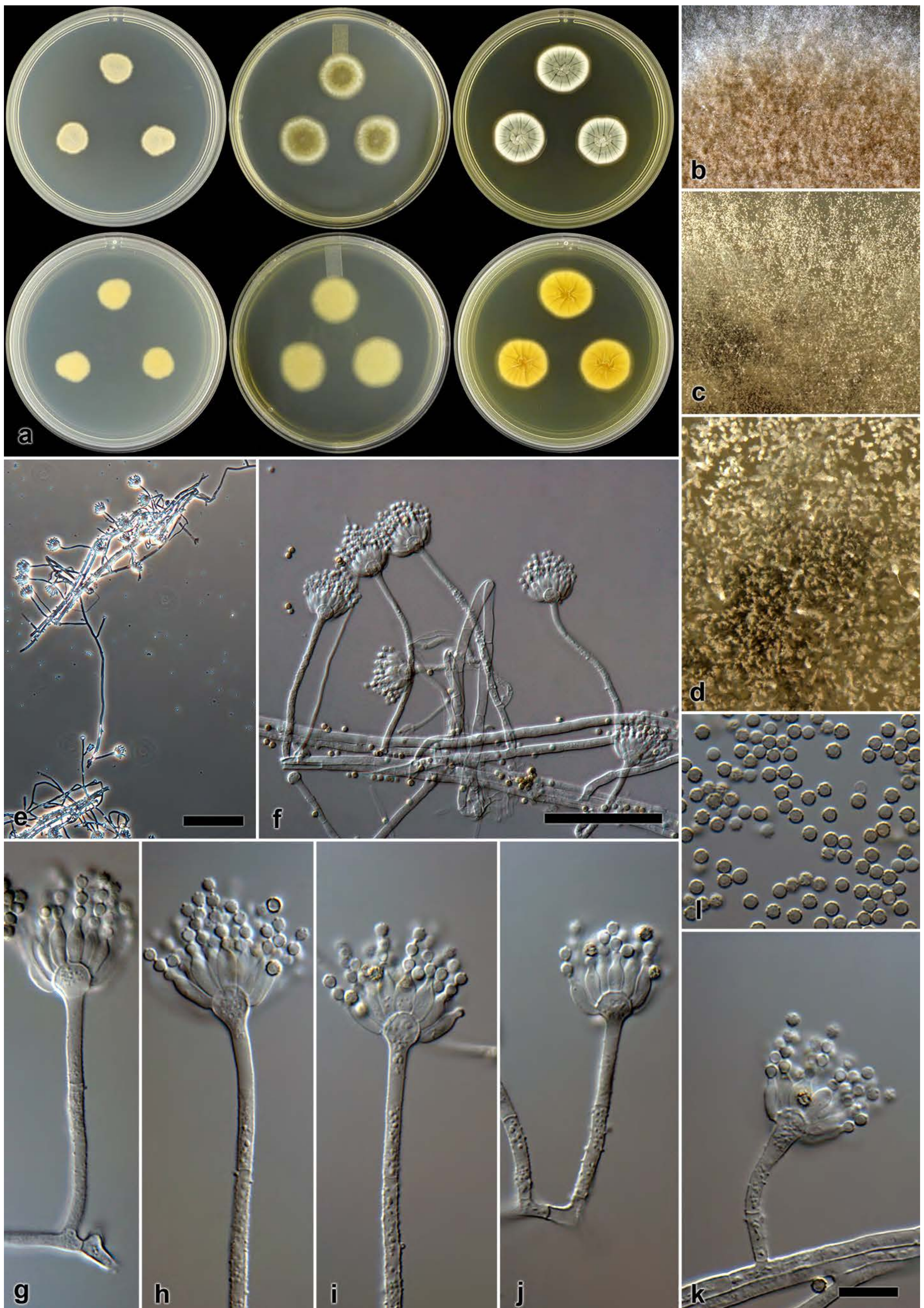

PLATE 44. *Penicillium clavistipa* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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25. ***Penicillium cumulacinatum*** Visagie **prov. nom.** PLATES 45, 55d

ETYMOLOGY: Latin, *cumulacinatum*: *cumulus* = pile; *acinus* = berry, referring to the long chains of conidia produced in culture
EX-TYPE: CV2847 = DTO180D9 = KAS3943 = DAOM241119
TYPE ISOLATED FROM: Soil, Malmesbury
SPECIMENS EXAMINED: CV997, CV1293, CV1299, CV1300, CV1301, CV209, CV214, CV1036, CV1247, CV1335, CV1339, CV1345, CV1547, CV1568, CV1585, CV1586, CV2779, CV2829, CV2840, CV2843, CV2859.
ISOLATED FROM: Soil, Mites and Bracts of *Protea repens* infructescences, Stellenbosch and Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 35–46 mm, low, radially and concentrically sulcate, sclerotia abundant underneath sporulation; margins low, narrow to wide (2–4 mm), entire tp irregular; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25E4–25E5); exudate absent to clear, soluble pigment yellow to dark yellow, reverse pigmentation light to dull yellow (3A4–3B4), with some orange (5B8–5C8) areas.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 20–33 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), irregular; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise to greyish green (24D5–25D5) at centre, greyish turquoise to greyish green (24B4–25B4) elsewhere; exudate absent, soluble pigment brownish orange with yellow halo surrounding colonies, reverse pigmentation brown (7E8) and greyish orange (6B6) areas, in some isolates the brown lacking, then greyish yellow (3B5).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 30–45 mm, low, plane, sclerotia abundant underneath sporulation; margins low, wide (3–4 mm), entire to irregular; mycelia white; texture velutinous with some floccose mycelia near centre; sporulation moderately dense, conidia *en masse* dull green (25D3–25D4) to greyish green (25E4–25E5); exudate absent, soluble pigment yellow in some isolates, mostly absent, reverse pigmentation yellowish white to pale yellow (1A2–1A3) at centre and margin, greyish yellow to greyish green (1B3–1C3) elsewhere, in some strains light yellow (4A4) at centre, fading into greyish yellow (1B3), orange (5B7) rings present.

YES, 25 °C, 7d: Colonies 45–50 mm, low to almost moderately deep, craterform, radially and concentrically sulcate; margins low, wide (2–3 mm), entire; mycelia white; texture velutinous, with some floccose near centre; sporulation moderately dense to sparse in some isolates, conidia *en masse* greyish green (25C4–25D5–25D6); exudate absent, soluble pigment brownish yellow in some isolates, mostly absent, reverse pigmentation greyish yellow (4B6) areas near centre, light yellow to greyish yellow (3A5–3B5) elsewhere.

G25N, 25 °C, 7d: Colonies 18–25 mm, low, radially and concentrically sulcate; margins low, wide (1–4 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dark green (25F4) near centre, greyish turquoise (24D4) near margin; exudate absent, soluble pigment sometimes very light yellow, mostly absent, reverse pigmentation yellowish white (1A2) at centre and margin, greenish grey to greyish green (30B2–30B5) or greenish white to greenish grey (30A2–30B2) elsewhere.

CREA, 25 °C, 7d: Colonies 13–18 mm, weak acid produced.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 60–330 × 2.5–3.5 µm, vesicle 4–9 [6±1.3] µm, average vesicle/stipe width 2.1; phialides ampulliform, 12–25 per stipe, 7–11 × 2.5–4 [8.6±0.6 × 3.1±0.3] µm; conidia finely rough walled, subspheroid, 2.5–3.5 × 2–3 [2.74±0.2 × 2.6±0.1] µm, average width/length = 0.93±0.1, n = 170; sclerotia produced on CYA and MEA, 50–250 × 40–250 µm.

Notes — *Penicillium cumulacinatum* produces abundant sclerotia on CYA and MEA. On CYA at 30 °C, a typical brown soluble pigment are consistently produced and colonies are surrounded by a yellow halo. To some degree, colonies resemble those of *P. thomii*. *Penicillium cumulacinatum*, however, commonly produce yellow to brown soluble pigments on CYA and MEA compared to *P. thomii* that lack soluble pigments. Based on micromorphology, *P. thomii* produce ellipsoid conidia compared to *P. cumulacinatum* that produce spheroid conidia. *Penicillium cumulacinatum* is phylogenetically distantly related to all previously described species (FIGURES 9, 10).

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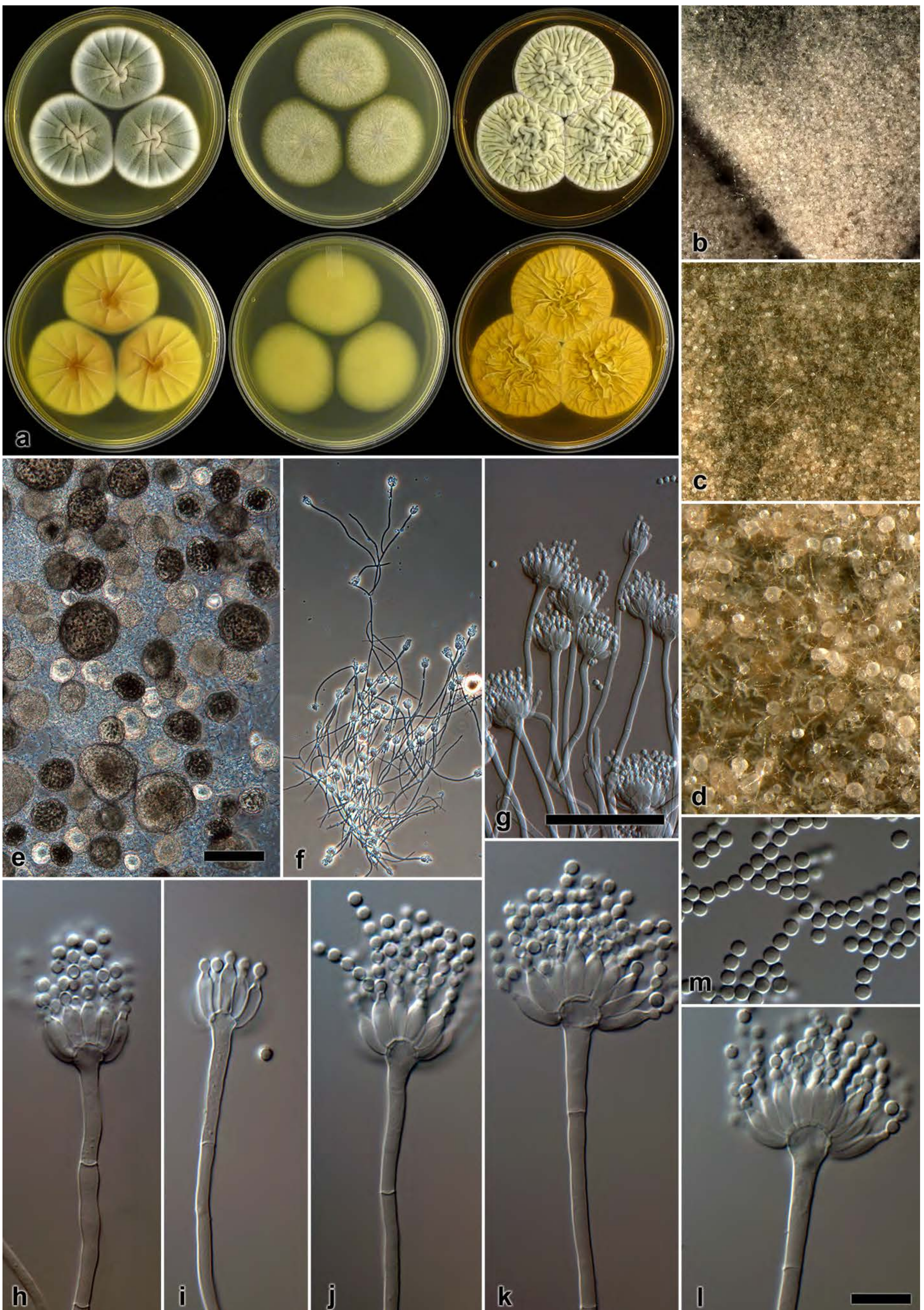

PLATE 45. *Penicillium cumulacinatum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia on MEA. f–l. Conidiophores. m. Conidia (— Scale bar in e = 100 µm, applies to e, f; — Scale bar in g = 50 µm; — Scale bar in l = 10 µm, applies to h–m).

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26. ***Penicillium flavosclerotia*** Visagie **prov. nom.** PLATES 46, 55e

ETYMOLOGY: Latin,
EX-TYPE: CV100 = DTO180I8 = KAS3958 = DAOM241157
TYPE ISOLATED FROM: Soil, Stellenbosch
SPECIMENS EXAMINED: CV65, CV70, CV73, CV74, CV76, CV80, CV84, CV94, CV97, CV99, CV108, CV109, CV537, CV545, CV553, CV839, CV895, CV924, CV925, CV938, CV955, CV971.
ISOLATED FROM: Soil, Stellenbosch and Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 15–25 mm, low, radially sulcate, yellow sclerotia present; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation absent to moderately dense, conidia *en masse* dull green (28E4); exudate absent, soluble pigment absent, reverse pigmentation pale to greyish yellow (2A3–2B3), some strains brownish orange (5C5).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 10–15 mm, conisisting of white mycelia, reverse pigmentation reverse pigmentation pale to greyish yellow (2A3–2B3).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 18–35 mm, low, plane, yellow sclerotia present; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to sparse, conidia *en masse* pale green (26A3); exudate absent, soluble pigment absent, reverse pigmentation light yellow (2A5) at centre, yellowish white (1A2) elsewhere.

YES, 25 °C, 7d: Colonies 20–30 mm, low, radially sulcate, yellow sclerotia present; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to sparse; exudate absent, soluble pigment absent, reverse pigmentation pale to greyish yellow (2A3–2B3), some strains brownish orange (5C5).

G25N, 25 °C, 7d: Colonies 2–4 mm, consisting of white mycelia

CREA, 25 °C, 7d: Colonies 8–12 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 23–80 × 2–3.5 µm, vesicle 4–5.5 [4.6±0.4] µm, average vesicle/stipe width 1.98; phialides ampulliform, 8–20 per stipe, 6–9 × 2.5–3.5 [7.7±0.6 × 3.1±0.2] µm; conidia heavy rough to spiny walls, some strains only finely rough walled, spheroid to somewhat subspheroid, 2–3.5 × 2–3.5 [2.85±0.4 × 2.84±0.4] µm, average width/length = 0.97±0.02, n = 30; sclerotia produced on CYA and MEA, 80–160 × 70–150 µm

Notes — Strains in *P. flavosclerotia* resolved in a clade together with *P. fuscum, P. montanense, P. ardesiacum, P. abeanum* and three other new Fynbos species (FIGURES 9, 11). The slow growth distinguishes this species from the previously described species. It produces abundant yellow sclerotia on all media. This character is not observed for the other species in the clade (*P. brunneoconidia, P. caseidecus, P. clavistipa*), except for *P. fuscum*. *Penicillium flavosclerotia* colonies are similar to *P. fuscum*. However, *Penicillium fuscum* grows faster on YES (35–40 mm) and grows poorly at 30°C (2–3 mm), compare to *P. flavosclerotia* (20–30 mm; 10–15 mm). For *P. flavosclerotia* sporulation is often absent and sometimes only occurs after two weeks of growth. Sclerotia never mature into ascospores, even after months of incubation. Conidiophores have short smooth walled stipes and produce conidia with heavy rough walls and is micromorphologically very similar to *P. fuscum*. The multigene phylogenies confirm this as a unique species (FIGURES 9, 11).

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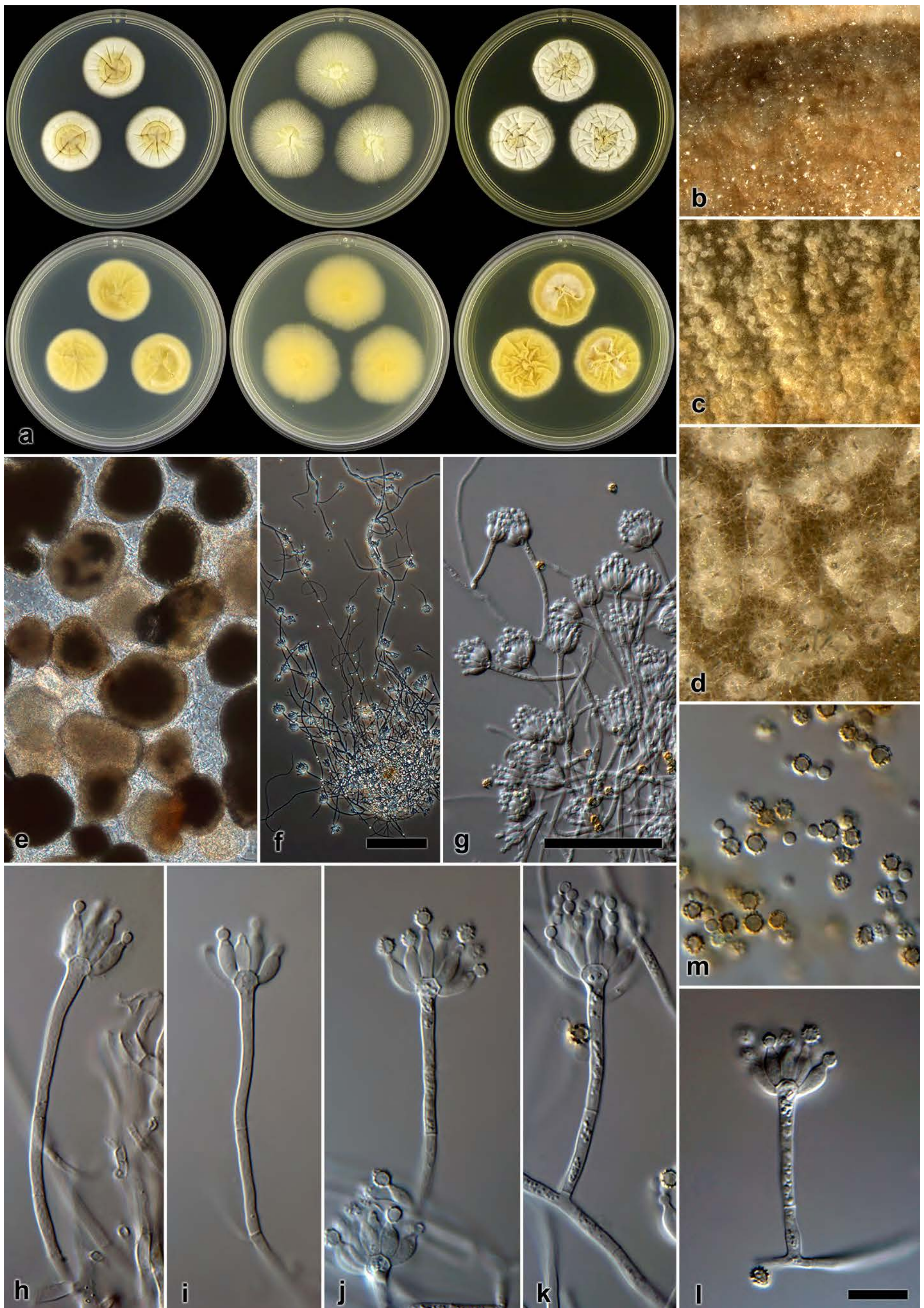

PLATE 46. *Penicillium flavosclerotia* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia on MEA. f–l. Conidiophores. m. Conidia (— Scale bar in f = 100 µm, applies to e, f; — Scale bar in l = 50 µm; — Scale bar in l = 10 µm, applies to h–m).

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27. *Penicillium fuscum* Biourge

PLATES 47, 55f

La Cellule 33: 315. 1923.

EX-TYPE: CBS295.62 = NRRL3008 = IMI094209
TYPE ISOLATED FROM: Pine forest soil, Vilas County, Wisconsin, USA
SPECIMENS EXAMINED: CV531.
ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 25–27 mm, low, sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dark green (30F6) to greyish turquoise (24B4); exudate absent, soluble pigment absent, reverse pigmentation pale to greyish green (30A3–30C3).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 2–3 mm, consiting of white mycelia.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 35–38 mm, low, plane, yellowish sclerotia only sometimes produced; margins low, wide (3–4 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dark green (30F5); exudate absent, soluble pigment absent, reverse pigmentation pale to greyish green (30A3–30C3).

YES, 25 °C, 7d: Colonies 35–40 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous to floccose; sporulation moderately dense, conidia *en masse* greyish green (30E6) to greyish green (25B4); exudate absent, soluble pigment absent, reverse pigmentation similar to CYA.

G25N, 25 °C, 7d: Colonies 5–8 mm, low, plane; margins low, narrow (<1 mm), entire; mycelia white; texture velutinous; sporulation sparse, conidia *en masse* greyish green (26C5); exudate absent, soluble pigment absent, reverse pigmentation greenish grey (26B2).

CREA, 25 °C, 7d: Colonies 8–12 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 14–70 × 2–3 µm, vesicle 3.5–5 [4.6±0.4] µm, average vesicle/stipe width 1.8; phialides ampulliform, 12–24 per stipe, 7.5–9 × 2.5–3.5 [8.1±0.4 × 2.98±0.2] µm; conidia finely rough walled, subspheroid, 2.5–3 × 2–2.5 [2.65±0.1 × 2.4±0.1] µm, average width/length = 0.92±0.04, n = 40.

Notes — *Penicillium fuscum* resolved in a clade together with, *P. montanense, P. ardesiacum, P. abeanum* and four new species described from Fynbos. This species produces abundant yellow sclerotia on all media. From this clade, this character was only observed in *P. flavosclerotia*. However, *Penicillium fuscum* grows faster on YES (35–40 mm) and grows poorly at 30°C (2–3 mm), compared to *P. flavosclerotia* (20–30 mm; 10–15 mm).

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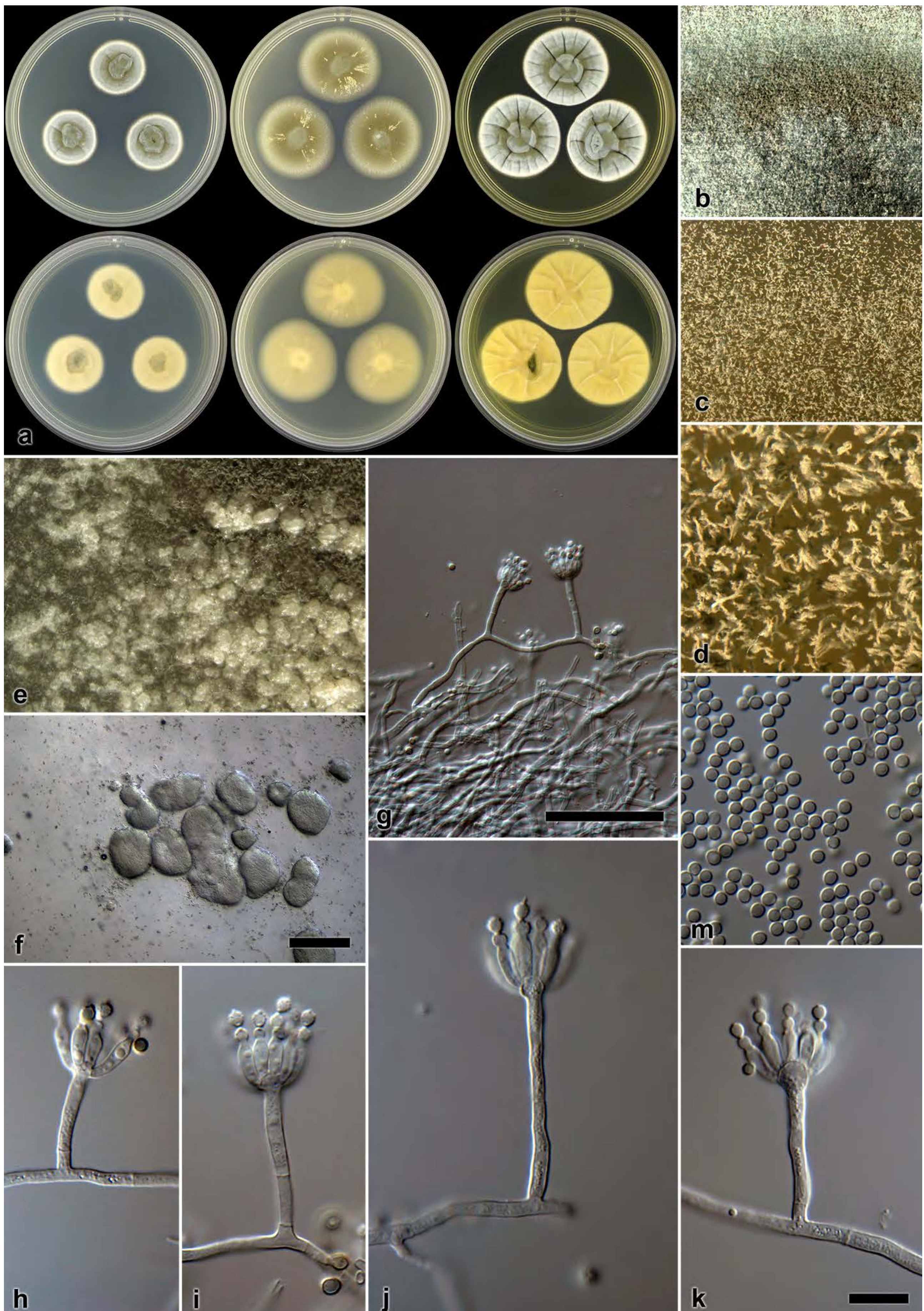

PLATE 47. *Penicillium fuscum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e, f. Sclerotia on MEA. g–k. Conidiophores. l. Conidia (— Scale bar in f = 100 µm; — Scale bar in g = 50 µm; — Scale bar in k = 10 µm, applies to h–m).

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28. *Penicillium glabrum* (Wehmer) Westling PLATES 48, 55g

Arkiv før Botanik 11: 131. 1911.

BASIONYM: *Citromyces glaber* (Beiträge zur Kenntnis Einheimischer Pilze 1: 92. 1893.

EX-TYPE: CBS125543 = IBT22658 = IMI91944

TYPE ISOLATED FROM: Unknown source

SPECIMENS EXAMINED: CV4, CV6, CV7, CV15, CV728, CV43, CV504, CV341, CV1038, CV188, CV1494, CV2082, CV36, CV1239, CV1181.

ISOLATED FROM: Soil, Air, Mites and Bracts of *Protea repens* infructescences, Stellenbosch, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 30–45 mm, low, sulcate, white sterile mycelia commonly produced at colony centre masking sporulation; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (26F4–26F6) to dull green (26D5) near margin; exudate absent, soluble pigment absent, some strains reddish yellow, some strains light reddish, reverse pigmentation range from orange to brownish orange (5A6–5C6), brownish orange (6C7), dark brown (7F8), pale yellow (1A3) and light yellow (3A4).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 16–30 mm, characters similar to that on CYA at 25°C.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 30–50 mm, low, plane; margins low, narrow to wide (2–4 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, some strains red, some strains yellow, reverse pigmentation range from dark brown (7F8), greyish orange (5B6), greyish yellow (2B7), greenish grey (1B2).

YES, 25 °C, 7d: Colonies 35–50 mm, low, randomly sulcate, often yellowish color near centre; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation colors similar to CYA at 25 °C.

G25N, 25 °C, 7d: Colonies 12–18 mm, low, lightly sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to sparse, conidia *en masse* greyish green (25C4) to dull green (28D4); exudate absent, soluble pigment absent, reverse pigmentation similar colors as on CYA at 25 °C.

CREA, 25 °C, 7d: Colonies 20–30 mm, weak acid production within colony periphery.

Micromorphology — Conidiophores monoverticillate, some strains have greenish olive pigment; stipes smooth to finely rough walled, 50–200 × 2.5–3.5 µm, vesicle 5–8 [6.8±0.7] µm, average vesicle/stipe width 2.3; phialides ampulliform, 14–24 per stipe, 7.5–10.5 × 2.5–4 [8.8±0.7 × 3.4±0.3] µm; conidia smooth finely rough walled, spheroid, some subspheroid, 2.5–3 × 2.5–3 [2.8±0.15 × 2.8±0.14] µm, average width/length = 0.98±0.02, n = 50.

Notes — *Penicillium glabrum* display characteristic fast growth on most media and produce strictly velutinous colonies. A large number of strains produced white sterile mycelia that mask the dark green conidial areas. *Penicillium glabrum* is closely related to *P. purpurescens*. *Penicillium glabrum*, however, produce smooth to finely rough walled conidia, compared to *P. purpurescens* that produce heavy rough walled conidia. Also, *P. purpurescens* produce larger conidia (3.5–4.5 µm) compared to *P. glabrum* that has smaller conidia (2.5–3 µm).

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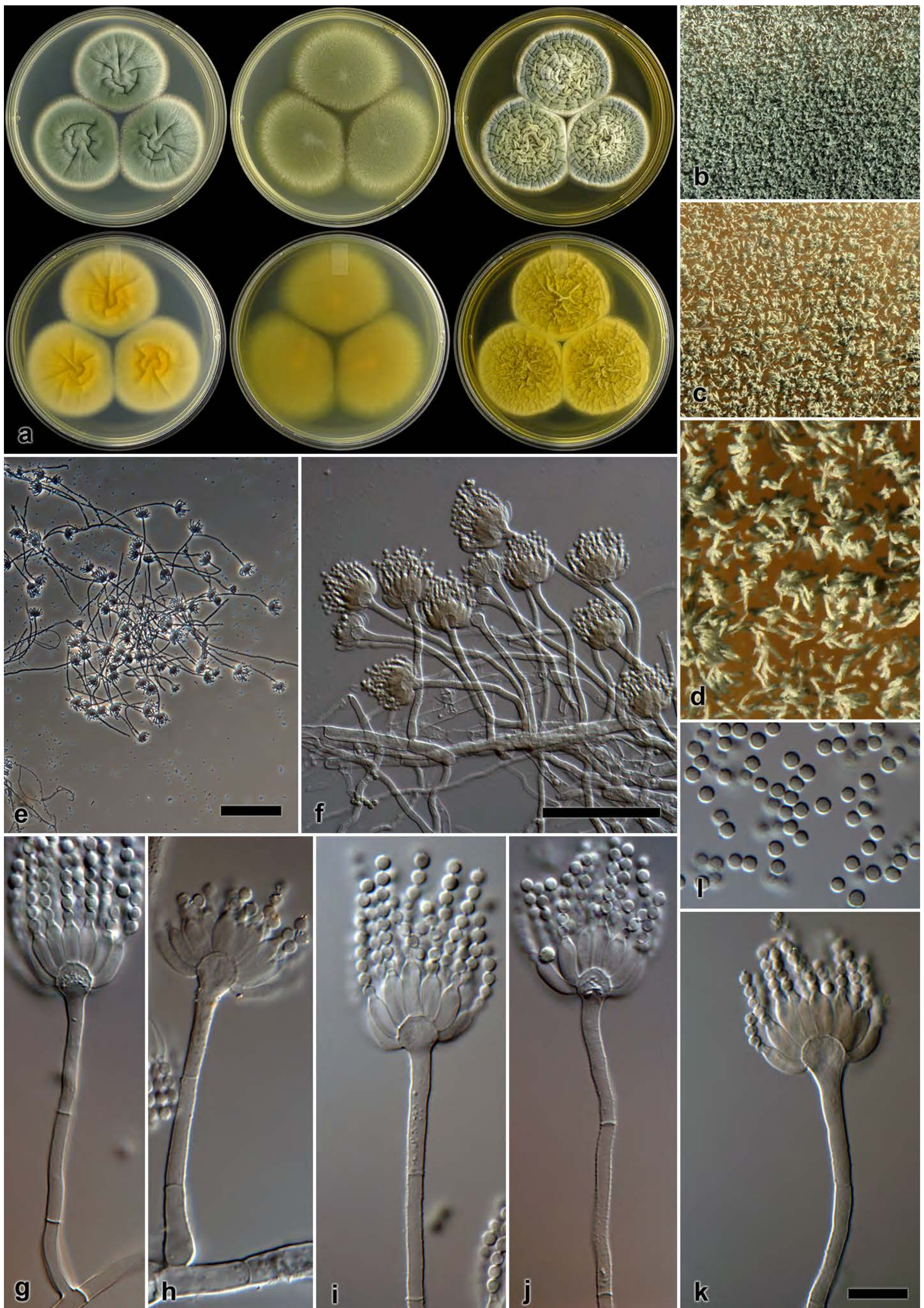

PLATE 48. *Penicillium glabrum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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29. ***Penicillium infra-aurantiacum*** Visagie **prov. nom.** PLATES 49, 55h

ETYMOLOGY: Latin, *infra-aurantiacum* = meaning underneath orange; named after the orange reverse pigmentation that is diagnostic in the species
EX-TYPE: CV1518 = DTO183C3 = KAS4022 = DAOM241145
TYPE ISOLATED FROM: Bracts of *Protea repens* infructescence, Malmesbury
SPECIMENS EXAMINED: CV362.
ISOLATED FROM: Bracts of *Protea repens* infructescence, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 29–30 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dark green (26F8); exudate orange, soluble pigment absent, reverse pigmentation orange (6B8) fading into light yellow (2C4) margin.

CYA, 5 °C, 7d: Germination to microcolonies.

CYA, 30 °C, 7d: Colonies 3–5 mm, having an almost orange color, reverse pigmentation brownish yellow (5C7)

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 33–40 mm, low, plane; margins low, wide (3 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (26F8); exudate absent, soluble pigment absent, reverse pigmentation greyish to dull green (28C3–28D3).

YES, 25 °C, 7d: Colonies 35–40 mm, low, radially and concentrically sulcate, brownish orange color at centre; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish turquoise (24B3); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (6C7) at centre, pale to greyish yellow (2A3–2B3) elsewhere.

G25N, 25 °C, 7d: Colonies 9–12 mm, raised at centre, radially sulcate, having an almost pinkish to orange color near centre; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to sparse, conidia *en masse* greyish green (25C4); exudate absent, soluble pigment absent, reverse pigmentation light orange (5A4–6A4).

CREA, 25 °C, 7d: Colonies 20–24 mm, weak acid production, only within colony periphery.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 100–230 × 2–3 µm, vesicle 4.5–6 [5.2±0.3] µm, average vesicle/stipe width 1.95; phialides ampulliform, 10–18 per stipe, 8.5–11 × 2.5–3 [9.7±0.6 × 3.1±0.2] µm; conidia rough walled, spheroid, 2.5–3.5 × 2.5–3.5 [3.0±0.2 × 3.0±0.14] µm, average width/length = 0.98±0.02, n = 32.

Notes — *Penicillium infra-aurantiacum* characteristically produce smooth walled stipes and rough walled conidia. Colonies consistently produce orange exudate and orange colors in colony reverses. This, together with its longer stipes (100–230 µm) distinguishes it from its closest relative *P. malmesburiensis* (stipes 35–115 µm). Morphologically it resembles *P. glabrum*. However, it shows restricted growth on CYA at 30 °C (3–5 mm) and also on average has bigger conidia (3.0±0.2 × 3.0±0.14 µm) compared to *P. glabrum* (16–30 mm; 2.8±0.15 × 2.8±0.14 µm).

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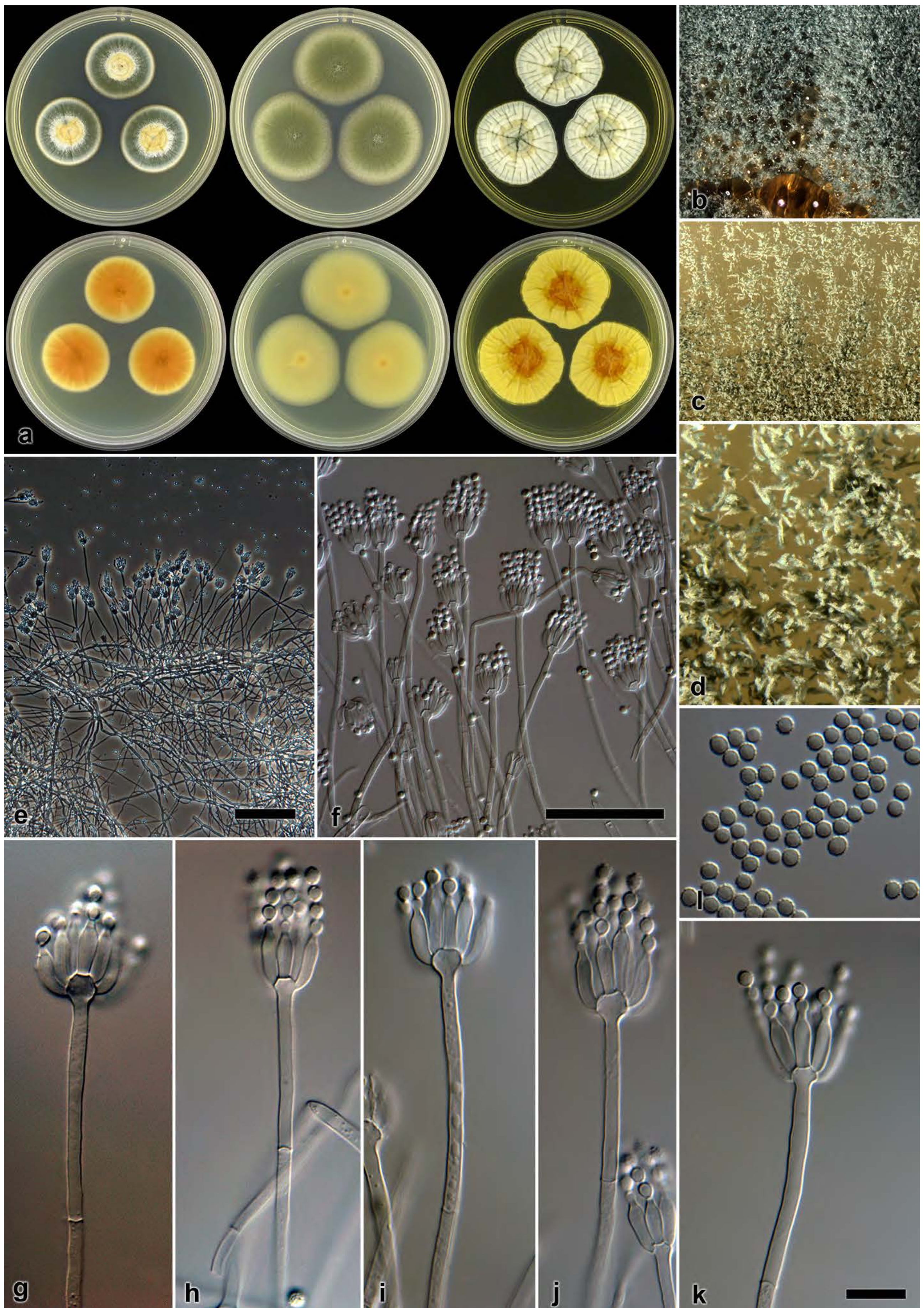

PLATE 49. *Penicillium infra-aurantiacum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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30. ***Penicillium malmesburiensis*** Visagie **prov. nom.** PLATES 50, 55i

ETYMOLOGY: Latin, *malmesburiensis*: named after the collection site, Malmesbury, it was isolated from

EX-TYPE: CV1180 = DTO182H5 = KAS3979 = DAOM241144

TYPE ISOLATED FROM: Mite from *Protea repens* infructescence, Malmesbury

SPECIMENS EXAMINED: CV1422.

ISOLATED FROM: Bracts from *Protea repens* infructescence, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 32–36 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (27F8); exudate absent, soluble pigment absent, reverse pigmentation greenish grey to greyish green (28B2–28B3).

CYA, 5 °C, 7d: Germination to microcolonies.

CYA, 30 °C, 7d: Colonies 8–10 mm, raised at centre, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to moderately dense, conidia *en masse* greyish turquoise (24D3–24D4); exudate absent, soluble pigment absent, reverse pigmentation olive brown (4D4).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 33–38 mm, low, plane; margins low, (2–3 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (27F8); exudate absent, soluble pigment absent, reverse pigmentation greyish to dull green (28C3–28D3).

YES, 25 °C, 7d: Colonies 32–36 mm, low, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* dark green (25F6–26F6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2C4) and dull green (29E3).

G25N, 25 °C, 7d: Colonies 10–15 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation absent to sparse, conidia *en masse* greyish green (25D5); exudate absent, soluble pigment absent, reverse pigmentation greenish grey (1B2).

CREA, 25 °C, 7d: Colonies 20–24 mm, weak acid production, only within colony periphery.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 35–115 × 2–3 µm, vesicle 4–6.5 [5.6±0.6] µm, average vesicle/stipe width 2.1; phialides ampulliform, 10–20 per stipe, 8.5–10 × 3–3.5 [9.5±0.5 × 3.3±0.2] µm; conidia finely rough walled, subspheroid, 2.5–3.5 × 2.5–3 [3.1±0.2 × 3.0±0.2] µm, average width/length = 0.96±0.04, n = 44.

Notes — *Penicillium malmesburiensis* produce smooth walled stipes and lightly rough walled conidia. Together with its closest relative *P. infra-aurantiacum*, it is phylogenetically resolved in a clade basal to the section. In general, conidiophores produced by *P. infra-aurantiacum* are more slender and have longer stipes (100–230 µm) compared to *P. malmesburiensis* (35–115 µm). Also, *P. malmesburiensis* lacks the orange exudate and the yellowish orange reverse pigmentation on CYA and YES present in *P. infra-aurantiacum*. *Penicillium malmesburiensis* is morphologically similar to *P. glabrum*. However, *P. malmesburiensis* grows slower on CYA at 30 °C (8–10 mm) and produces bigger conidia (average 3.1±0.2 × 3.0±0.2 µm) compared to *P. glabrum* (16–30 mm; 2.8±0.15 × 2.8±0.14 µm).

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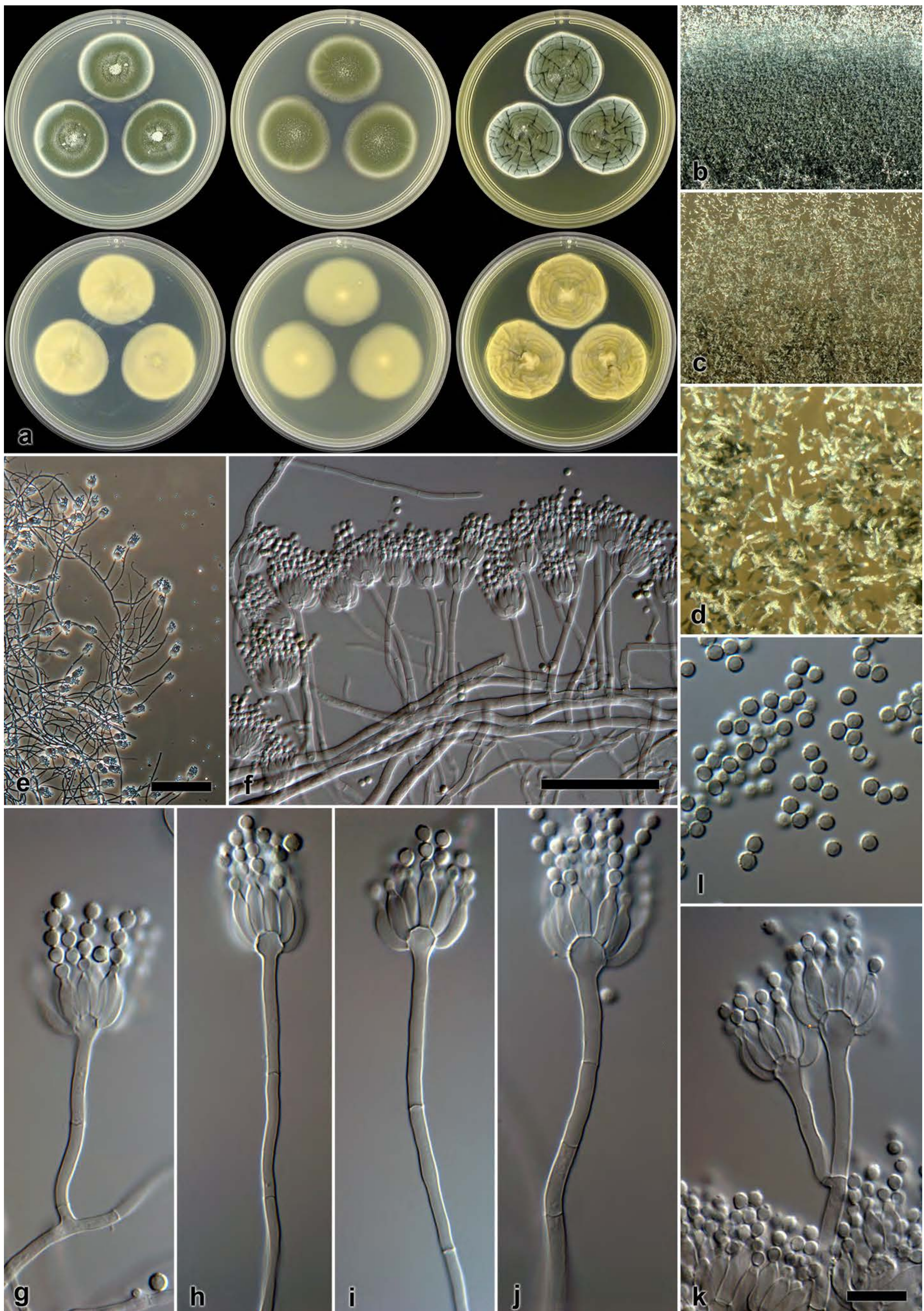

PLATE 50. *Penicillium malmesburiensis* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 10 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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31. *Penicillium purpuroides* Visagie **prov. nom.** PLATES 51, 55j

ETYMOLOGY: Latin, *purpuroides*: named after its close relative *P. purpurescens*
EX-TYPE: CV26 = DTO180G4 = KAS4104 = DAOM241136
TYPE ISOLATED FROM: Air sample, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 43–46, low, very lightly sulcate; margins low, wide (3–4 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (26F8–27F8); exudate clear, soluble pigment absent, reverse pigmentation greyish yellow to greyish orange (4B6–5B6) at centre, fading into light yellow (4A5) to greyish green (30B3) near margin.

CYA, 5 °C, 7d: Germination to microcolonies.

CYA, 30 °C, 7d: Colony characters similar to CYA at 25 °C, diameters 33–35 mm.

CYA, 37 °C, 7d: Colonies 10–15 mm, raised at centre, randomly sulcate; margin low, narrow (<1 mm), entire; mycelia white, texture velutinous; sporulation sparse, conidia *en masse* greyish green (26C3); exudate absent, soluble pigment absent, reverse pigmentation light to pale yellow (3A3–3A4).

MEA, 25 °C, 7d: Colonies 45–50, low, plain; margins low, wide (4 mm), entire; mycelia white; texture velutinous with some floccose mycelia near centre; sporulation dense, conidia *en masse* dark green (27F8); exudate absent, soluble pigment absent, reverse pigmentation (1A2) at centre, greyish green (30B3–30C3) elsewhere.

YES, 25 °C, 7d: Colonies 45–48 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense near margin, conidia *en masse* dark green (26F8–27F8); exudate clear, soluble pigment absent, reverse pigmentation orange (6A8) in areas of colonies facing each other, light yellow (4A5) to pale yellow (3A4) elsewhere.

G25N, 25 °C, 7d: Colonies 18–22 mm, low, lightly radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation moderately dense near margin, conidia *en masse* greyish green (26E5); exudate absent, soluble pigment absent, reverse pigmentation orange (6A8) in areas of colonies facing each other, light yellow (4A5) to pale yellow (3A4) elsewhere.

CREA, 25 °C, 7d: Colonies 15–18 mm, no acid produced.

Micromorphology — Conidiophores monoverticillate, minor proportion biverticillate; stipes smooth to finely rough walled, 80–215 × 2.5–3 µm, vesicle 4–7.5 [5.3±0.7] µm, average vesicle/stipe width 1.9; branches when present 2, 15–30 × 2.5–3 µm, phialides ampulliform, 8–16 per stipe, 9–10 × 3–4 [9.5±0.3 × 3.5±0.2] µm; conidia very heavy spiny walls, spheroid, 3–3.5 × 3–3.5 [3.26±0.2 × 3.26±0.2] µm, average width/length = 0.98±0.02, n = 33.

Notes — *Penicillium purpuroides* is characterized by fast growth on most media. A unique feature is its ability to grow at 37 °C, which is not typical for the section. It produces conidiophores with smooth vesiculate stipes and spheroid conidia that have heavy and spiny walls. The species is closely related to *P. purpurescens* and *P. glabrum*. *Penicillium glabrum*, however, produce smooth to finely rough walled conidia of similar dimensions. On the other hand, *P. purpurescens* produces similar heavy rough walled spiny conidia, but its conidia are larger (3.5–4.5 µm) compared to *P. purpuroides* (3–3.5 µm). All the genes studied resolved *P. purpuroides* as a distinct species (FIGURES 9, 10).

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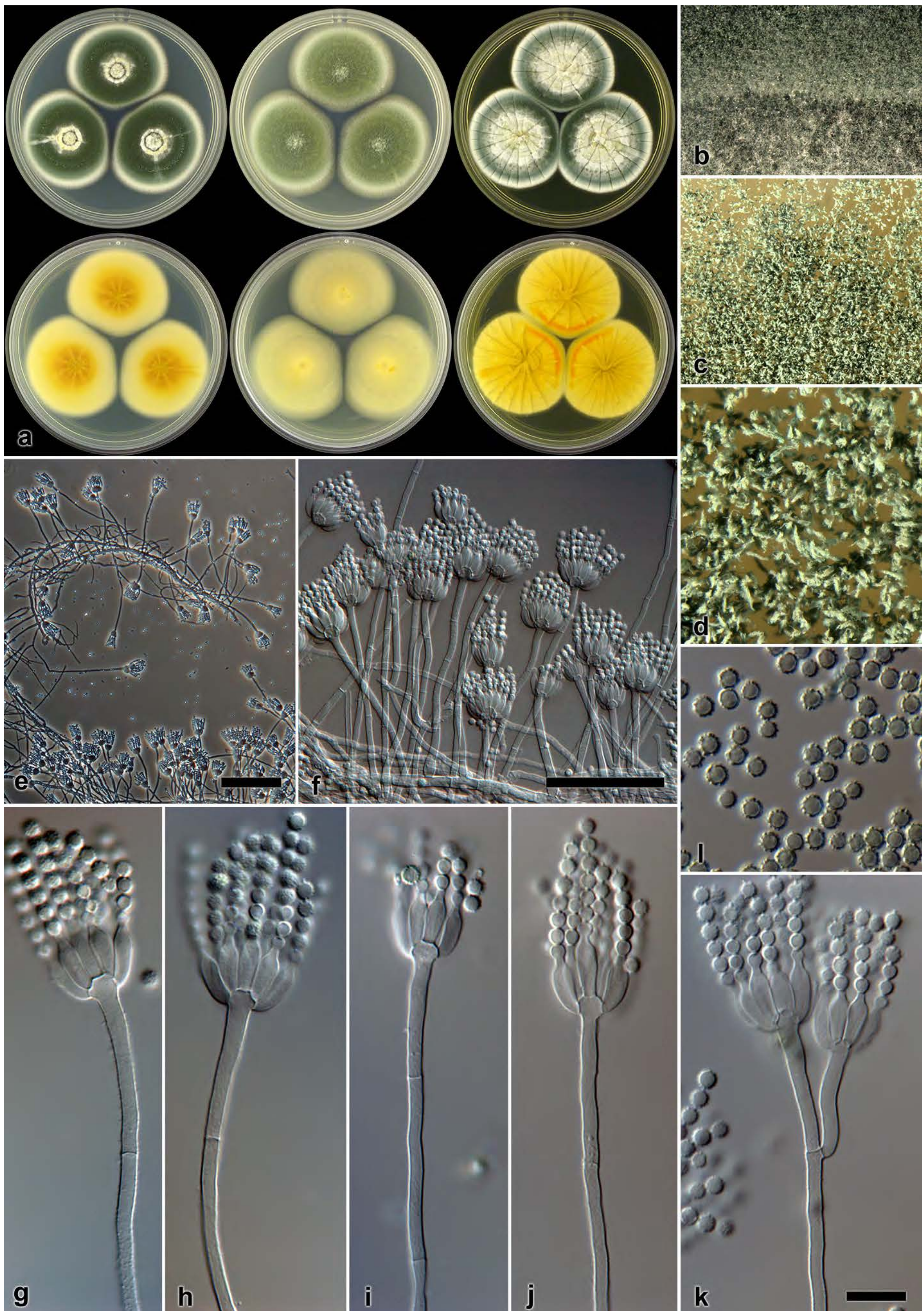

PLATE 51. *Penicillium purpuroides.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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32. *Penicillium thomii* Maire (morphogroup 1) PLATES 52, 55k

Bulletin de la Société d´Histoire naturelle de l'Afrique du Nord 8: 134. 1917.

EX-TYPE: CBS225.81 = IMI189694 = NRRL2077
TYPE ISOLATED FROM: Pine cone, USA
SPECIMENS EXAMINED: CV1189.
ISOLATED FROM: Mite of *Protea repens* infructescence, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 45–51 mm, low, radially and concentrically sulcate, brown sclerotia abundant; margins low, wide (3–4 mm), entire; mycelia white; texture velutinous with some floccose; sporulation moderately dense, conidia *en masse* greyish green (27D5–27E5); exudate clear, soluble pigment absent, reverse pigmentation yellowish white (2A2), with light yellow (2A4) regions.

CYA, 5 °C, 7d: Germination to microcolonies.

CYA, 30 °C, 7d: Colonies 10–15 mm, consisting of white mycelia.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 39–42 mm, low, plane, sclerotia present centrally; margins low to subsurface, wide (5 mm), entire; mycelia white; texture velutinous with floccose mycelia present; sporulation moderately dense, conidia *en masse* greyish green (27E5–27E6), (28E5) in less dense areas; exudate absent, soluble pigment absent, reverse pigmentation yellowish white (1A2) at point of inoculation and margins, greyish yellow (1B3) elsewhere.

YES, 25 °C, 7d: Colonies 47–53 mm, low to moderately deep, randomly sulcate, sclerotia present centrally; margins low, wide (3 mm), entire; mycelia white; texture mostly velutinous, with some floccose present; sporulation moderately dense, conidia *en masse* greyish green (26E4), greyish green (25C5) near margin; exudate clear, soluble pigment absent, reverse pigmentation yellow (3A6) areas near centre, yellowish white to pale yellow (2A3–2A4) elsewhere.

G25N, 25 °C, 7d: Colonies 15–23 mm, low, radially and concentrically sulcate; margins low, wide (3 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (26E4–26E6); exudate absent, soluble pigment absent, reverse pigmentation yellowish white (2A2) at centre and margin, greenish white (27A2) elsewhere.

CREA, 25 °C, 7d: Colonies 16–18 mm, acid not produced.

Micromorphology — Conidiophores monoverticillate; stipes rough walled, 65–250 × 2.5–3.5, vesicle 5–8 [6.6±0.9] µm, average vesicle/stipe width 2.1; phialides 14–18 per stipe, ampulliform, 8.5–11 × 3–4.5 [9.5±0.5 × 3.3±0.3] µm; conidia rough walled, ellipsoidal, 2.5–3.5 × 2–3 [3±0.2 × 2.5±0.1], average width/length = 0.8±0.03, n = 71; sclerotia produced on CYA and MEA, 130–320 × 125–240 µm.

Notes — Strain CV1189 are identified here as morphogroup1 of the *P. thomii* species complex. Morphologically, it does not differ from the description of *P. thomii* (Pitt 1979), as well as a couple of reference strains isolated from Madagascar that matches the ex-type sequences of the species. It is characterized by the production of abundant sclerotia on all media. However, phylogenetically this strain represents a possible new species (FIGURES 9, 10). Conidiophores have rough walled stipes and produce rough walled ellipsoid conidia. This species differ from strains identified here as *P. thomii* (morphogroup 2), by the lack of acid production and yellow reverse colors that lack.

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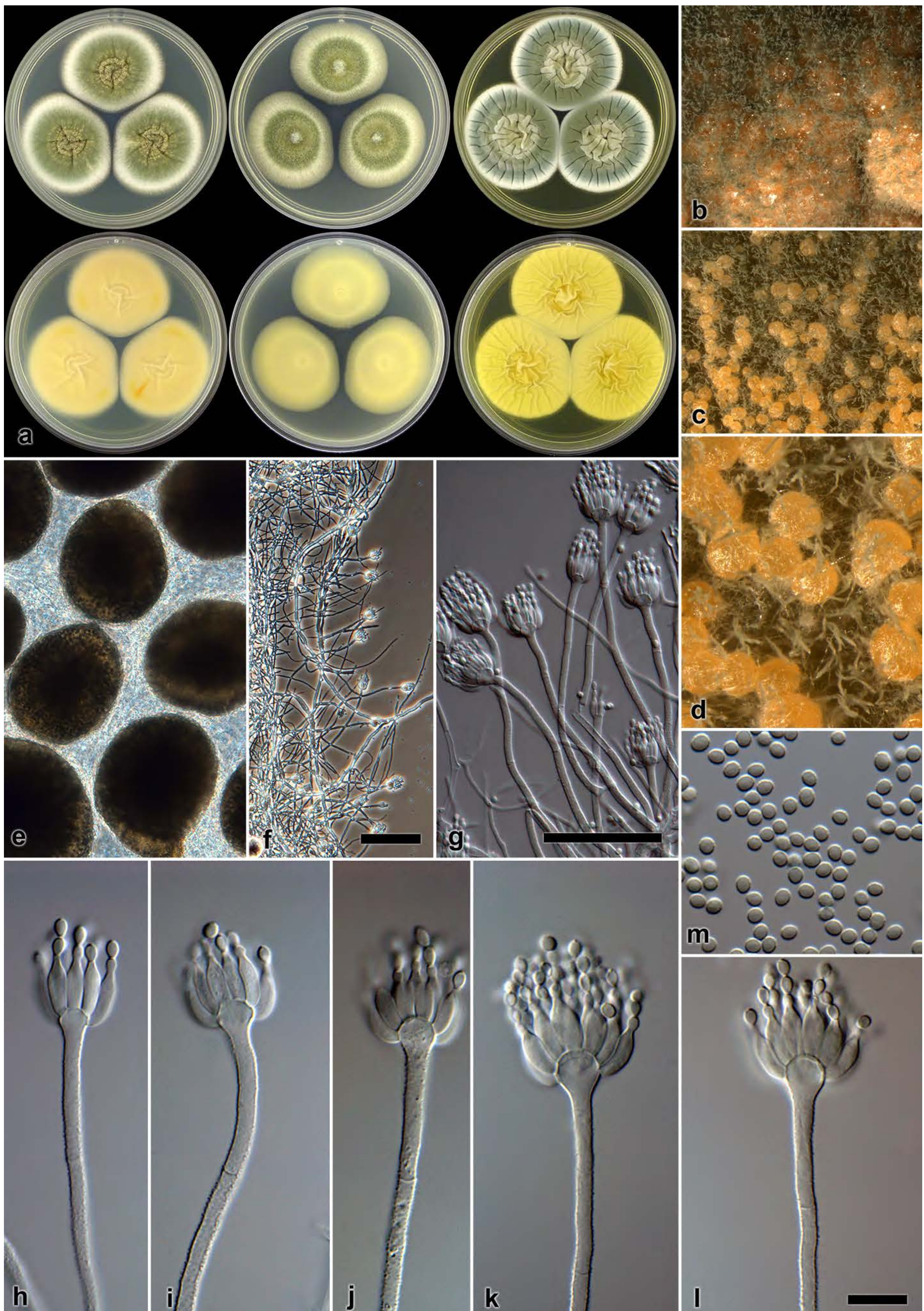

PLATE 52. *Penicillium thomii* morphogroup1 a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia on MEA. f–l. Conidiophores. m. Conidia (— Scale bar in f = 100 µm, applies to e, f; — Scale bar in g = 50 µm; — Scale bar in l = 10 µm, applies to h–m).

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33. *Penicillium thomii* Maire (morphogroup 2) PLATES 53, 55l

Bulletin de la Société d´Histoire naturelle de l'Afrique du Nord 8: 134. 1917.

EX-TYPE: CBS225.81 = IMI189694 = NRRL2077
TYPE ISOLATED FROM: Pine cone, USA
SPECIMENS EXAMINED: CV851, CV905, CV1145, CV1148, CV2850, CV2851.
ISOLATED FROM: Air, Soil, Mites and Bracts of *Protea repens* infructescences, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 40–55 mm, low, concentrically sulcate; margins low, wide (2–3 mm), entire; mycelia white; texture velutinous with some floccose regions; sporulation dense, conidia *en masse* greyish green (28D4–28E4), greyish green (25D5) at margin; exudate absent, soluble pigment absent, reverse pigmentation greenish yellow (1A6) at centre of some isolates, mostly white at centre and margin, elsewhere pale grey (1B1).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 20–35 mm, low, radially and lightly concentrically sulcate; margins low, narrow (2–3 mm), entire; mycelia white; texture velutinous with some floccose areas; sporulation moderately dense, in some isolates absent, conidia *en masse* dull green (28D4–29D4) at centre, greyish to greyish to dull green (25C4–25D4) near margin; exudate absent, soluble pigment absent, reverse pigmentation pale yellow (4A3) at centre, yellowish grey (4B2) elsewhere.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 40–50 mm, low, plane; margins low, wide (2–3 mm), entire; mycelia white; texture velutinous, some floccose centrally; sporulation moderately dense, conidia *en masse* dull to greyish green (29E3–29E6); exudate absent, soluble pigment absent, reverse pigmentation light yellow (2A5) at centre, fading into greenish grey to greyish yellow (1B2–1B4).

YES, 25 °C, 7d: Colonies 38–55 mm, low to moderately deep, randomly sulcate; margins low, wide (2–3 mm), entire; mycelia white; texture velutinous with floccose mycelia near centre; sporulation moderately dense, conidia *en masse* dull to greyish green (26E4–26E5), dull green (25D4) near margin; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3B6–4B6) at centre, dull yellow (3B4) and greyish yellow (1B3) elsewhere.

G25N, 25 °C, 7d: Colonies 10–20 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull to greyish green (28E3–28E6), greyish green (27D5) near margin; exudate absent, soluble pigment absent, reverse pigmentation greyish green (29C3), some isolates orange (5A6) at centre, greenish grey to greyish green (29B2–29B3) elsewhere.

CREA, 25 °C, 7d: Colonies 25–35 mm, strong acid production.

Micromorphology — Conidiophores monoverticillate; stipes finely rough walled, 45–220 × 2–3.5 µm, vesicle 4–7.5 [5.7±0.8] µm, average vesicle/stipe width 2.2; phialides 13–24 per stipe, ampulliform, 8–10.5 × 2.5–3.5 [9.1±0.6 × 2.9±0.2] µm; conidia smooth to finely rough walled, broadly ellipsoidal, 2.5–4 × 2.5–3 [3.2±0.2 × 2.7±0.14] µm, average width/length = 0.87±0.05, n = 66; sclerotia produced on MEA, 200–400 × 180–250 µm.

Notes — Strain CV461 is phylogenetically similar to *P. crocicola,* a species considered synonymous with *P. thomii* (Pitt 1974). Strain CV461, however, does not have morphological differences from strains resolved in the clade together with CV1145 (FIGURES 9, 10). These strains differ from morphogroup 1 by the acid produced on CREA, abundant sporulation on CYA and MEA with conidia often breaking off in crusts. The bright yellow reverse colors are also not present in morphogroup 1.

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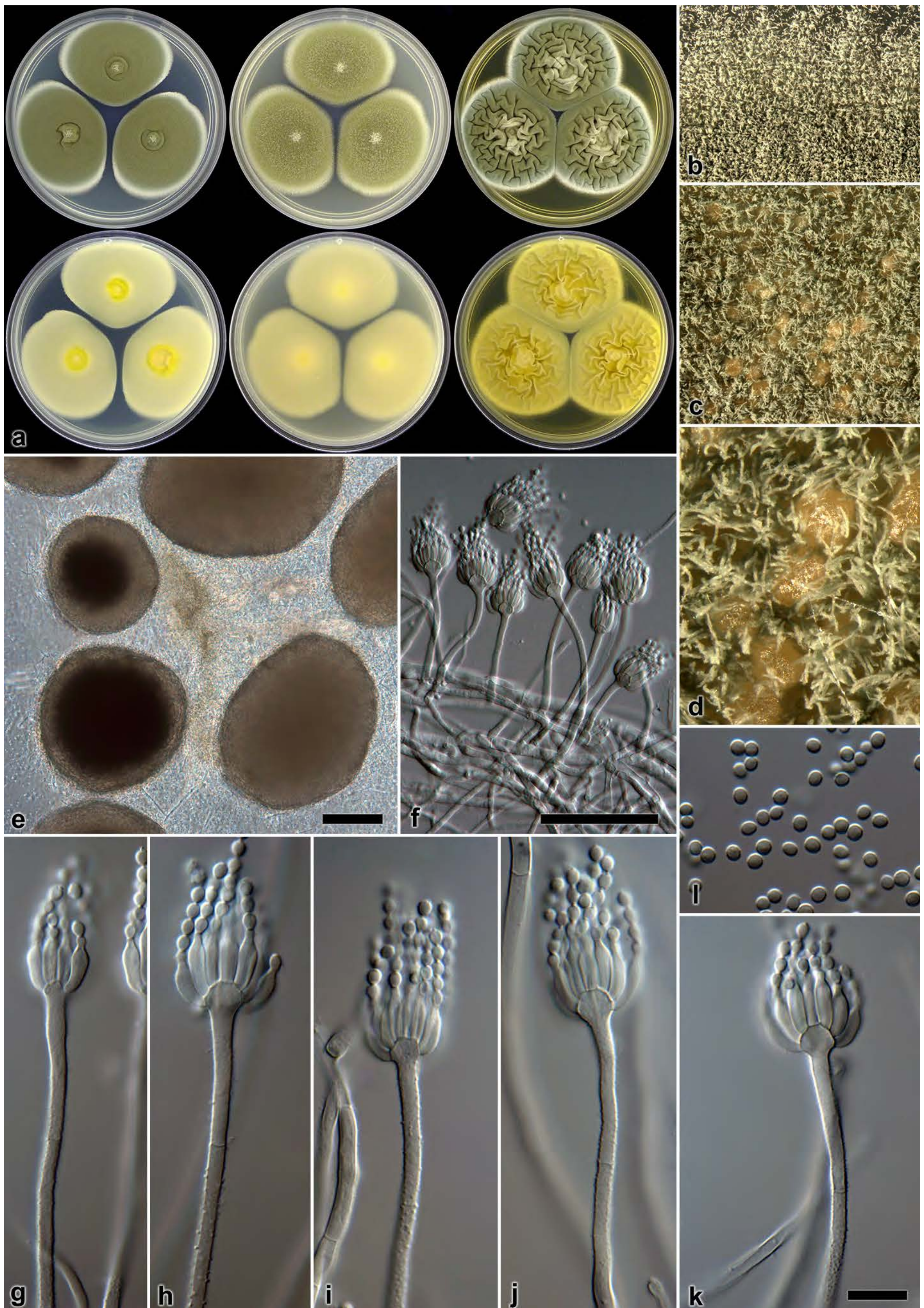

PLATE 53. *Penicillium thomii* morphogroup2 a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia on MEA. f–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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34. *Penicillium vagum* Visagie **prov. nom.** PLATES 54, 55m

ETYMOLOGY: Latin, *vagum* = meaning wanderer; named after this species phylogenetic relationships that changes between different genes analyzed
EX-TYPE: CV25 = DTO180G3 = KAS4100 = DAOM241357
TYPE ISOLATED FROM: Air sample, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 22–24 mm, low, radially and concentrically sulcate; margins low, narrow (<1 mm); mycelia white; texture velutinous; sporulation dense, conidia *en masse* dark green (27F4–27F7); exudate abundant clear, soluble pigment absent, reverse pigmentation yellowish white to greenish grey (1A2–1B2).

CYA, 5 °C, 7d: Germination to microcolonies.

CYA, 30 °C, 7d: Colonies 22–24 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation dense to sometimes moderately dense, conidia *en masse* dark green (26F8), when less dense greyish green (26E6); exudate absent, soluble pigment absent, reverse pigmentation dull to greyish yellow (3B4–3C4), yellowish white (2A2).

CYA, 37 °C, 7d: No germination to sometimes colonies up to 15 mm, raised at centre, randomly sulcate; mycelia white; sporulation sparse, conidia *en masse* greyish green (26C3); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow to olive (3B5–3D5).

MEA, 25 °C, 7d: Colonies 15–17 mm, moderately deep, plane; margins low, very narrow (<1 mm), irregular; mycelia white; texture velutinous, floccose mycelia centrally; sporulation dense, conidia *en masse* dark green (26F8–26F8); exudate absent, soluble pigment absent, reverse pigmentation greenish white (30A2) at point of inoculation, greenish grey to greyish green (30B2–30B3) elsewhere.

YES, 25 °C, 7d: Colonies 32–36 mm, low to moderately deep, radially and concentrically sulcate, craterform; margins low, narrow (1–2 mm), irregular; mycelia white; texture floccose and velutinous; sporulation moderately dense, sometimes sparse, conidia *en masse* dark green (25F8), greyish green (24C4) in less dense areas; exudate absent, soluble pigment absent, reverse pigmentation yellow (3A6) areas near centre, yellowish white to pale yellow (3A2–3A3) elsewhere.

G25N, 25 °C, 7d: Colonies 18–22 mm, low, lightly radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous with some floccose mycelia near centre; sporulation moderately dense, conidia *en masse* greyish green (27E5) at centre, dark green (27F7) elsewhere; exudate absent, soluble pigment absent, reverse pigmentation light yellow (2A4) at centre and margin, greyish green (29B4) elsewhere.

CREA, 25 °C, 7d: Colonies 18–21 mm, moderately deep, raised towards centre, plane; margins low, narrow (1–2 mm), irregular; mycelia white to deep yellow near centre; texture floccose; sporulation moderately dense near margin, conidia *en masse* greyish turquoise (24C5), strong acid production.

Micromorphology — Conidiophores monoverticillate; stipes smooth walled, 36–310 × 2.5–3.5 µm, vesicle 4.5–7 [5.8±0.5] µm, average vesicle/stipe width 2.1; phialides ampulliform, 15–20 per stipe, 8.5–11 × 3–4 [9.6±0.5 × 3.5±0.3] µm; conidia heavy spiny walled, spheroid, 2.5–3.5 × 2.5–3.5 [3±0.1 × 3±0.1] µm, average width/length = 0.98±0.02, n = 62.

Notes — *Penicillium vagum* show restricted growth on most media, especially on MEA. Sporulation is dense and the abundant exudate on CYA was consistently produced. Similar to *P. purpuroides*, it produces spheroid conidia with heavy and spiny walls. Strong acid production on CREA is observed for *P. vagum*, a character not often observed in the Fynbos strains from this section. Morphologically this species does not resemble any other species from this clade. This is mainly due to it sharing a mixture of characters typical of both the *P. glabrum* (FIGURE 10) and *P. fuscum* (FIGURE 11) clade. For instance, *P. vagum* produce dense sporulation with a dark green color on all media and its conidiophores have smooth walled vesiculated stipes, similar to species in the *P. glabrum* clade. However, its slow growth on most media is similar to species from the *P. fuscum* clade. Its phylogenetic position also shifts between these two clades depending on the gene studied. Phylogenetically the species is consistently resolved separate from all previously described species (FIGURES 9, 10).

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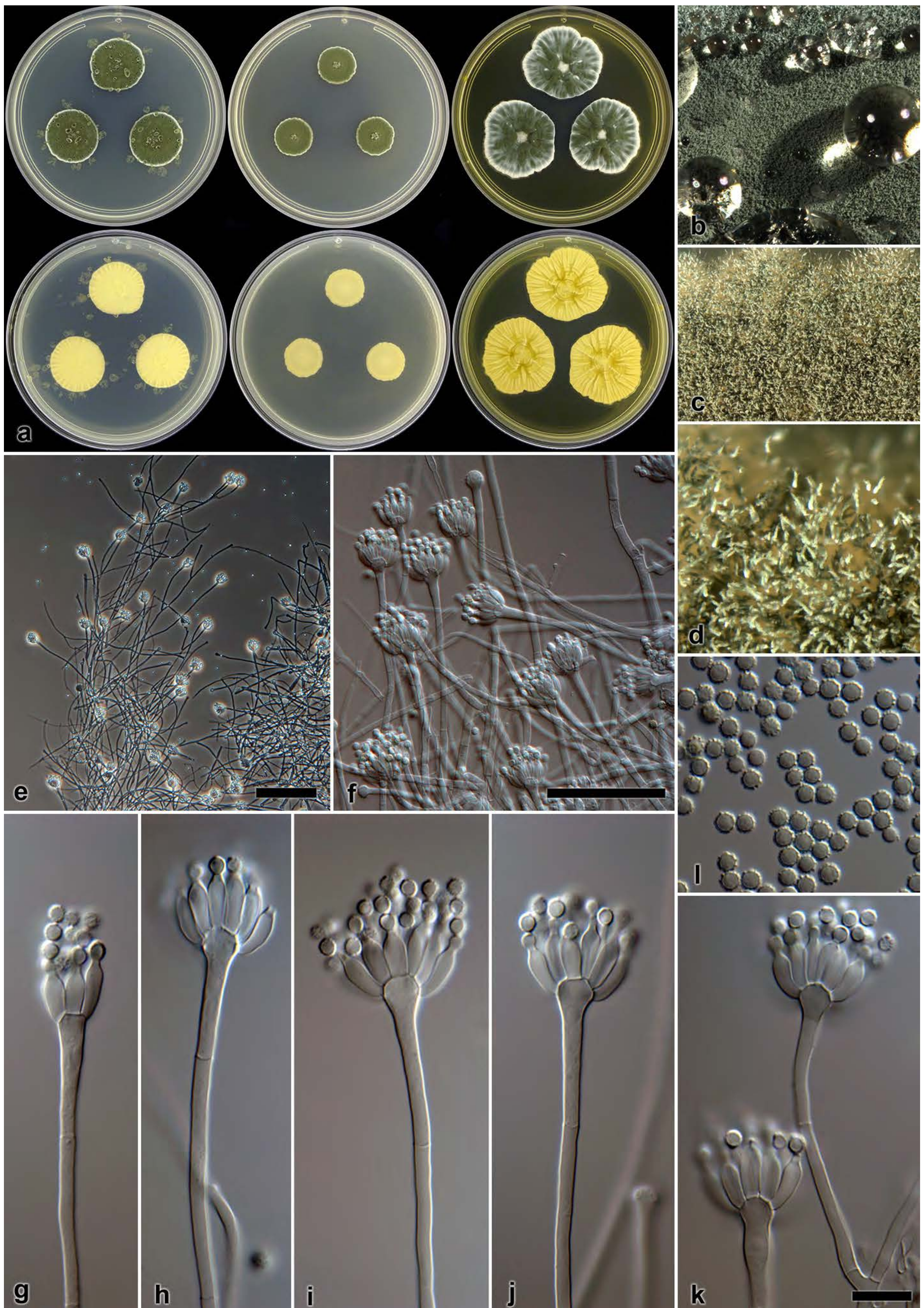

PLATE 54. *Penicillium vagum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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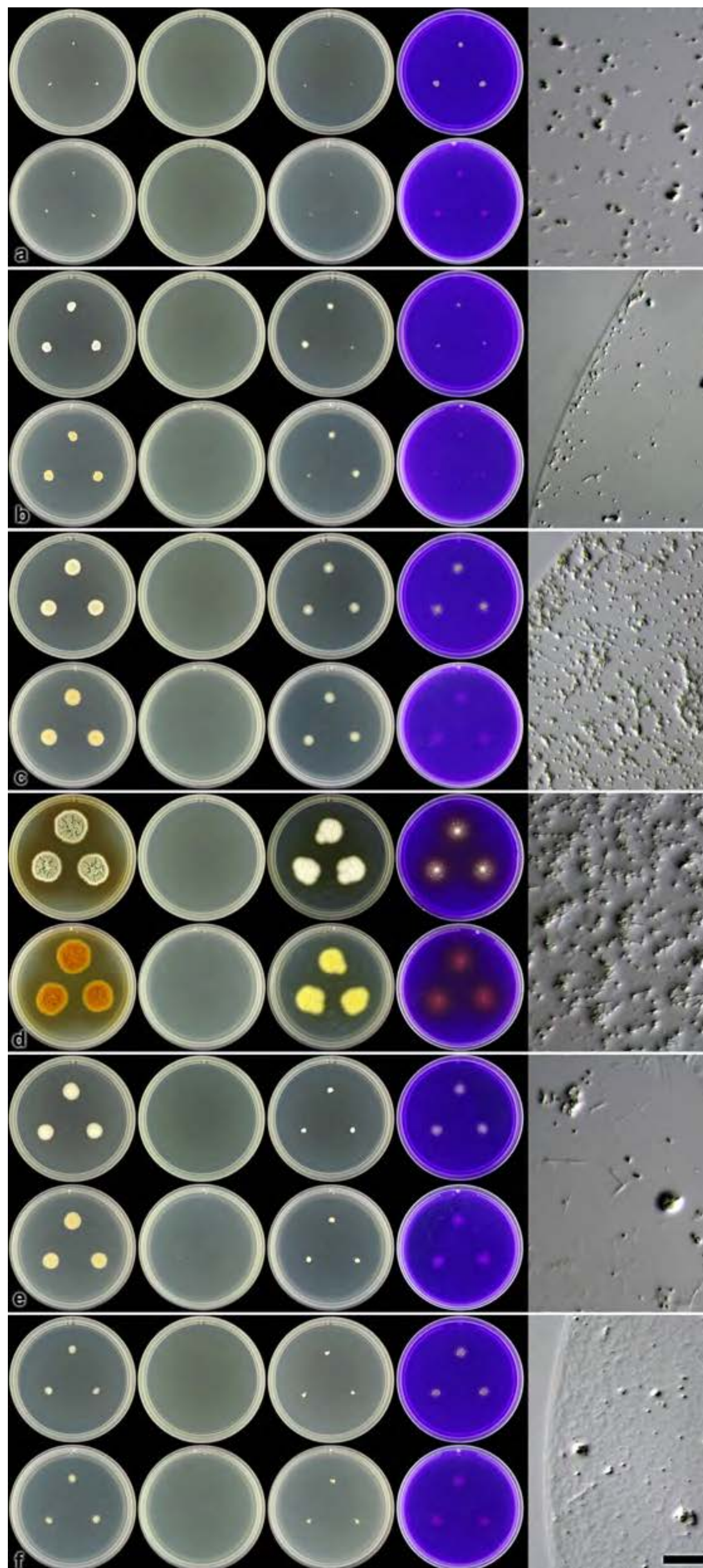

PLATE 55. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium brunneoconidium.* b. *P. caseidecus*. c. *P. clavistipa*. d. *P. cumulacinatum*. e. *P. flavosclerotia*. f. *P. fuscum*.

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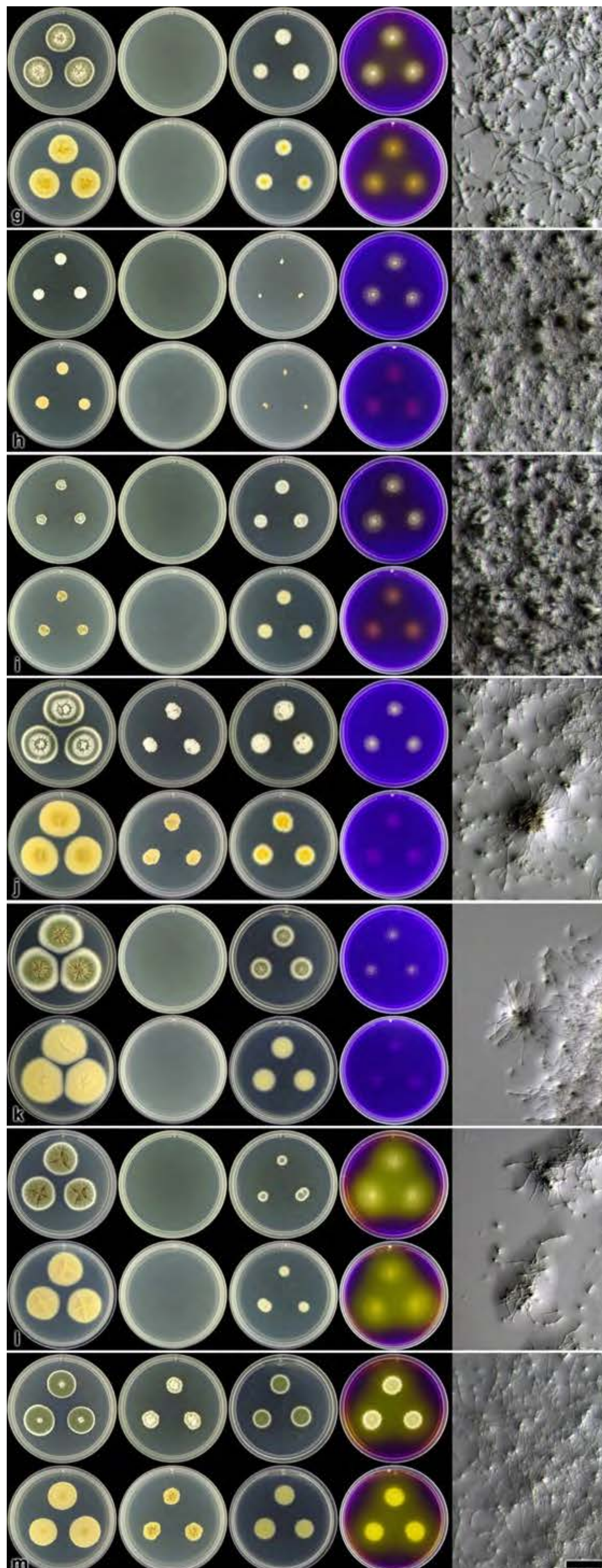

PLATE 55. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). g. *Penicillium glabrum.* h. *P. infra-aurantiacum*. i. *P. malmesburiensis.* j. *P. purpuroides.* k. *P. thomii* morphogroup1 CV1189. l. *P. thomii* morphogroup2 CV1148. m. *P. vagum*.

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The subgenus *Penicillium* Pitt (sections *Fasciculata* Thom, *Penicillium, Chrysogena* Frisvad & Samson, *Brevicompacta* Thom)

The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*: 316. 1979

Section *Fasiculata* Thom, The Penicillia: 374. 1930

Section *Chrysogena* Frisvad & Samson, Studies in Mycology 49: 17. 2004

Section *Brevicompacta* Thom, The Penicillia: 289. 1930

SPECIES TREATED: *Penicillium aurantiogriseum, P. brevicompactum, P. crustosum, P. expansum, P. griseofulvum, P. melanoconidium, P. rubens*

Penicillium subgenus *Penicillium* species are the best-studied group in the genus and are of great economic importance (Thom 1910, Thom 1930, Raper & Thom 1949, Samson *et al*. 1976, Pitt 1979, Frisvad & Filtenborg 1990a, Seifert & Louis-Seize 2000, Frisvad & Samson 2004). The majority of species in the section are associated with animals, plants or indoor environments, where they can cause great damage and produce a variety of toxins (Frisvad & Samson 2004). Subgenus *Penicillium* represents a group of species, characterized by typical terverticillate conidiophores. They have the ability to grow at low temperatures, at low water activity and pH, and typically grow poorly at high temperatures (Pitt 1979, Frisvad & Samson 2004). Samson *et al*. (2004) identified six sections in subgenus *Penicillium* based on a polyphasic approach, which included morphology, β-tubulin phylogeny and secondary metabolite profiles. Even though their phylogenies did not fully support the clades of Samson *et al*. (2004), Houbraken & Samson more or less accepted the sectional classification of subgenus *Penicillium*. Sections confirmed include *Digitata*, *Penicillium*, *Roquefortorum* and *Chrysogena*. Section *Coronata* of Samson *et al*. (2004) was renamed as section *Brevicompacta* and section *Ramosa* introduced for a clade of species basal to the latter section. Houbraken & Samson (2011) placed section *Viridicata* and *Lanata-typica* as synonyms into the section *Fasciculata*. The ITS phylogeny from this study confirmed that subgenus *Penicillium* forms a monophyletic clade as found in other studies that used alternative genes (Peterson 2000, Samson *et al*. 2004, Houbraken & Samson 2011). However, the sectional classification of Houbraken & Samson (2011) could not be confirmed with the ITS phylogeny. This is most probably due to the lack of enough variation in this gene region, which result in poor species separations. Because of this and subgenus *Penicillium* that is monophyletic, the current study will not differentiate between sections. Thus the Fynbos species are treated under the subgenus *Penicillium* collective. It is important to note that many more subgenus *Penicillium* species occur in the western Cape and may be isolated in future. Studies of fungal communities from indoor environments in the Stellenbosch region resulted in the isolation of *ca*. 30 subgenus *Penicillium* species (pers. obs). These species will be treated in a future study and included into identification keys.

The present study isolated seven subgenus *Penicillium* species. Except for *P. brevicompactum* isolated from most samples, strains represented a small percentage of the total strains isolated. Strains were mostly isolated from *Protea repens* infructescences, with only two species, *Penicillium aurantiogriseum* and *P. expansum* found in soil from Stellenbosch and Malmesbury. Although these two species are better known from the damage they cause in the cereal (*P. aurantiogriseum*) and apple (*P. expansum*) industries, they have been reported as common in soils from around the world (Frisvad & Samson 2004). *Penicillium brevicompactum* was one of the most common species isolated from *Protea repens* infructescences and were found at all three sampling sites. The presence of this species was not surprising since it has been reported from a wide range of habitats that range from soil, conifers, mushrooms, maple syrup, coffee beans and even cosmetic products (Frisvad & Samson 2004). The low number of isolates found for the species isolated implies that species from this group are not common in the oligotrophic Fynbos habitat and was possibly dispersed via air currents from other habitats. *Penicillium brevicompactum* is the obvious exception. This species was also commonly found from the indoor environments from Stellenbosch and it will be interesting to know why it is so common in the Western Cape region. This is unfortunately very difficult to determine, since it has been reported from such a wide range of habitats.

In total, this study characterized and identified seven subgenus *Penicillium* species. The strains conformed to morphological descriptions given by Frisvad and Samson (2004), with identifications confirmed by phylogenetic analyses. Species isolated include *P. aurantiogriseum, P. brevicompactum, P. crustosum, P. expansum, P. griseofulvum, P. melanoconidium* and *P. rubens. Penicillium aurantiogriseum* and *P. melanoconidium* did, however, vary slightly from the descriptions provided by Frisvad & Samson (2004). *Penicillium melanoconidium* consistently produced smooth to very finely rough walled stipes, compared to the rough walled stipes observed in *P. aurantiogriseum* isolates. However, this is contradictory to the Frisvad & Samson (2004) study, which reported the smooth to finely rough walled stipes in *P. aurantiogriseum* and not in *P. melanoconidium*. Unfortunately, these two species was only represented by three Fynbos strains. This makes it

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difficult to alter the concept of both these species. As such, we consider stipe ornamentation as a poor character to distinguish these species. However, colony morphology separate between these two similar species, with *P. aurantiogriseum* that produce a dark blue-green conidial color, compared to the dark green conidia of *P. melanoconidium*.

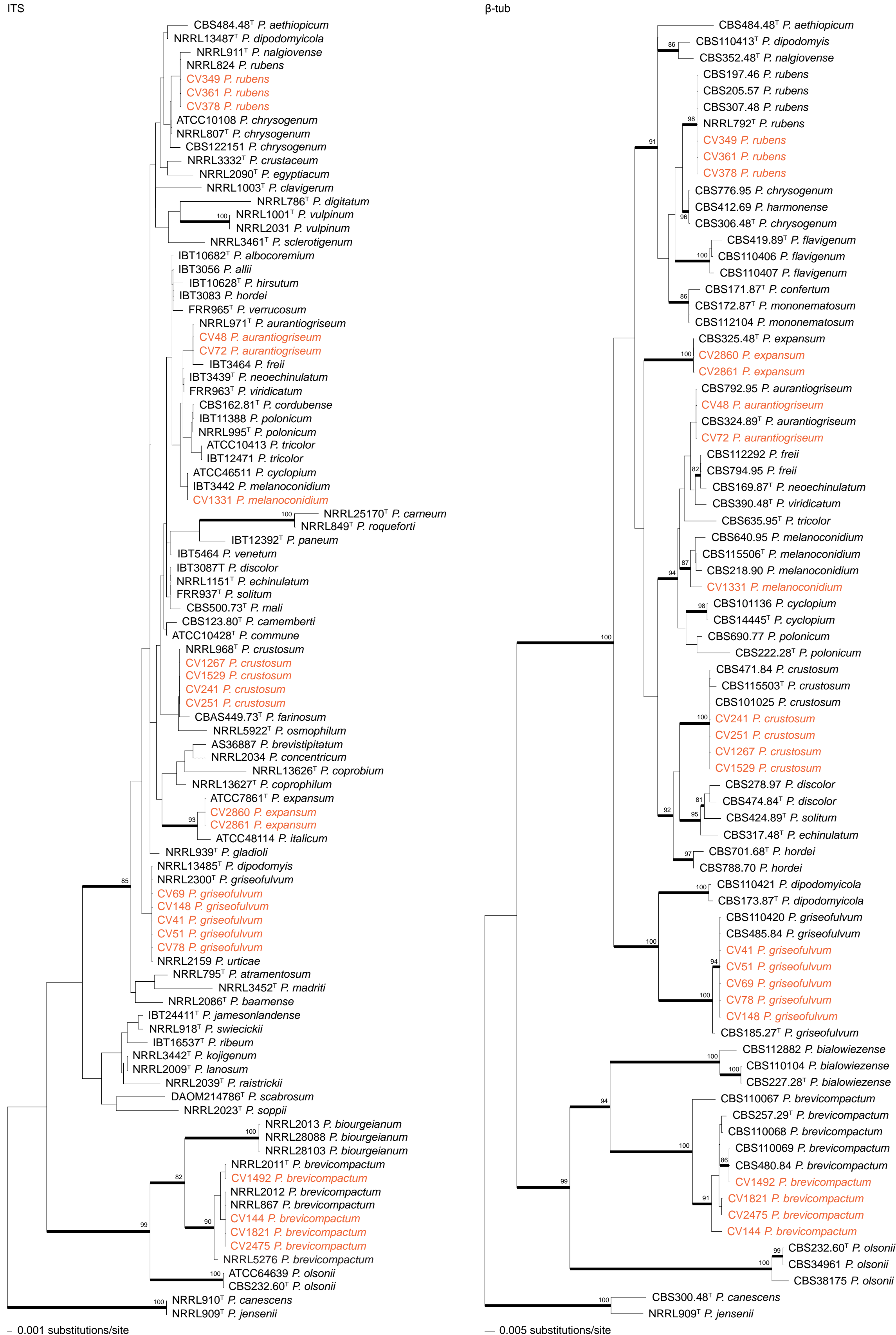

FIGURE 12: Phylogenetic trees based on ITS and β-tubulin, showing relationship of Fynbos *Penicillium* spp. to other species in subgenus *Penicillium*. *Penicillium canescens* and *P. jensenii* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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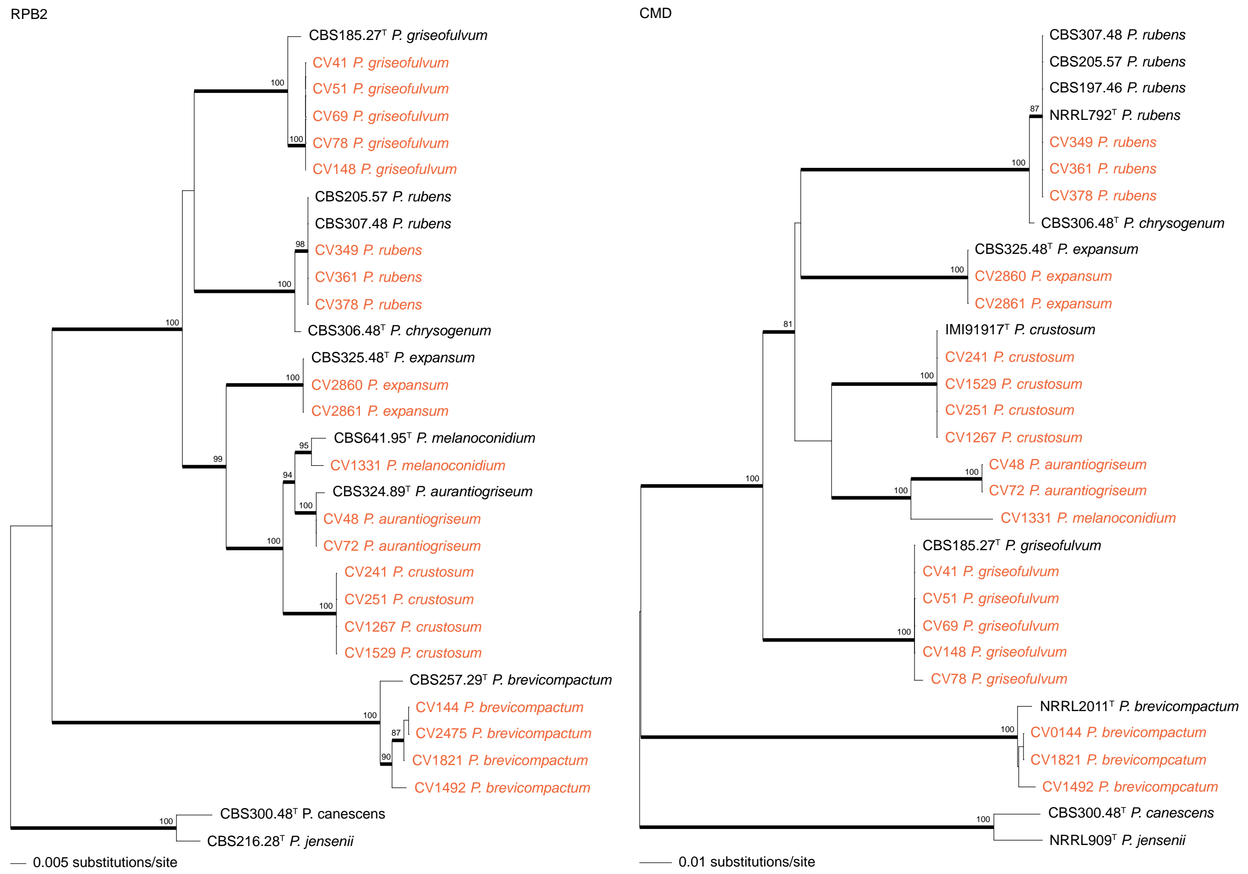

FIGURE 13: Phylogenetic trees based on RPB2 and Calmodulin, showing relationship of Fynbos *Penicillium* spp. to other species in subgenus *Penicillium*. *Penicillium canescens* and *P. jensenii* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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35. *Penicillium aurantiogriseum* Dierckx

PLATES 56, 57, 70a

Annales de la societe scientifique de bruxelles 25: 88. 1901.

EX-TYPE: CBS324.89 = NRRL971 = ATCC48920 = IBT14016 = IMI195050 = MUCL29090
TYPE ISOLATED FROM: Unrecorded source
SPECIMENS EXAMINED: CV48, CV72.
ISOLATED FROM: Soil, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 25–28 mm, low to moderately deep, radially sulcate; margins low, narrow (1 mm), entire, yellow edge surrounding margin; mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* greyish turquoise (24D5–24E5); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at point of inoculation, fading from orange (5A6) into brownish yellow (5C8), with orange (5A6) margin.

CYA, 5 °C, 7d: Microcolonies, 2–5 mm.

CYA, 30 °C, 7d: All features similar to colonies on CYA at 25 °C except for more restricted growth, 20–22 mm.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 28–31 mm, low, plane; margins subsurface to low, wide (3 mm), entire; mycelia white; texture velutinous, fasciculate and granular; conidiogenesis moderate to dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation light yellow (2A5) at centre, greyish green to yellowish green (30C7–30C8), with greyish green (30B4) margin.

YES, 25 °C, 7d: Colonies 23–26 mm, deep, radially sulcate; margins deep, narrow (<1 mm), irregular; mycelia white; texture velutinous to fasciculate; conidiogenesis dense, conidia *en masse* similar to CYA; exudate sometimes clear, soluble pigment absent, reverse pigmentation orange (5A8–5B8) at centre, fading into deep yellow (4A8), with orange yellow (4B7) margin.

G25N, 25 °C, 7d: 19–23 mm, low, plain; margin low, narrow (3 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse greyish yellow (2B5) at centre, greyish yellow (2C5) elsewhere, light yellow (2A5) at margin.

CREA, 25 °C, 7d: Colonies 20–23 mm, moderate to good acid production.

Micromorphology — Conidiophores mostly terverticillate, biverticillate also present; stipes heavy rough walled, 200–400 × 2.5–4 µm; rami 2, appressed, with some divergent, 13–24 × 2.5–4 [18.1±3] µm; metulae 3–5, appressed, 28–62° [41.7±11.2°], 9–15 × 2.5–4 [11.1±1.3 × 3.3±0.33] µm, vesicle 3.5–5 [3.9±0.43] µm; phialides ampulliform, 5–8 per metula, 7–9 × 2.5–3.5 [8±0.56 × 2.8±0.19) µm; conidia smooth, subspheroidal to broadly ellipsoidal, 3–4 × 2.5–3 [3.3±0.19 × 2.7±0.16] µm, average width/length = 0.83±0.04, n = 76.

Notes — *Penicillium aurantiogriseum* strains from this study are characterized by a typical bluish conidial color, rough walled stipes and strong acid production on CREA. Morphologically it is very similar to *P. melanoconidium*. The latter species, however, produces a very dark conidial color and on average produce smaller conidia. For the strains studied, *P. aurantiogriseum* also produced smaller colonies on YES (23–26 mm), compared to *P. melanoconidium* (36–39 mm).

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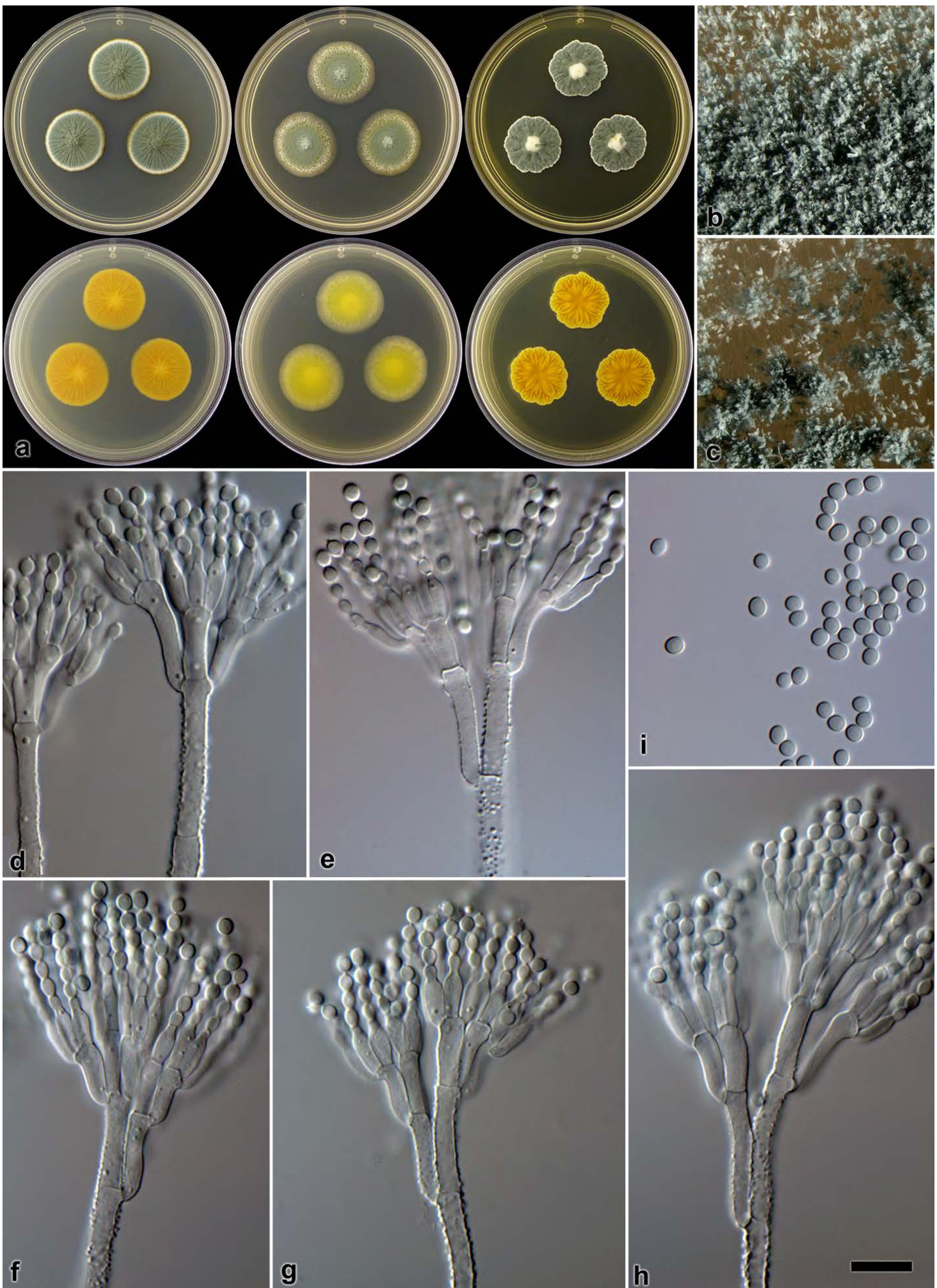

PLATE 56. *Penicillium aurantiogriseum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to d–i).

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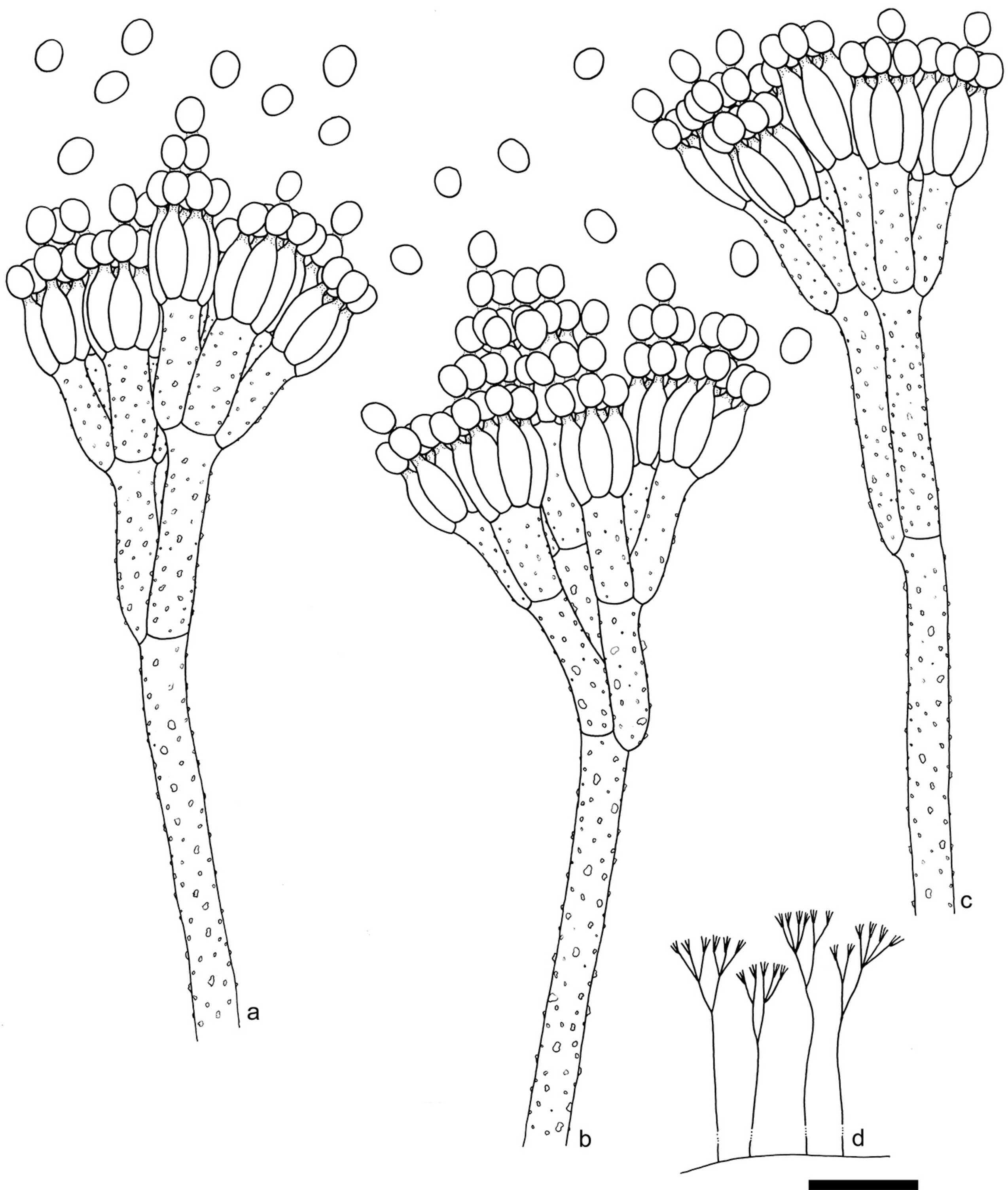

PLATE 57. Line drawing of *P. aurantiogriseum*. a–c. Conidiophores (— Scale bar = 10 μm). d. Conidiophore branching (— Scale bar = 50 μm).

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36. *Penicillium brevicompactum* Dierckx PLATES 58, 59, 70b

Annales de la societe scientifique de bruxelles 25: 88. 1901.

EX-TYPE: CBS257.59 = ATCC10418 = NRRL2011 = IBT23045
TYPE ISOLATED FROM: Unrecorded source
SPECIMENS EXAMINED: CV144, CV342, CV1492, CV1821, CV2020, CV2475.
ISOLATED FROM: Air sample, Stellenbosch; Mites and bracts from *Protea repens* infructescence, Malmesbury, Stellenbosch and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 23–30 mm, low, radially and lightly concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous; conidiogenesis dense, conidia *en masse* dull to greyish green (28E3–28E6; 29E3–29E6), but olive (1E4–1E5) at centre; exudate mostly absent with brownish orange sometimes produced, soluble pigment absent, reverse pigmentation yellow (3A6–3B6) at centre fading into a greyish yellow (2B4–2B6).

CYA 5 °C, 7d: Microcolonies, 1–2 mm.

CYA, 30 °C, 7d: Microcolonies to colonies of 13 mm and thus showing great variation in colony appearance. Colonies craterform,, radially sulcate, yellowish to grey color; margins very narrow (<1 mm), irregular; mycelia white; conidiogenesis absent; exudate absent, soluble pigment absent, reverse pigmentation olive (3D5), other strains greyish yellow (2B3).

CYA 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 14–16 mm, some up to 25 mm, moderately deep, plain; margins deep, very narrow (<1 mm), slightly wider in faster growing isolates, irregular; mycelia white; texture velutinous; conidiogenesis dense, conidia *en masse* deep to dark green (29E8–29F8), some isolates greyish green (30E6); exudate absent, soluble pigment absent, reverse pigmentation yellow (3B6) at centre fading into olive to olive yellow (2D5–2D6).

YES, 25 °C, 7d: Colonies 26–30 mm, low, rising towards centre, radially sulcate; margins low, very narrow (<1 mm), irregular; mycelia white; texture velutinous; conidiogenesis dense, conidia *en masse* similar to that on CYA; exudate absent, soluble pigment absent, reverse pigmentation similar to that on CYA.

G25N, 25 °C, 7d: Colonies 15–18 mm, low, lightly radially and concentrically sulcate; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B5) at point of inoculation, fading into greyish yellow (1B3–1B4).

CREA, 25 °C, 7d: Colonies 7–10 mm, weak acid production.

Micromorphology —

Conidiophores mostly terverticillate, bi- and quaterverticillate less common; stipes smooth, very long, 400–800 × 4–6 µm; rami 2–4, divergent and appressed, 11–30 × 4–5 [19.3±3.9] µm; metulae 3–7, divergent and appressed, 40–125° [77.6±21.8°], 9–15 × 3–6(–11.5) [11.7±1.4 × 4.6±1.7] µm, vesicle swollen in fresh isolates, 4–12 [5.6±1.3] µm; phialides ampulliform, 5–10 per metula, 7.5–10.5 × 2.5–4 [8.7±0.78 × 3±0.29] µm; conidia finely rough walled, broadly ellipsoidal with minor proportion subspheroidal, 2.5–3.5 × 2.5–3 [3±0.15 × 2.6±0.13] µm, average width/length = 0.86±0.05, n = 87.

Notes — *Penicillium brevicompactum* produces broad and compact conidiophores, which is its most striking feature. In fresh isolates, the metulae are commonly swollen at the apex, sometimes up to 12 µm. Interestingly it does not grow at 30 °C and have restricted growth on MEA. This species was very common in all of the samples collected. It was isolated from soil, air, *Protea repens* infructescences and the mites inside infructescences. Literature has reported this species from a wide host range.

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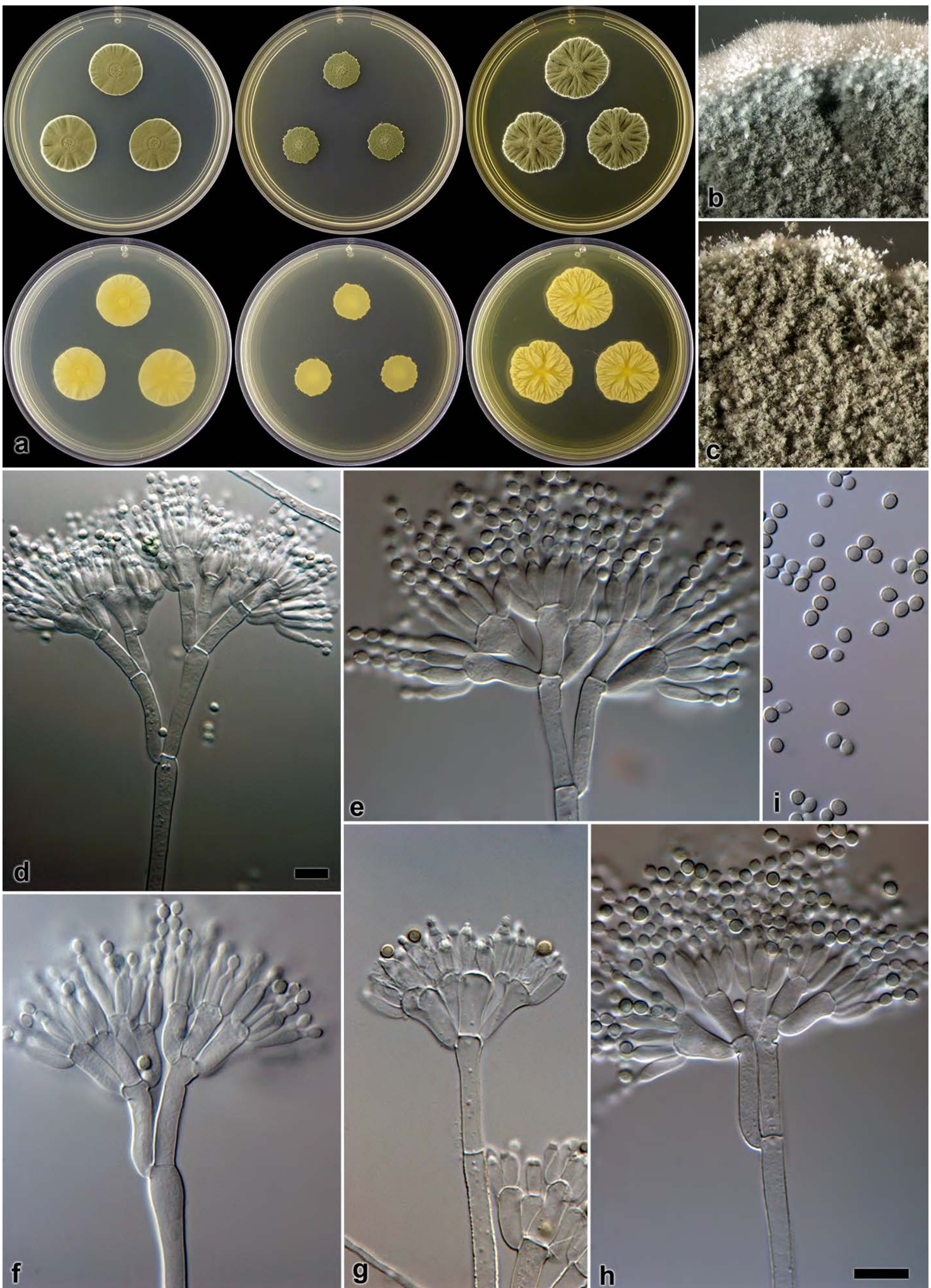

PLATE 58. *Penicillium brevicompactum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in d = 10 µm; — Scale bar in h = 10 µm, applies to e–i).

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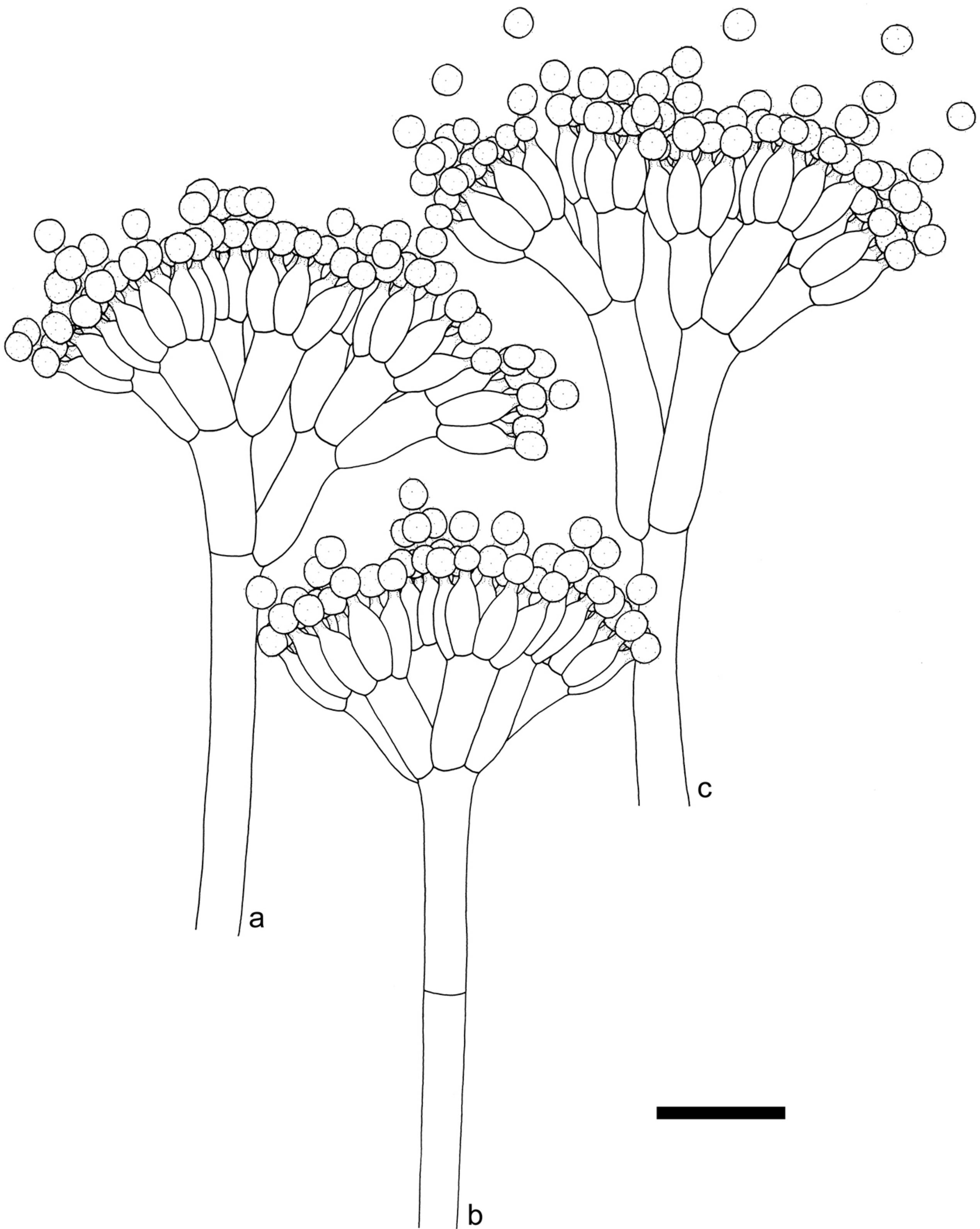

PLATE 59. Line drawing of *P. brevicompactum*. a–c. Conidiophores. (— Scale bar = 10 µm).

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37. *Penicillium crustosum* Thom

PLATES 60, 61, 70c

The Penicillia. 399. 1930.

EX-TYPE: NRRL968 = CBS115503 = IMI091917 = IBT6175
TYPE ISOLATED FROM: Lemon, Aberdeen, Scotland.
SPECIMENS EXAMINED: CV241, CV251, CV1266, CV1267, CV1529.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescences, Stellenbosch and Malmesbury; Apples and guavas, Western Cape

Macromorphology — CYA, 25 °C, 7d: Colonies 41–48 mm, low, radially and sometime lightly concentrically sulcate; margins low, narrow (1–2 mm), irregular; mycelia white; texture velutinous and granular; conidiogenesis dense to almost powdery, conidia *en masse* dull to greyish green (26D4–26D6–27D6–27D4); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at point of inoculation, fading into greyish yellow (4B5–4B6) and into greyish orange (5B5–5B6), greenish grey (26D2) at margin of colonies facing each other.

CYA 5 °C, 7d: Microcolonies, 3–5 mm.

CYA, 30 °C, 7d: Variation in colonies, ranging from 24–33 mm, low, raised towards centre, radially sulcate; margins low, narrow (1–2 mm), entire to irregular, yellow edge surrounding margin; mycelia white; texture velutinous; conidiogenesis moderate to dense, more restrict conidiogenesis observed in isolate CV1529, conidia *en masse* similar to CYA (25 °C); exudate absent, soluble pigment absent, reverse pigmentation dull yellow (3B3) at point of inoculation, greyish yellow (4B4–4B5), fading to olive brown (4D6) to olive (2D4) margin, isolate CV1529 lacking the yellowish nature of other isolates.

CYA 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 45–52 mm, low, plain; margins subsurface to low, wide (3 mm), irregular; mycelia white; texture velutinous to granular, becoming crustose; conidiogenesis dense, conidia *en masse* dull to greyish green (26D4–26D6); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A3) at centre, greyish yellow (1B3) fading into greyish green (29C3) to greenish grey (29B2) margin.

YES, 25 °C, 7d: Colonies 51–55 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire to somewhat irregular; mycelia white; texture velutinous; conidiogenesis dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse greyish yellow (3B7–3B8) to yellow (3A8) at margin.

G25N, 25 °C, 7d: Colonies 23–27 mm, low, lightly radially and concentrically sulcate; margins low, narrow (2–3 mm), entire, yellow edge surrounding margin; mycelia white; texture velutinous, somewhat fasciculate; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation light grey (1B1) at centre, greyish yellow to olive (2C5–2D5), vivid yellow (3A8) margin.

CREA, 25 °C, 7d: Colonies 30–38 mm, moderate acid production, followed by base production.

Micromorphology — Conidiophores mostly terverticillate, biverticillate present; stipes heavily rough walled, 200–400 × 2.5–4 µm; rami 2, appressed, 12.5–25 × 2.5–4 µm; metulae 3–5, appressed, 9–47° [27±8.4°], 10–17 × 2.5–4 [13.5±1.5 × 3.3±0.36] µm, vesicle 3–5 [3.9±0.43] µm; phialides ampulliform, 5–8 per metula, dimensions 7–10 × 2.5–3.5 [9.2±0.89 × 3.1±0.23] µm; conidia smooth walled, broadly ellipsoidal to subspheroidal, 3–4 × 2.5–3.5 [3.5±0.18 × 3±0.19] µm, average width/length = 0.87±0.04, n = 64.

Notes — *Penicillium crustosum* produces closely appressed conidiophores with roughened stipes and smooth walled conidia. It grows well on CREA on which it produces acid that is followed by base production. After 10 days of growth, colonies on CYA and MEA become crustose. It is morphologically closely related to *P. expansum*, which displays similar growth on for instance CREA. *Penicillium expansum* colonies, however, are never crustose and produce closely packed determinate synnemata. Furthermore, the conidiophores of these species may appear similar, except for *P. expansum* that have smooth walled stipes compared to the roughened stipes of *P. crustosum.*

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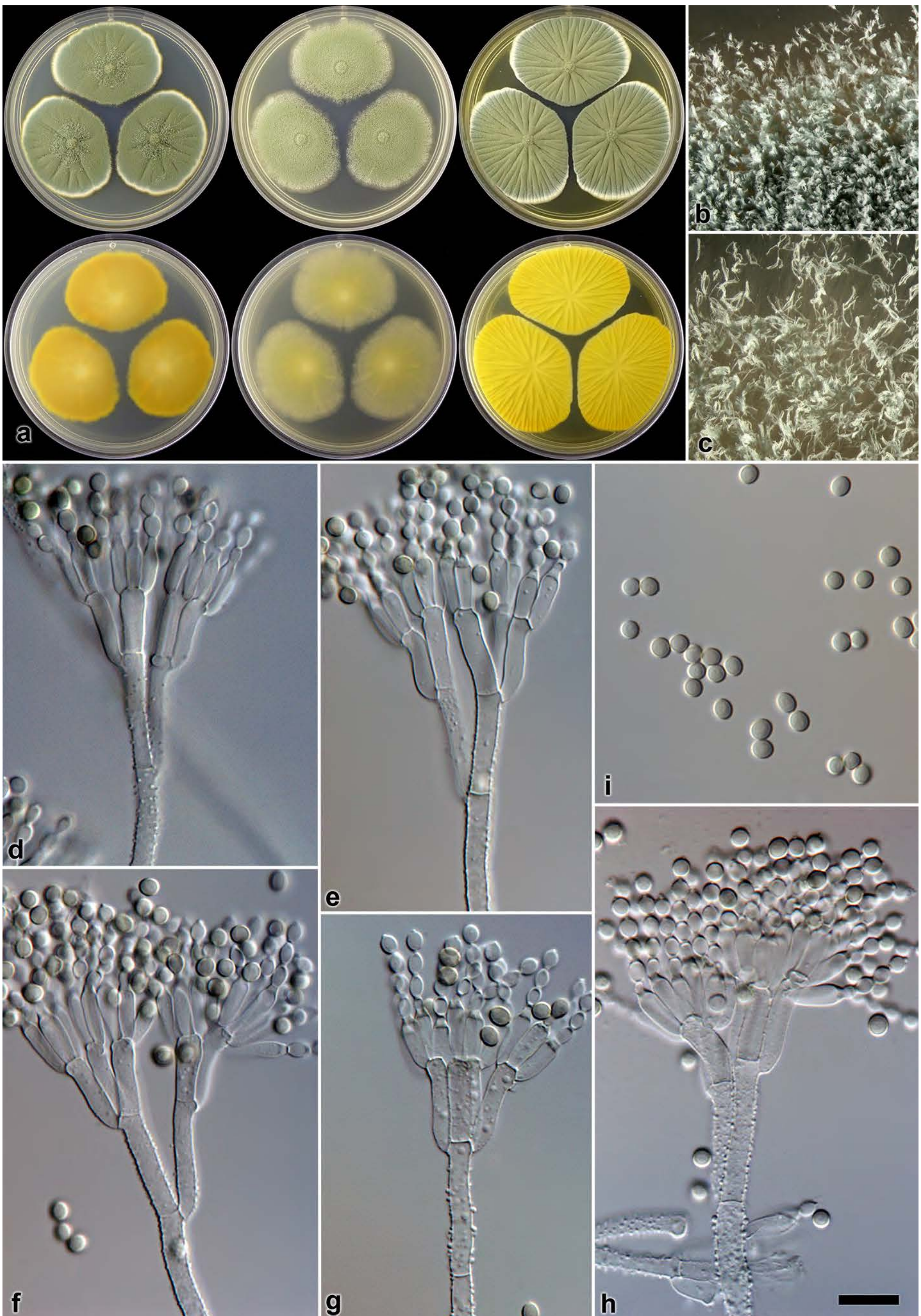

PLATE 60. *Penicillium crustosum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to d–i).

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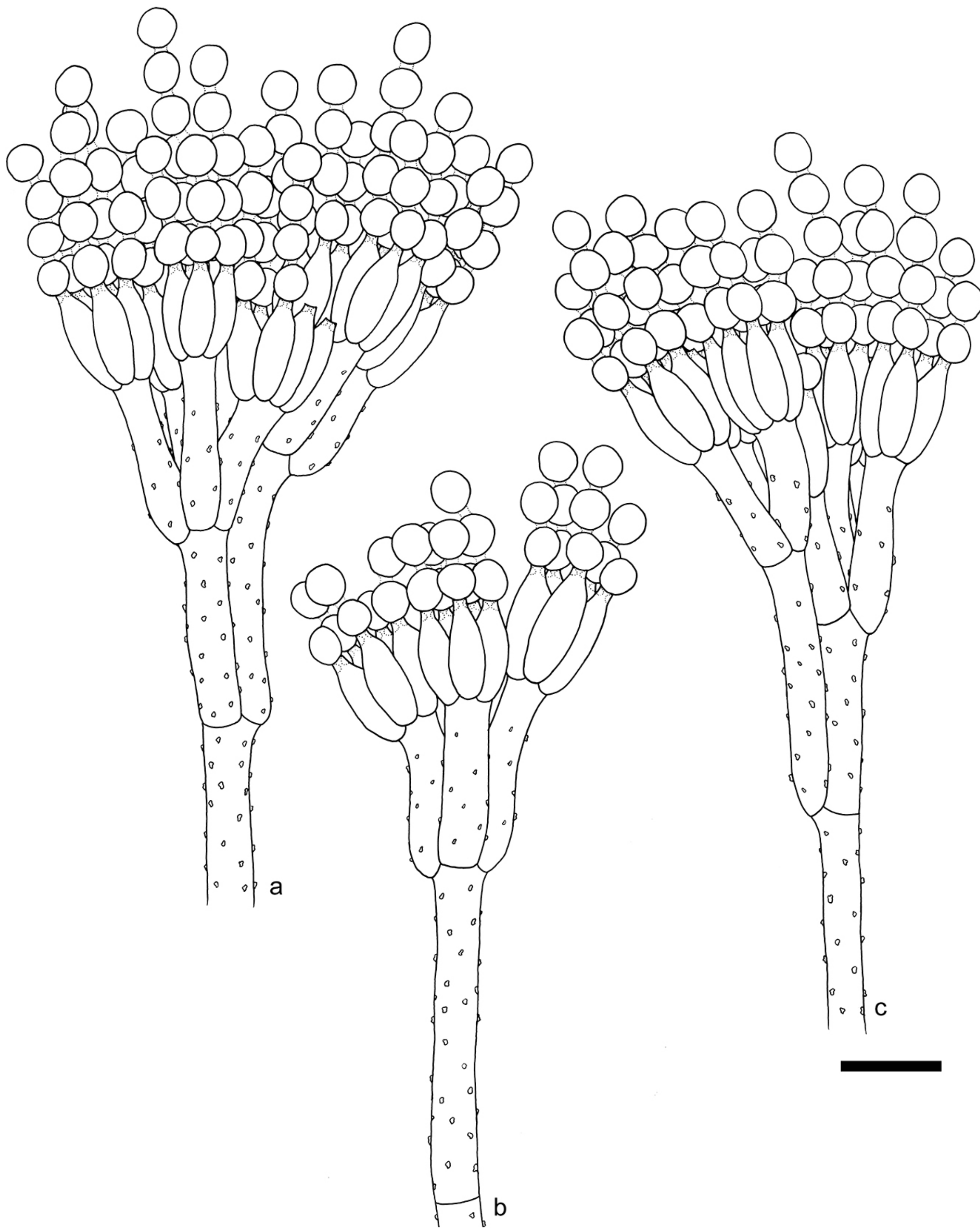

PLATE 61. Line drawing of *P. crustosum*. a–c. Conidiophores. (— Scale bar = 10 µm for a–c).

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38. *Penicillium expansum* Link

PLATES 62, 63, 70d

Magazin der Gesellschaft Naturforschenden Freunde Berlin 1: 16. 1809.

EX-NEOTYPE: CBS325.48 = ATCC7861 = IBT3486 = FRR976
TYPE ISOLATED FROM: *Malus sylvesitris*, USA
SPECIMENS EXAMINED: CV2860, CV2861, STEU6561, STEU6562, STEU6563, STEU6564.
ISOLATED FROM: Soil, Malmesbury; Apples, Apple orchards in Western Cape of South Africa

Macromorphology — CYA, 25 °C, 7d: Colonies 45–55 mm, deep, radially and lightly concentrically sulcate; margins low to deep, narrow, entire; mycelia white; texture mostly densely packed determinate synnema, floccose and velutinous areas also present; conidiogenesis dense, conidia *en masse* dull to greyish green (27E4–27E7); exudate clear and brownish droplets, some isolates absent, soluble pigment brownish orange in areas where colonies face each other, reverse pigmentation brownish orange to brown (6C6–6C7–6D7–6D6) at colony centre spreading towards margins facing each other, at margins not facing each other pale yellow (3A3–4A3).

CYA 5 °C, 7d: Colonies developed in areas close to inoculation point.

CYA 30 °C, 7d: Colonies 16–20 mm, very deep, radially sulcate; margins low, narrow, somewhat irregular; mycelia white; texture floccose and velutinous; conidia *en masse* greyish green (25C5–25D5); exudate clear and brownish droplets, soluble pigment light yellow halo surrounding colonies, reverse pigmentation ranging from greyish yellow to brownish orange (4C6–6D6) at centre, fading into light yellow (4A5) margin.

CYA 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 40–55 mm, low, plain; margins subsurface to low, narrow to wide, somewhat irregular; mycelia white; texture velutinous, with indeterminate synnema giving colonies granular look; conidiogenesis dense, conidia *en masse* dull to greyish green (26C4–26E4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (2A3) at point of inoculation, then fading from greyish yellow (1B4) to greyish green (29C3) to greenish grey (29B2) at margin.

YES, 25 °C, 7d: Colonies 60–68 mm, deep, radially and concentrically sulcate; margins deep, narrow to wide, entire; mycelia white; texture similar to CYA; conidiogenesis dense, conidia *en masse* similar to CYA; exudate clear and brownish droplets present, soluble pigment absent, reverse pigmentation dull to greyish yellow (3B4–3B5).

G25N, 25 °C, 7d: Colonies 11–15 mm, moderately deep, radially sulcate; margins low, narrow; mycelia white; texture velutinous and floccose; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish green (30B3–30B4), with pale green (30A3) margin.

CREA, 25 °C, 7d: Colonies 25–30 mm, good acid production leading to base production.

Micromorphology — Conidiophores mostly terverticillate; stipes smooth walled, 200–500 × 3–4 µm; rami 2, appressed, 17–27 × 3–4 µm; metulae 3 to 5, appressed, 13.5–48° [28.5±9.2°], 11.5–16 × 3–3.5 [13.7±1.0 × 3.0±0.28] µm; phialides ampulliform, 3 to 5, 9.5–12 × 2.5–3 [10.6±0.6 × 2.9±0.16] µm; conidia smooth walled, broadly ellipsoidal, 3–4 × 2.5–3.5 [3.7±0.2 × 3.1±0.19] µm, average width/length = 0.82±0.04, n = 44.

Notes — *Penicillium expansum* is characterized by its smooth walled, closely appressed conidiophores. Determinate synnemata are typical on CYA and MEA. It grows very well on CREA, on which it produces a strong acid followed by base production. Colonies often have a fruity odor. This species is morphologically similar to *P. crustosum*, but the latter species produce rough walled stipes.

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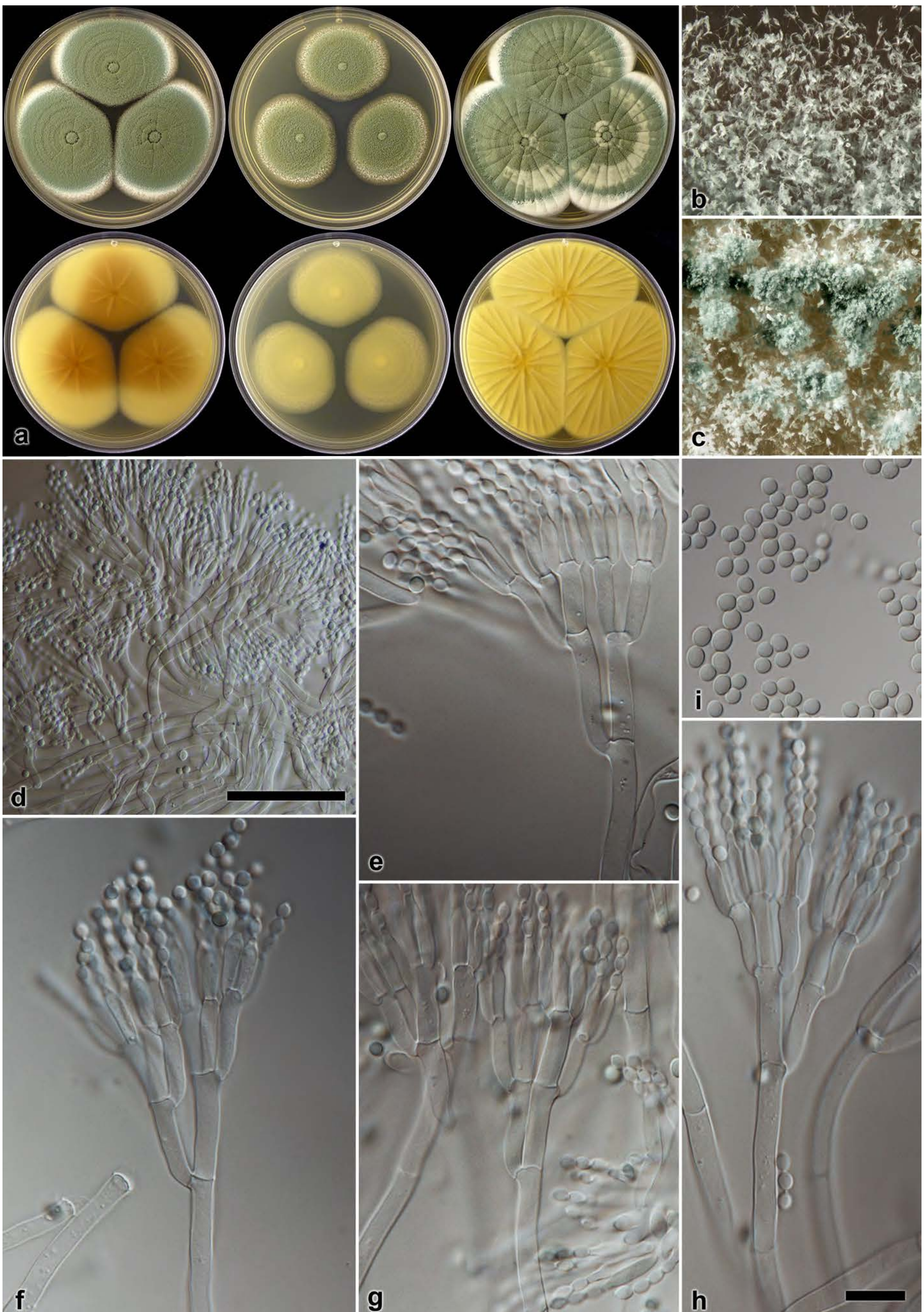

PLATE 62. *Penicillium expansum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in d = 50 µm; — Scale bar in h = 10 µm, applies to e–i).

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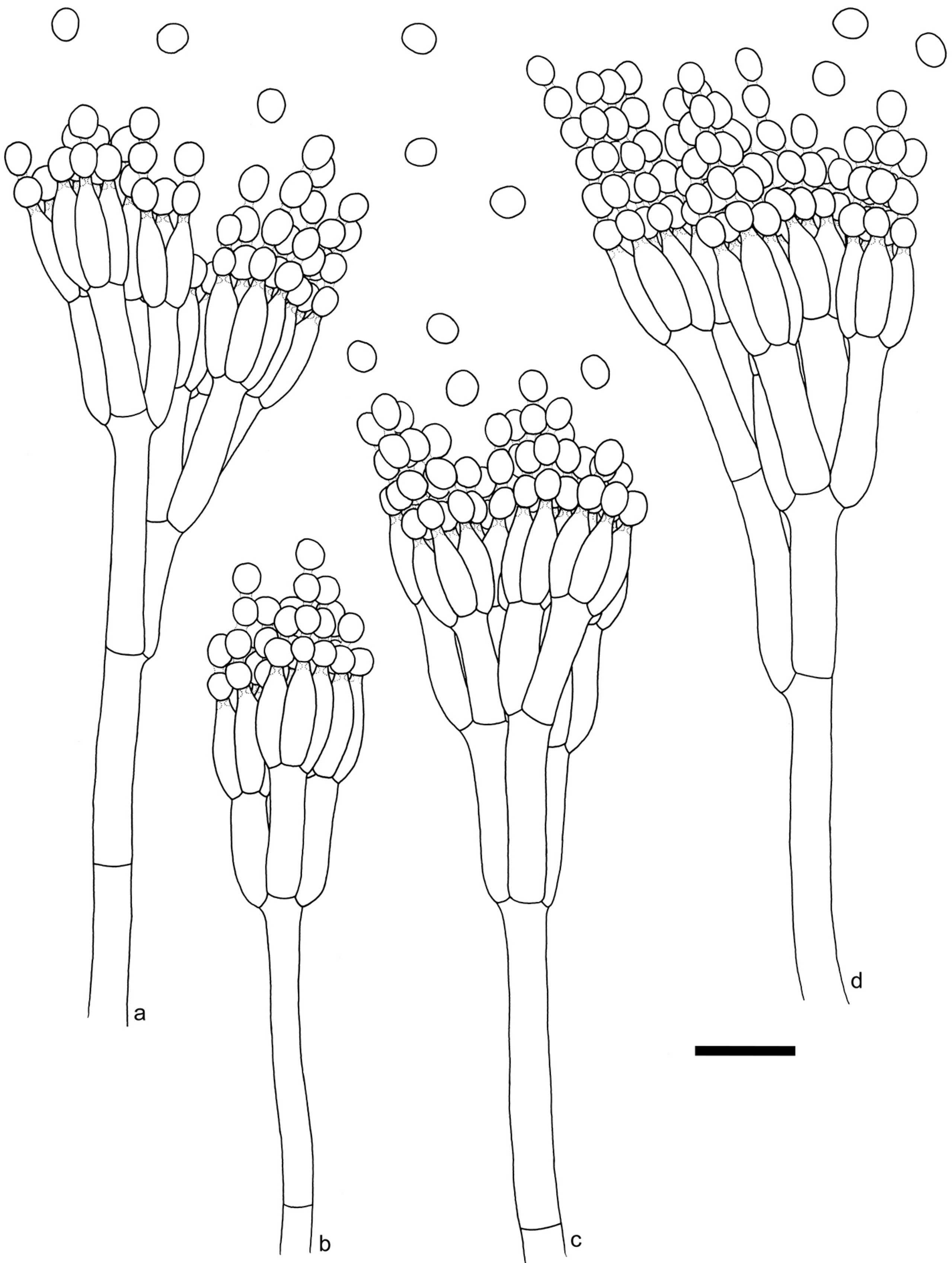

PLATE 63. Line drawing of *P. expansum*. a–d. Conidiophores (— Scale bar = 10 µm).

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39. *Penicillium griseofulvum* Dierckx PLATES 64, 65, 70e

Annales de la societe scientifique de bruxelles 25: 88. 1901.

EX-TYPE: CBS185.27 = ATCC11885 = IBT6740 = NRRL2300
TYPE ISOLATED FROM: Unknown source
SPECIMENS EXAMINED: CV41, CV51, CV69, CV78, CV146, CV148.
ISOLATED FROM: Air, Soil, *Protea repens* infructescence, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 25–32 mm, moderately deep, sulcate; margins low, very narrow (<1 mm); mycelia white; texture velutinous to fasciculate; conidiogenesis dense, conidia *en masse* greyish green (25C3–25C4–26C4–26C3); exudate mostly absent, clear when present, soluble pigment absent, reverse pigmentation olive brown to olive brown (4D4–4C4–4C5).

CYA 5 °C, 7d: Microcolonies, 2–4 mm.

CYA, 30 °C, 7d: All features similar to colonies on CYA at 25 °C, except for slightly darker reverse color.

CYA 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 26–31 mm, low, plain; margins low, narrow (1 mm), irregular; mycelia white; texture velutinous to fasciculate; conidiogenesis dense, conidia *en masse* greyish green (25C3–25C4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A3) at centre, greyish green (1C3) elsewhere, sometimes dull yellow (3C3–3C5) to olive (3D4–3D5).

YES, 25 °C, 7d: Colonies 29–32 mm, low, radially and lightly concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous to fasciculate; conidiogenesis dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3B7) at centre, greyish yellow to olive yellow (3C4–3C6).

G25N, 25 °C, 7d: Colonies 15–18 mm, low, plain, slightly raised towards centre; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2C3) at point of inoculation fading into yellowish grey (2C2).

CREA, 25 °C, 7d: Colonies 21–25 mm, no acid production.

Micromorphology — Conidiophores mostly ter- and quaterverticillate, typically with additional subterminal branches forming very complex conidiophores; stipes smooth, 400–500 × 2.5–3.5 µm; rami/branches 2–3, divergent, 9–27 × 2–3 [15.5±4.8] µm; metulae 3–4, divergent, 41–76° [59±8.9°], 6–12 × 2–3 [8.44±1.1 × 2.5±0.24] µm, vesicle 2.5–4 [3.4±0.35] µm; phialides, ampulliform, 5–9 per metula, 4.5–6.5 × 2–3 [5.5±0.37 × 2.3±0.2] µm; conidia smooth, broadly ellipsoidal, 2.5–3.5 × 2–3 [3±0.18 × 2.6±0.16] µm, average width/length = 0.86±0.05, n = 74.

Notes — *Penicillium griseofulvum* is one of the easier species to recognize as it produce characteristic grey conidia on all media. Also, conidiophores are typically divergent and often branched more complex than terverticillate. Conidiophores end in very short phialides, which is unique in this species.

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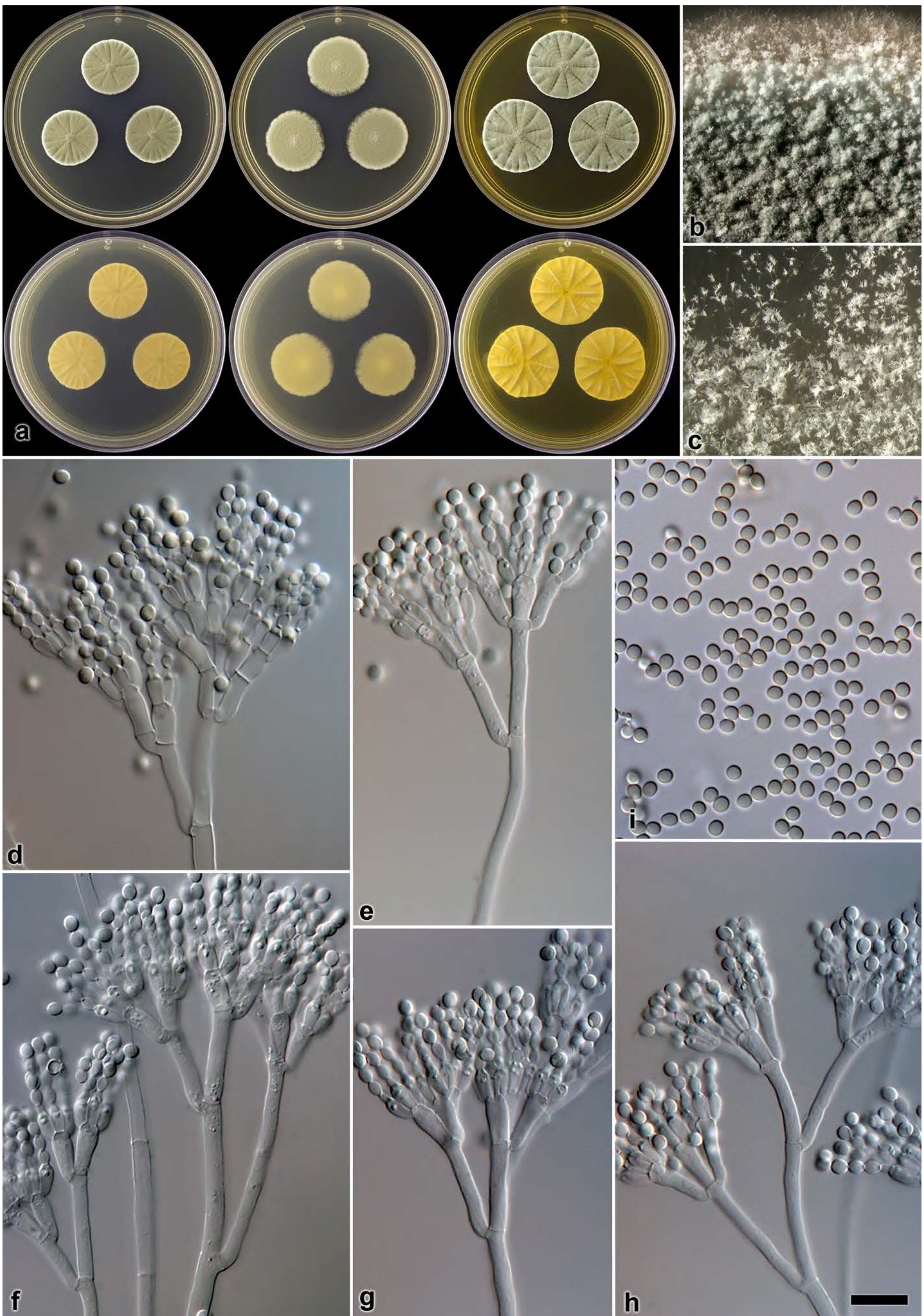

PLATE 64. *Penicillium griseofulvum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to d–i).

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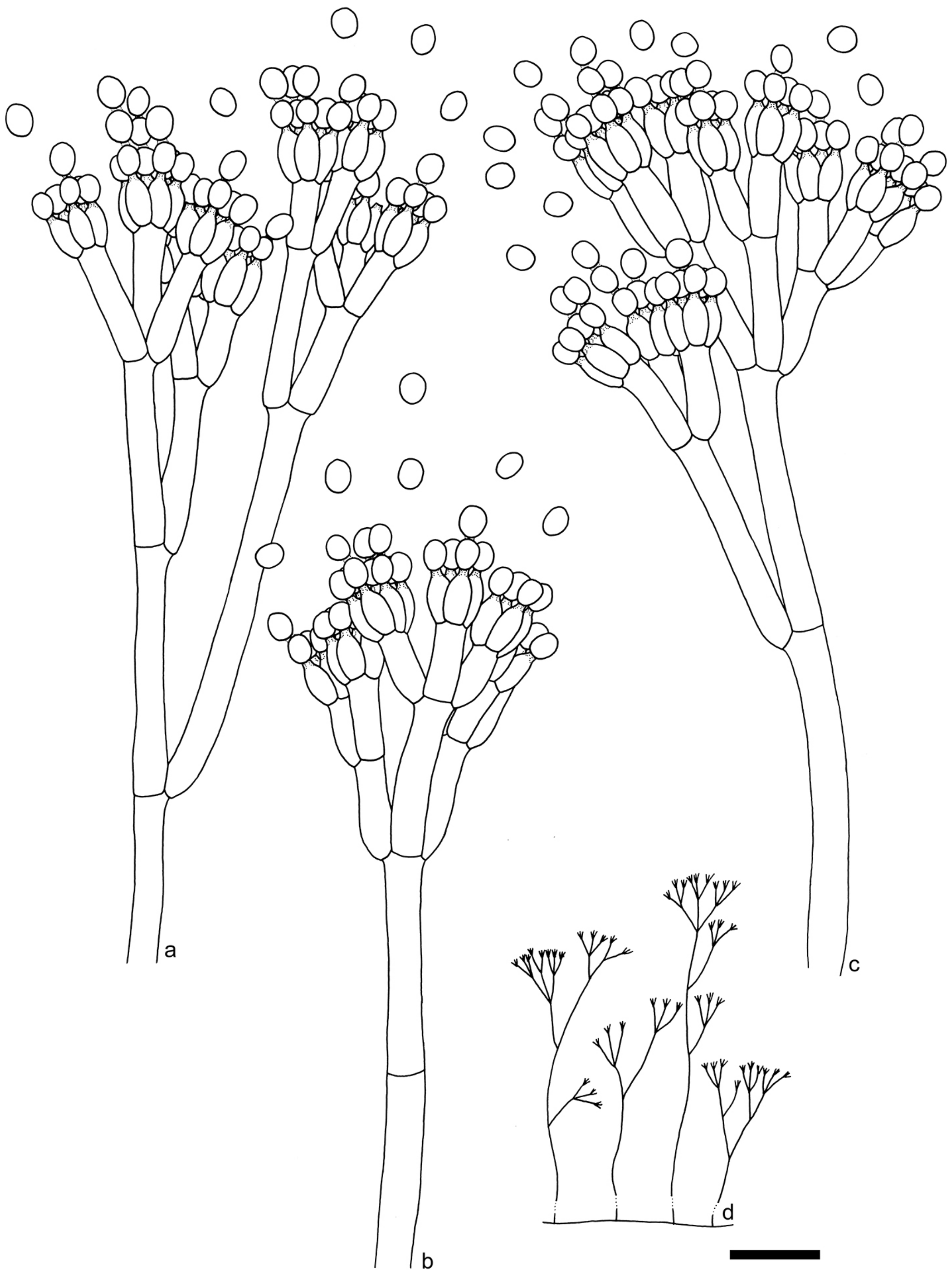

PLATE 65. Line drawing of *P. griseofulvum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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40. *Penicillium melanoconidium* (Frisvad) Frisvad & Samson PLATES 66, 67, 70f

Studies in Mycology 49: 28. 2004.

BASIONYM: *P. aurantiogriseum* var. *melanoconidium* Frisvad (1989. Mycologia 81: 849)
EX-TYPE: IBT3444 = IMI321503 = CBS115506
TYPE ISOLATED FROM: Wheat, Denmark
SPECIMENS EXAMINED: CV1331, IMI321503.
ISOLATED FROM: *Protea repens* infructescence, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 29–31 mm, low to moderately deep, radially and lightly concentrically sulcate; margins low, narrow (1 mm), entire, yellow edge surrounding margin; mycelia white; texture velutinous; conidiogenesis moderate to dense, conidia *en masse* dark green (25F4–25F8); exudate clear, soluble pigment absent, reverse pigmentation light yellow (3A4) at centre, light brown (6B8) and orange (6A6) elsewhere.

CYA, 5 °C, 7d: Microcolonies, 2–5 mm.

CYA, 30 °C, 7d: All features similar to colonies on CYA at 25 °C except for more restricted growth, 20–22 mm.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 25–26 mm, low, plane; margins subsurface to low, narrow (1 mm), irregular, yellow edge surrounding margin; mycelia white; texture fasciculate to crustose; conidiogenesis dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greenish yellow (1A6) at point of inoculation, greyish yellow (1B5) and greyish green (1D7) elsewhere.

YES, 25 °C, 7d: Colonies 36–39 mm, deep, radially and lightly concentrically radiate; margins deep, narrow (1–2 mm), somewhat irregular; mycelia white; texture velutinous to fasciculate near margin; conidiogenesis dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation orange yellow (4A6) at centre, fading into orange (5B7), with margin orange yellow (4A6).

G25N, 25 °C, 7d: Colonies 19–22 mm, low, lightly radially and concentrically sulcate; margins low, narrow (2–3 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B5) at centre, greyish yellow (2C5) elsewhere, light yellow (2A5) at margin.

CREA, 25 °C, 7d: Colonies 18–20 mm, moderate to good acid production.

Micromorphology — Conidiophores terverticillate; stipes smooth to finely roughened, 100–400 × 2.5–4 µm; rami 2, divergent, 12–24 × 2.5–3.5 [17±3.2] µm; metulae 3–5, appressed with some divergent, 22–50° [38±6.7°], 8–15 × 2–3.5 [10.6±1.3 × 2.7±0.29] µm, vesicle 3–5 [3.8±0.4] µm; phialides ampulliform, 5–8 per metula, 6.5–9.5 × 2–3 [7.9±0.6 × 2.6±0.18] µm; conidia smooth, broadly ellipsoidal to subspheroidal, 2.5–3.5 × 2.5–3 [3±0.13 × 2.5±0.12] µm, average width/length = 0.84±0.04, n = 58.

Notes — *Penicillium melanoconidium* are characterized by dark green conidia produced, as well its orange–yellow reverse color on CYA. Morphologically it is closely related to *P. aurantiogriseum*, also isolated in this study. However, the latter species has a typical blue-green conidial color, which distinguishes it from *P. melanoconidium*. For the strains studied, *P. aurantiogriseum* produced smaller colonies on YES (23–26 mm), compared to *P. melanoconidium* (36–39 mm). In addition, *P. melanoconidium* on average produce smaller conidia (3±0.13 µm) compared to *P. aurantiogriseum* (3.3±0.19 µm).

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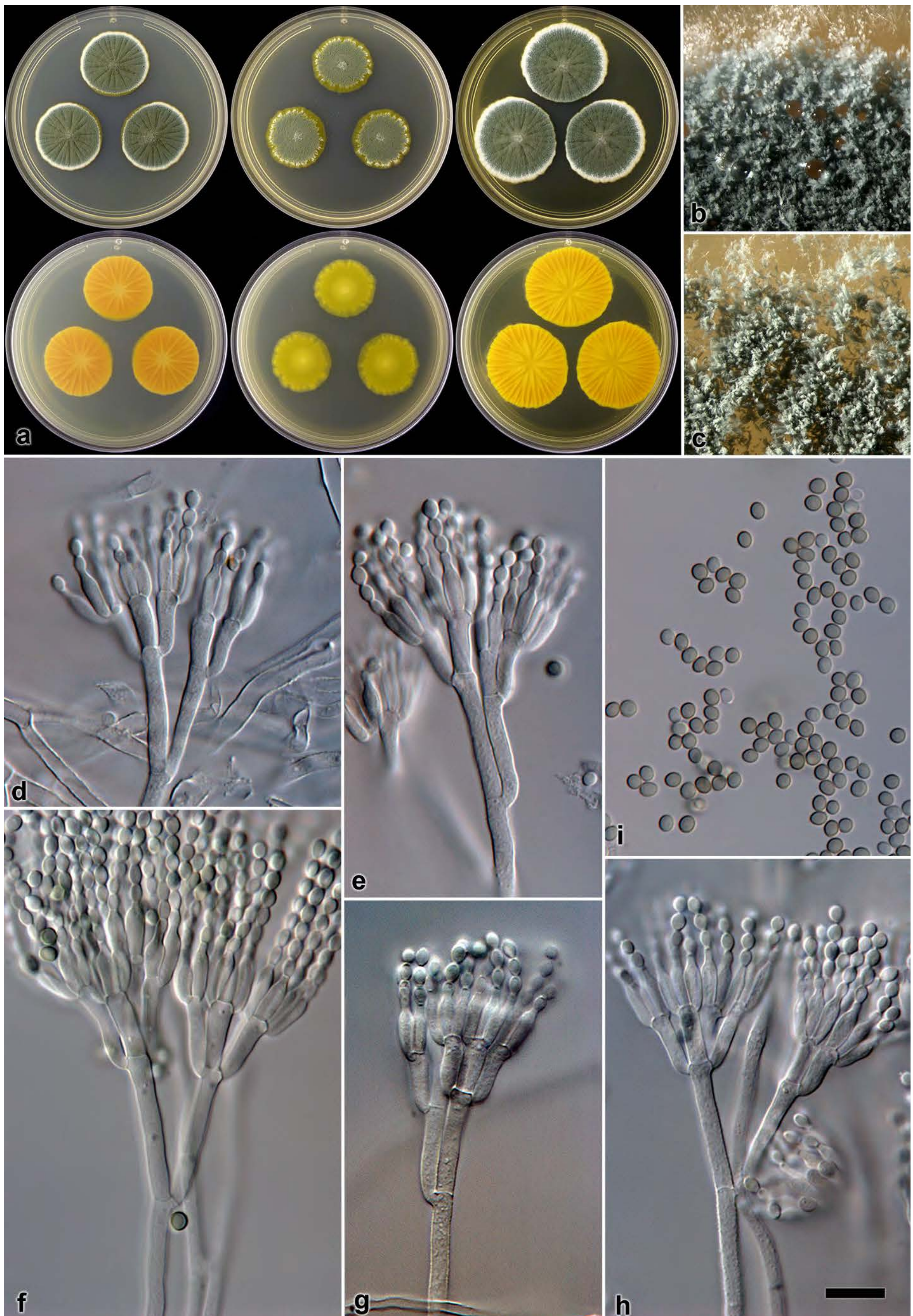

PLATE 66. *Penicillium melanoconidium* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to d–i).

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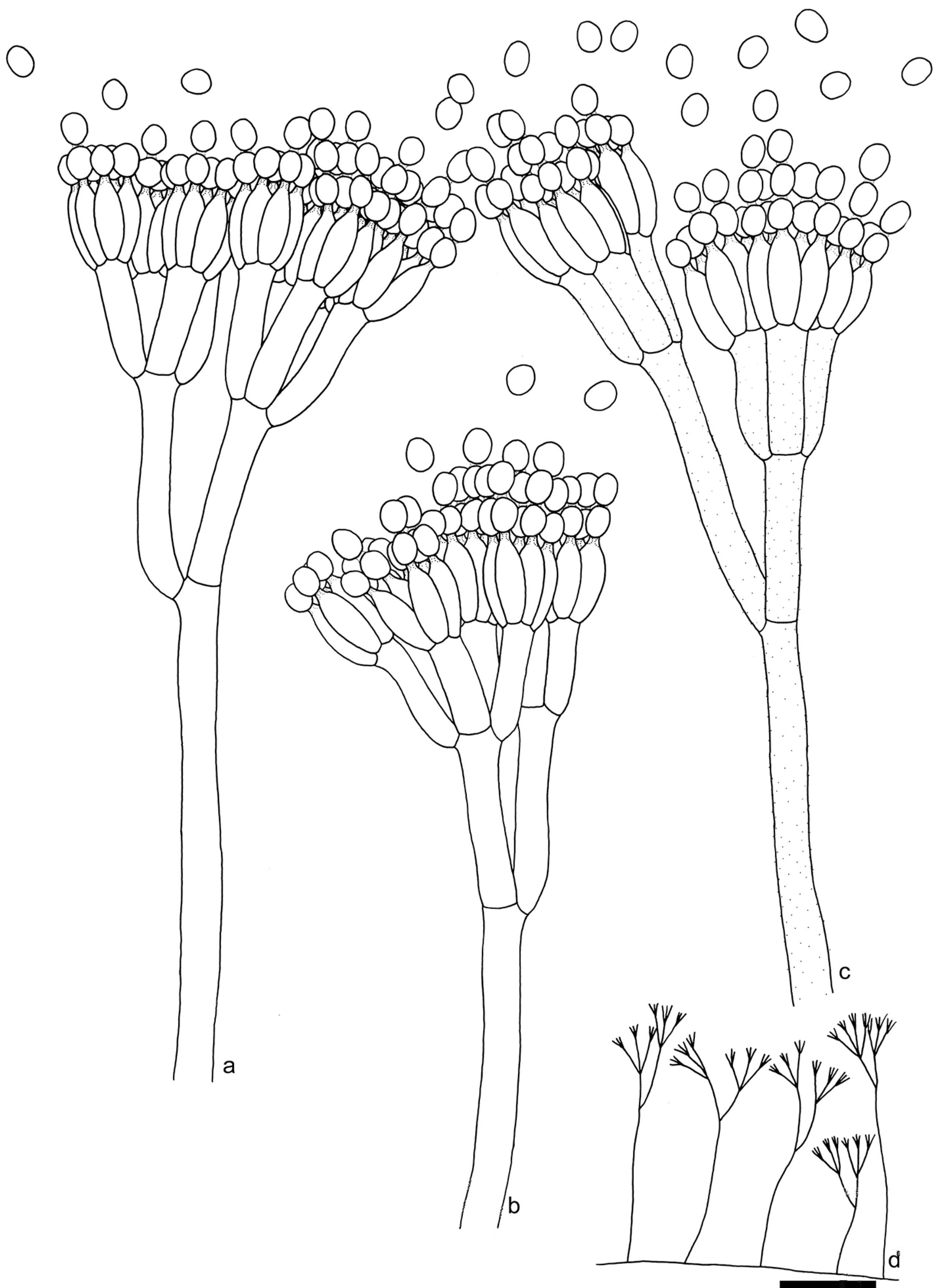

PLATE 67. Line drawing of *P. melanoconidium*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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41. *Penicillium rubens* Biourge PLATES 68, 69, 70g

La Cellule 33: 265. 1923.

EX-LECTOTYPE: NRRL792 = IBT30129 = CBS129667
TYPE ISOLATED FROM: Unrecorded source
SPECIMENS EXAMINED: CV349, CV361, CV378.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescence, Stellenbosch; House dust, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 54–58 mm, low, radially and concentrically radiate; margins subsurface to low, wide (4–5 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate to dense, conidia *en masse* greyish green (25E5–26E6); exudate sometimes absent, mostly yellow droplets produced, soluble pigment bright yellow, sometimes absent, reverse pigmentation greyish yellow (2B6) at centre, fading into pastel to greyish yellow (2A4–2B4), into greyish green (29B3) near margin.

CYA, 5 °C, 7d: Microcolonies, 2–3 mm.

CYA, 30 °C, 7d: All features similar to colonies on CYA at 25 °C, except for more restricted growth of 37–40 mm.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 49–54 mm, low, plain; margin low, wide (4–5 mm), entire; mycelia white; texture velutinous; conidiogenesis moderate to dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation yellow (3A7) at centre, fading into greyish green (28D4–28D5) elsewhere.

YES, 25 °C, 7d: Colonies 64–67 mm, low, radially and concentrically sulcate; margins low, wide (4–5 mm), entire; mycelia white; texture velutinous to granular; conidiogenesis moderate to dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation pastel yellow (2A4–3A4).

G25N, 25 °C, 7d: Colonies 24–26 mm, low, plain, raised at centre; margins low, wide (3 mm); mycelia white; texture velutinous; conidiogenesis moderate, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2B4) at point of inoculation, greenish grey (1B2) near center fading into greyish green (27C3–27C4) elsewhere.

CREA, 25 °C, 7d: Colonies 23–25 mm, weak acid production.

Micromorphology — Conidiophores mostly terverticillate, bi- and quarterverticillate conidiophores also present, with subterminal branches regularly formed; stipes smooth walled, 200–300 × 2.5–4 µm; rami 2, divergent, 11.5–26 × 2.5–4 [17.6±3.65] µm; metulae 3–5, divergent and appressed, 25–73° [51±10.4°], 7–13.5 × 2.5–4 [10.8±1.2 × 3.1±0.3) µm, vesicle 3–5 [4±0.41] µm; phialides ampulliform, 4–8 per metula, 7–10 × 2.5–3.5 [7.9±0.61 × 2.9±0.25] µm; conidia smooth walled, broadly ellipsoidal to subspheroidal, 3–4 × 2.5–3.5 [3.2±0.16 × 2.8±0.19] µm, average width/length = 0.88±0.04, n = 60.

Notes — *Penicillium rubens* typically produces fast growing colonies on most media. Conidiophores are terverticillate, with bi- and quaterverticillate conidiophores often observed. It is closely related to *P. chrysogenum* from which it morphologically cannot be consistently distinguished (Houbraken *et al*. 2011). However, phylogenetic data and secondary metabolite profiles do distinguish between these two species. Strains isolated from Fynbos produce abundant yellow exudate on MEA, but lost this ability after time.

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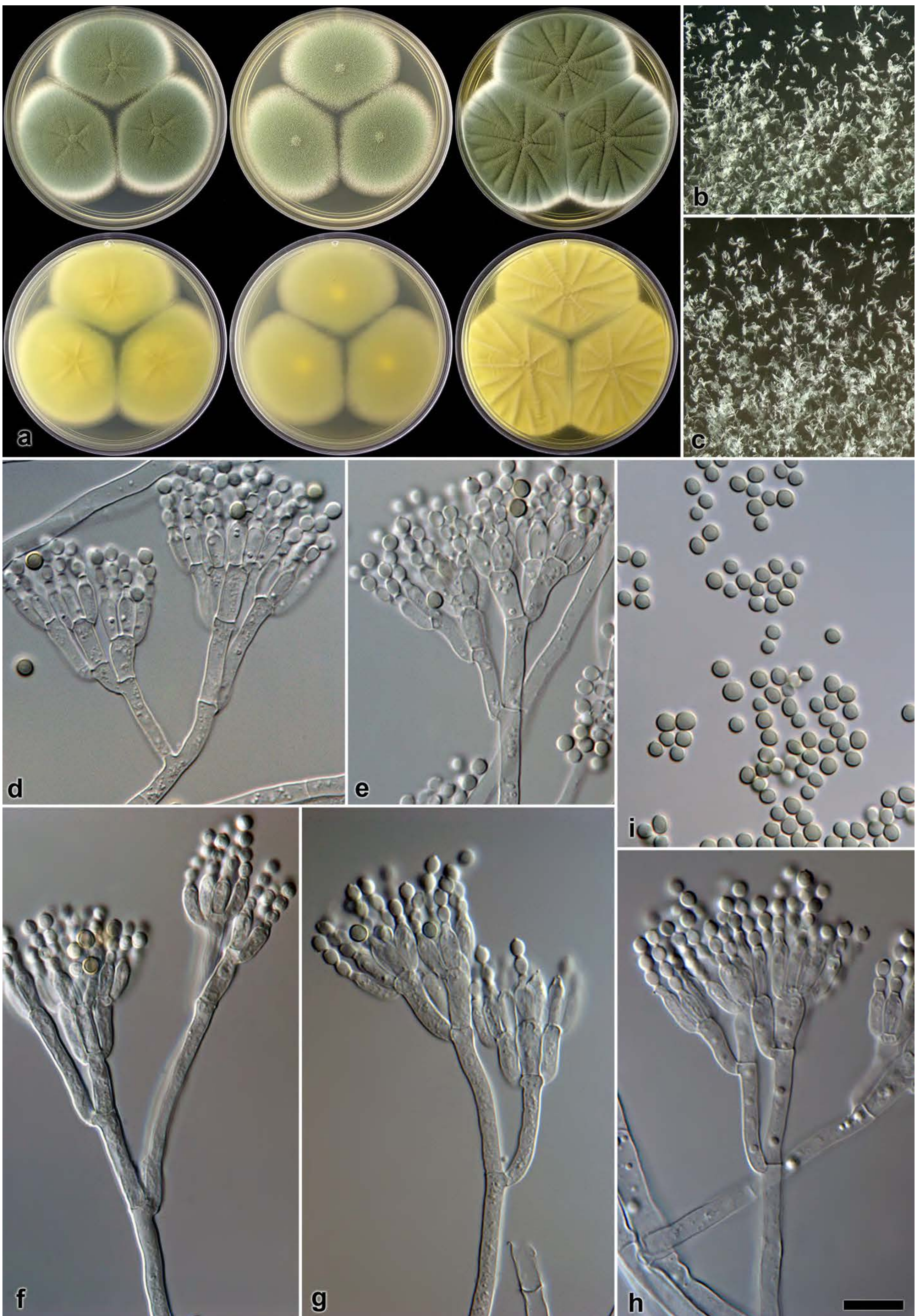

PLATE 68. *Penicillium rubens* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to d–i).

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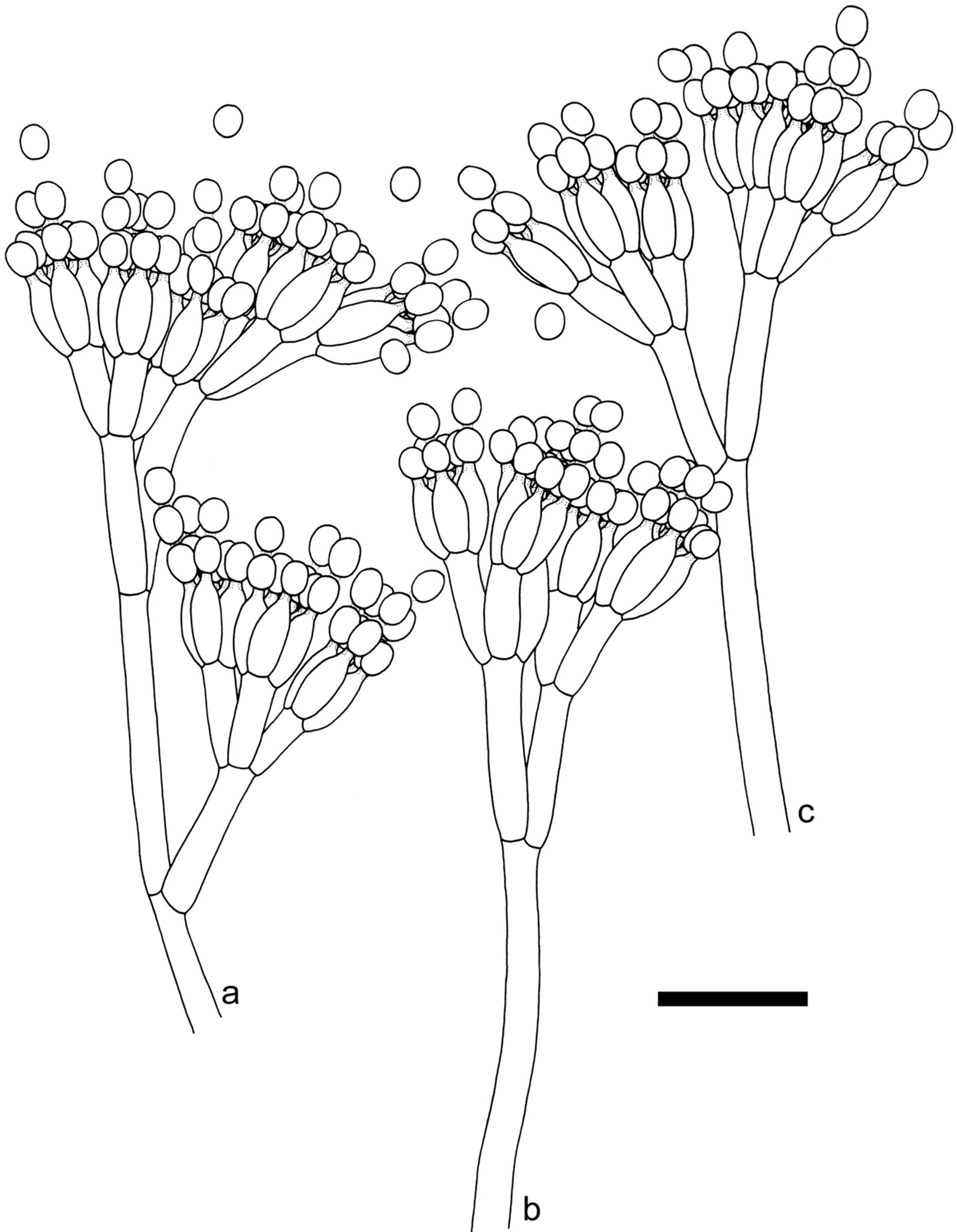

PLATE 69. Line drawing of *P. rubens*. a–c. Conidiophores. (— Scale bar = 10 µm).

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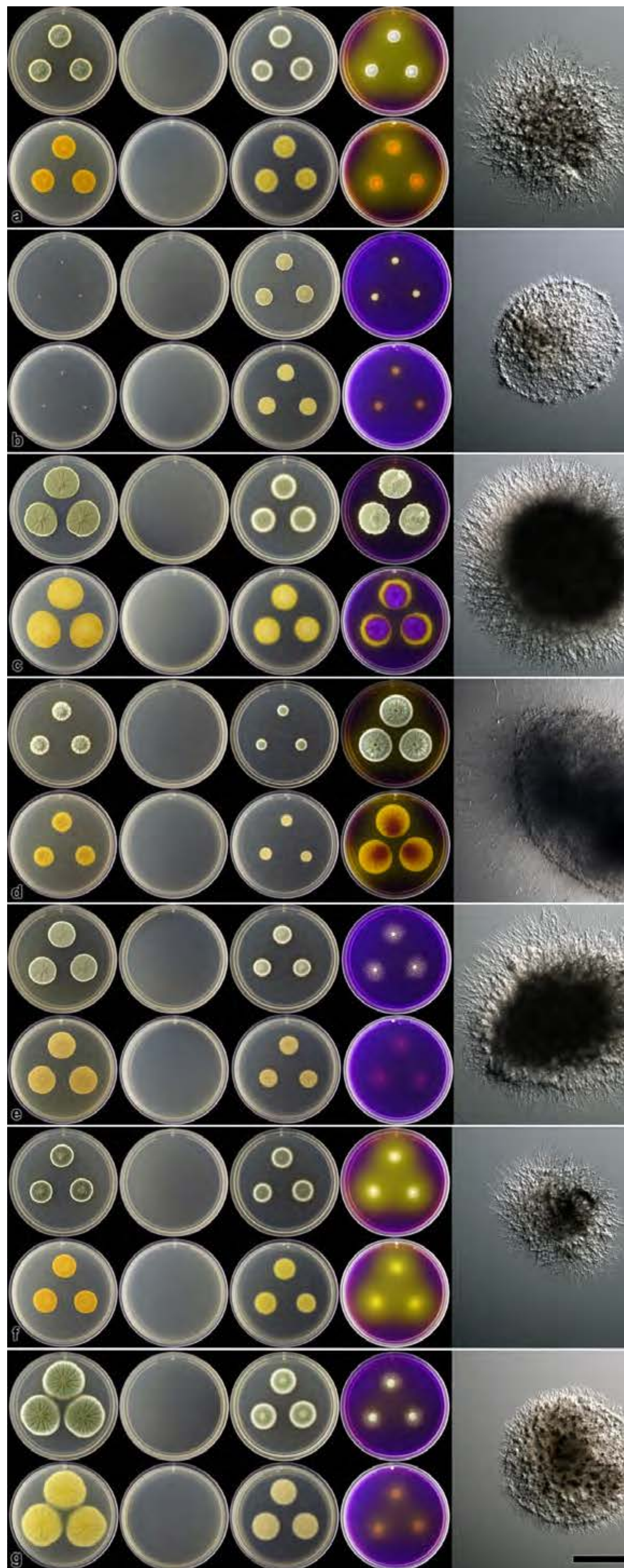

PLATE 70. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 1000 µm). a. *Penicillium aurantiogriseum.* *b. Penicillium brevicompactum.* c. *Penicillium crustosum.* d. *Penicillium expansum.* a. *Penicillium griseofulvum.* f. *Penicillium melanoconidium.* g. *Penicillium rubens.*

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The section *Canescentia* Houbraken & Samson

Studies in Mycology 70: 46. 2011.

TAXONOMIC NOVELTIES: *Penicillium fynbosense* prov. nom., *P pseudoantarcticum* prov. nom., *P. pseudoatrovenetum* prov. nom., *P. pseudocanescens* prov. nom.

SPECIES TREATED: *Penicillium novae-zeelandiae, P. radiatolobatum*

Soil-borne species in *Penicillium* often belong to the section *Canescentia*. Species are typically characterized by their terminal symmetrical biverticillate conidiophores, with subterminal branching commonly observed. Phialides are often short, somewhat broad and swollen (Houbraken & Samson 2011). The section includes *P. canescens, P. jensenii, P. janczewskii, P. yarmokense, P. antarcticum, P. atrovenetum, P. novae-zeelandiae* and *P. coralligerum* (Houbraken & Samson 2011). However, a review of this section is needed since there are a number of taxonomic questions (Houbraken & Samson 2011), which makes species identification difficult. In a separate study, extrolite data for a large number of strains was analyzed and results indicate that 60 new species belong in this section (Frisvad, personal communication). Added to this, is the four new species isolated in this study. The large number of strains examined in both these studies will provide a great platform for a revision of section *Canescentia* and thus define species concepts for problematic taxa in the section.

Problematic taxa include for instance *P. canescens* and *P. janczewskii*. Pitt (1979) synonymized a number of species with *P. canescens* (= *P. raciborskii, P. kapuscinksii, P. novae-caledoniae* and *P. yarmokense*) and *P. janczewskii* (= *P. echinatum, P. swiecickii, P. nigricans* and *P. nigricans var. sulphuratum*). These species were all found to produce somewhat similar colony morphologies and were distinguished based on conidiophore morphology. On CYA colonies are 25–32 mm wide, are dense and have white to yellow colored mycelia (Pitt 1979). On MEA colonies are typically 15–25 mm wide, typically floccose and conidia *en masse* that is a bluish to greenish grey (Pitt 1979). In general the long, rugose stipes and smooth walled conidia of *P. canescens*, distinguish it from the smooth walled stipes and rough walled conidia of *P. janczewskii* (Pitt 1979). However, Pitt (1979) also reported on the existence of strains that bridge these characters and form intermediates between these species. *Penicillium canescens* strains IMI149218 and FRR97, for example, produce rough walled stipes, rough walled conidia and are irregularly branched. These strains may thus have lost the distinct nature of the typical *P. canescens* conidiophore, even though phylogenetically they are similar to the ex-type of *P. canescens* (CBS300.48). Phylogenetic analysis of many of the ex-types cultures of these species (FIGURES 15, 16) show that they should probably be reinstated as distinct species. Houbraken & Samson (2011) showed that *P. raciborskii* belong in section *Exilicaulis*, *P. swiecickii* in section *Ramosa* and *P. kapuscinskii* in section *Citrina* as a synonym of *P. godlewskii*. The phylogenies also confirmed *P. nigricans var. sulphuratum* and *P. granatense* as synonyms of *P. janczewskii*, with *P. echinatum* and *P. nigricans* closely related.

Morphologically section *Canescentia* species are very similar and have to be distinguished based on minute differences. This is complicated even more by intraspecies variation reported for *P. canescens* (Pitt 1979) and also observed in this study for *P. fynbosense*. A phylogenetic species concept is also not advisable here. In a clade where variation seems normal, sequence differences or similarities might not reflect the true relationship between strains. Extrolite data may, therefore, provide vital information for species delineation in this section. However, preliminary secondary metabolite data of the Fynbos strains also show this variation (Frisvad personal communication). Thus, for species delimitation a polyphasic approach that include morphology, sequence and extrolite data are needed. Species boundaries should then be determined based on concordance between all data, similarly to the concept of the congruence of gene genealogies (Taylor *et al*. 2000). It is thus important to note that the results given here will form part of an extensive review of section *Canescentia*. However, the species from the Fynbos are well defined morphologically and phylogenetically.

Isolations from Fynbos resulted in six species that belong in section *Canescentia*. Two major clades were formed based on phylogeny (FIGURES 14, 15, 16). The one contained *P. canescens* and its close relatives and the other *P. novae-zeelandiae* and its close relatives. The sequence variation observed between the different genes resulted in a broad species concept applied for the Fynbos strains. As a result, this allows for variation within a species and prevents the acceptance of a large number of species that is distinguished based on minor differences. As such, the taxonomy of this group will be more stable. Intraspecies variation was more pronounced in the case of *P. fynbosense*. Based on the β-tubulin and Calmodulin phylogenies (FIGURE 15), Fynbos species were identified as *P. radiatolobatum* and *P. novae-zeelandiae*. These identifications were confirmed by extrolite data (Frisvad personal communication). However, four species resolved in distinct clades separate from all previously described species and is described here as new.

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P. pseudocanescens resolved in a clade with a couple of strains previously identified as *P. canescens*. This clade is, however, distinct from the ex-type (CBS300.48) and produces unique extrolites, although it is morphologically very similar to the ex-type culture of *P. canescens*. The main difference is the rough walled conidia of *P. pseudocanescens* compared to the smooth walled conidia of *P. canescens.* Based on this and the unique sequence and extrolite data it is described as a new species. *Penicillium radiatolobatum*, also found in the Fynbos, is a close relative. The latter species generally displayed faster growth on most media, especially at 37 °C. *Penicillium radiatolobatum* has previously been considered a synonym to *P. canescens* (Frisvad *et al*. 1990c). However, phylogenetically and morphologically it represents a unique species (FIGURES 14, 15, 16).

The *Penicillium atrovenetum* and *P. antarcticum* clade present a unique challenge. Morphological and chemical data suggest that strain CV1758 should be identified as *P. atrovenetum* and strains CV2189 and CV2198 as *P. antarcticum* (Smith 1957, Pitt 1979, McRae *et al*. 1999, Frisvad personal communication). The ITS, β-tubulin, Calmodulin and RPB2 phylogenies resolve the Fynbos strains in clades distinct from these two described species (FIGURES 14, 15, 16). *Penicillium antarcticum* and *P. atrovenetum* is morphologically two distinct species, showing differences in growth rates and very different conidiophore morphologies, with conidiophore elements and colonies bigger in *P. antarcticum*. Under the genealogical concordance species concept, identifying the Fynbos strains as these two species would thus result in synonymizing *P. antarcticum* with *P. atrovenetum*. For the stable taxonomy of these two distinct species, the Fynbos strains are described as two new species based on phylogenetic data.

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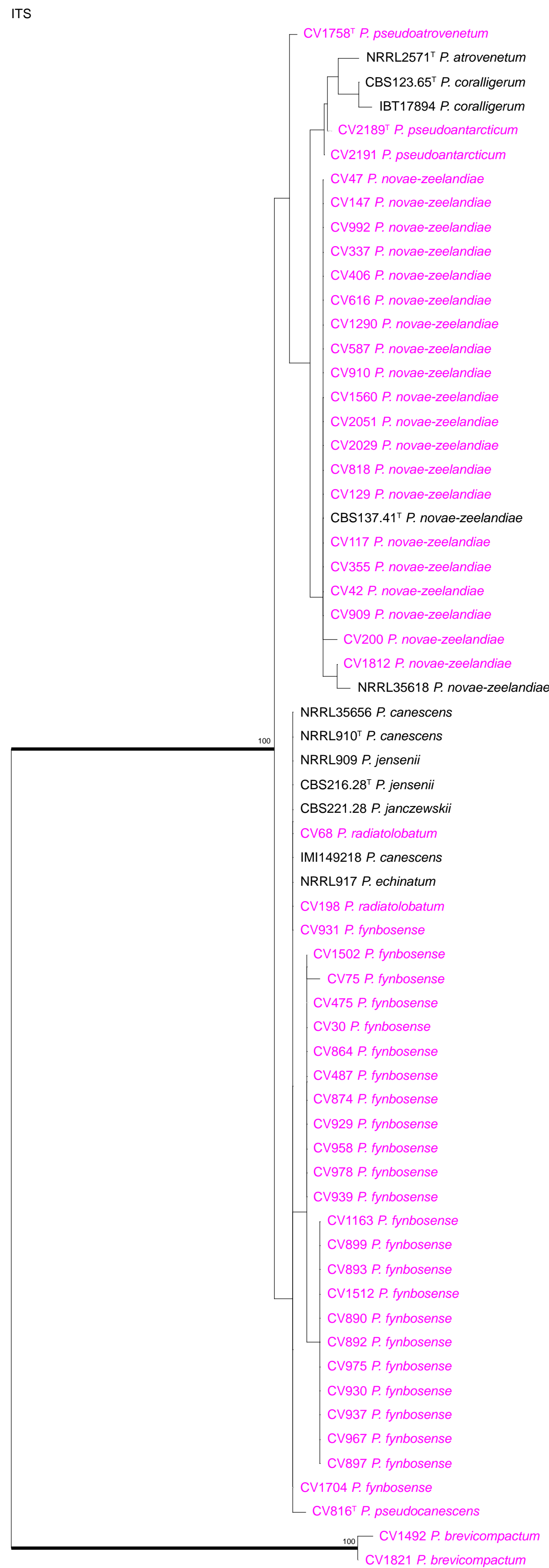

FIGURE 14: Phylogenetic tree based on ITS showing relationship of species in the section *Canescentia*. *Penicillium brevicompactum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type). Colored names indicate strains isolated from Fynbos.

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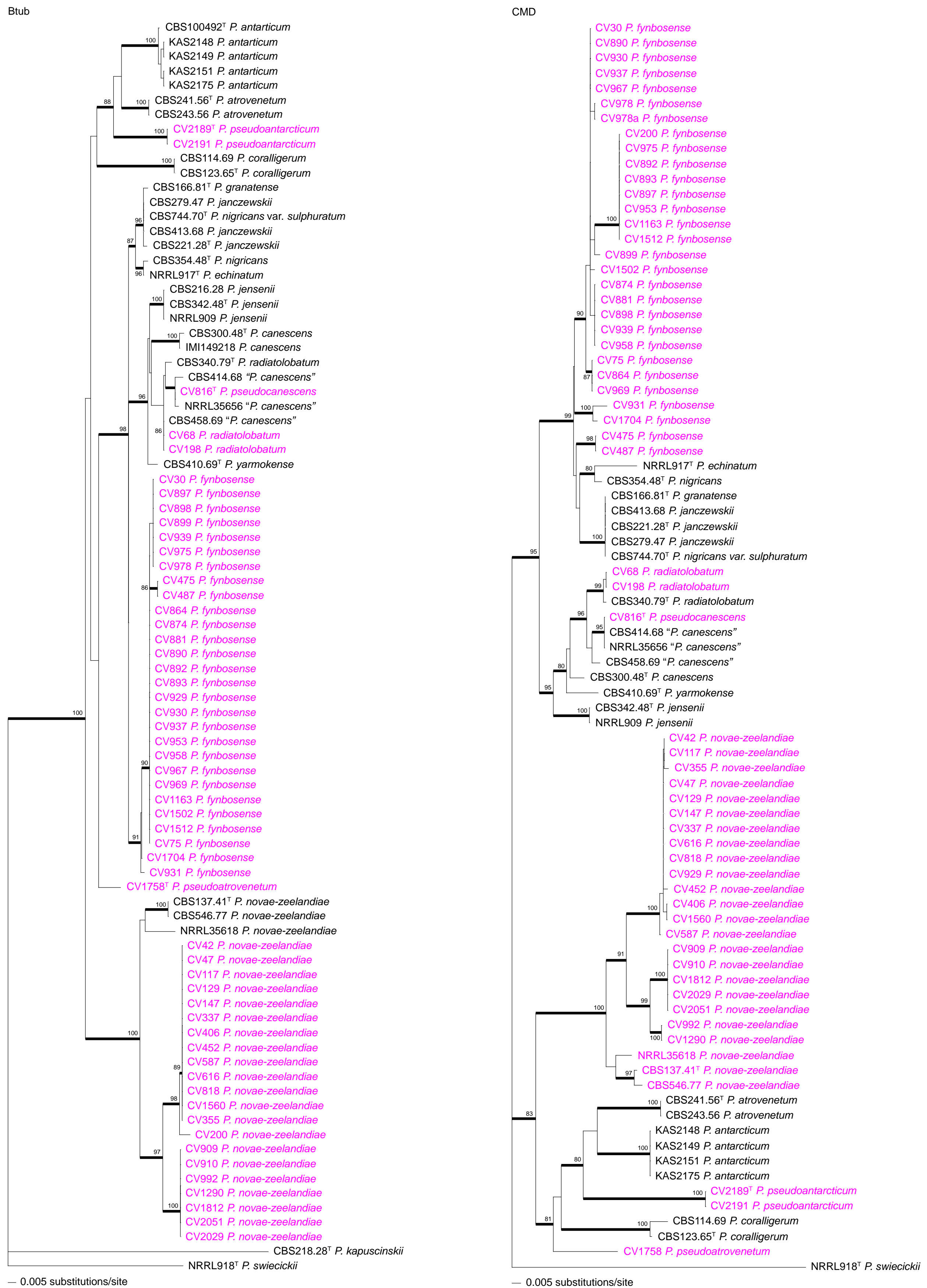

FIGURE 15: Phylogenetic trees based on β-tubulin and Calmodulin showing relationship of species in the section *Canescentia*. *Penicillium swiecickii* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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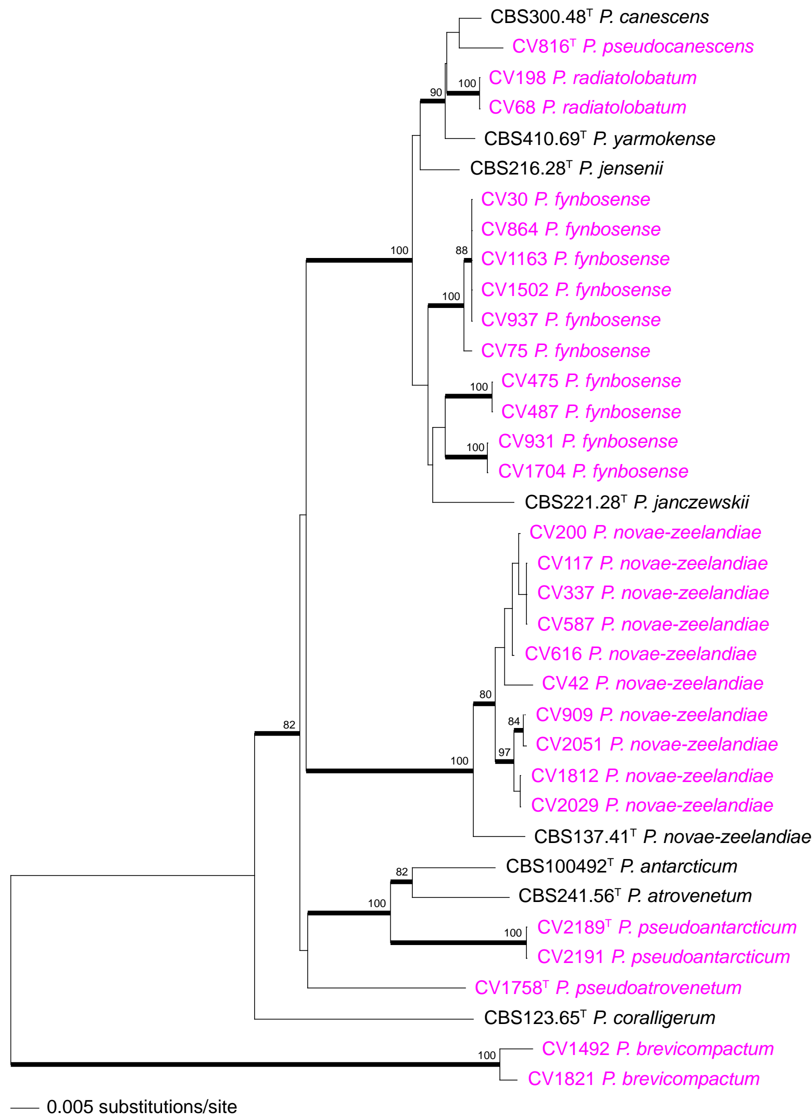

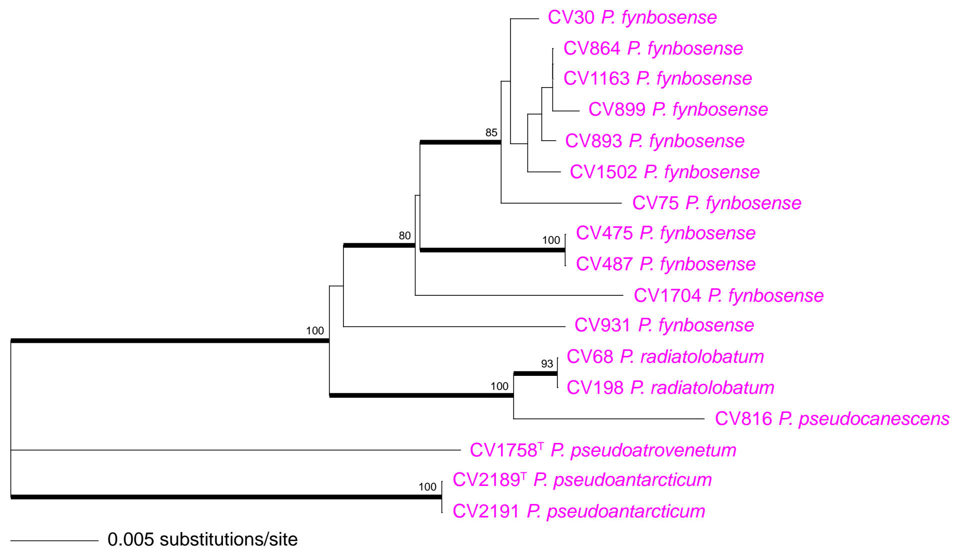

FIGURE 16: Phylogenetic trees based on RPB2 and Elongation Factor-1α, showing relationship of species in the section *Canescentia*. *Penicillium brevicompactum* was chosen as outgroup for RPB2 and *P. pseudoantarcticum* for EF-1α. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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42. *Penicillium fynbosense* Visagie **prov. nom.** PLATES 71, 72, 83a

ETYMOLOGY: Latin: Reference to the species that is common in soil in the Fynbos biome
EX-TYPE: CV931 = DTO182D5 = KAS4026 = DAOM241348
TYPE ISOLATED FROM: Soil, Malmesbury
SPECIMENS EXAMINED: CV1163, CV1502, CV1512, CV1704, CV30, CV475, CV487, CV75, CV864, CV874, CV881, CV890, CV892, CV893, CV897, CV898, CV899, CV929, CV930, CV931, CV937, CV939, CV953, CV958, CV967, CV969, CV975, CV978
ISOLATED FROM: Soil, air, Mites and Bracts from *Protea repens* infructescences, Stellenbosch, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 30–40 mm, moderately deep, radially and concentrically sulate, group C yellow margins; margins moderately deep, wide (2–3 mm), entire; mycelia white, yellowish in group C; texture floccose; sporulation moderately dense, sparse in group B, conidia *en masse* dull to greyish green (25D4–27D4–27D5); exudate mostly absent, clear in group A, soluble pigment absent, reverse pigmentation mostly greyish orange (5B5), some isolates brownish orange (7C7), greyish yellow (1B6) or white.

CYA, 5 °C, 7d: Sometimes germination.

CYA, 30 °C, 7d: Colonies 25–30 mm, all characters similar to CYA at 25 °C.

CYA, 37 °C, 7d: Colonies 4–11 mm, irregular; colors ranging from white to bluish grey (24C2) to pinkish grey (7C2); soluble pigment brown in some isolates, reverse pigmentation white to greyish green (1C3) to brownish orange (5C4) to dark brown (6F8).

MEA, 25 °C, 7d: Colonies 30–40 mm, group A 19–20mm, moderately deep, some isolates have yellow color in mycelia at margin; margins low to moderately deep, narrow to wide, somewhat irregular; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (25E4–27E4); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B4) at centre, greenish grey to greyish yellow to greyish green (1B2–1B4–1C4–1C2) elsewhere, some isolates olive brown to brown (4D5–5D5).

YES, 25 °C, 7d: Colonies 35–48 mm, moderately deep, radially and concentrically sulcate, random furrows also common, with light pinkish brown color in non-sporulating areas, some isolates yellowish at centre; margins difficult to determine; mycelia white; texture floccose; sporulation sparse, absent in some isolates, conidia *en masse* greyish green (25B3); exudate absent, soluble pigment some isolates brownish, some isolates absent, reverse pigmentation dark brown (6F8) and brownish orange (5C5) areas, mostly greyish to brownish orange (6B6–6C6), group B pale to light yellow (3A3–3A4)

G25N, 25 °C, 7d: Colonies 17–20 mm, low, lightly radially sulcate, brownish yellow color at centre, some isolates yellow at margin; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at centre, greyish green (1C3) elsewhere, margin white.

CREA, 25 °C, 7d: Colonies 13–20 mm, acid mostly not produced, very weak in some isolates.

Micromorphology — Conidiophores mostly biverticillate, with subterminal conidiophores common, terverticillate also present; stipes smooth to finely rough to distinctly rough walled, 200–800 × 3–4 µm; branches 10–38 × 3–4 µm; metulae 2–6, divergent, 60–130° [86±17.5°], 10–19 × 2–4 [13.2±1.76 × 3.4±0.38] µm, vesicle 4–8.5 [5.2±0.86] µm; phialides ampulliform, 8–22 per metula, 6.5–9.5 × 2.5–3.5 [7.5±0.6 × 2.9±0.2] µm; conidia finely rough to rough walled, spheroid, 2–2.5 × 2–2.5 [2.2±0.1 × 2.2±0.1] µm, average width/length = 0.98±0.02, n = 100.

Notes — *Penicillium fynbosense* is characterized by moderately deep, dense, floccose colonies. Conidiophores are typically divaricate. However, stipe walls range from smooth to finely rough to rough walled. Conidia are finely rough to rough walled. Metulae are often swollen at the apex, a character not observed in other Fynbos species found in this section. Morphological variation is reflected in the phylogeny and extrolite data. *Penicillium fynbosense* strains displayed intraspecies variation in morphological, sequence and preliminary extrolite data. This variation was, however, not congruent between the different data sets and as such, a rather broad species concept is applied. The new species is closely related to *P. janczewskii* and species previously reduced to synonymy by Pitt (1979). Faster growth on all media distinguishes this species from the *P. janczewskii* species complex.

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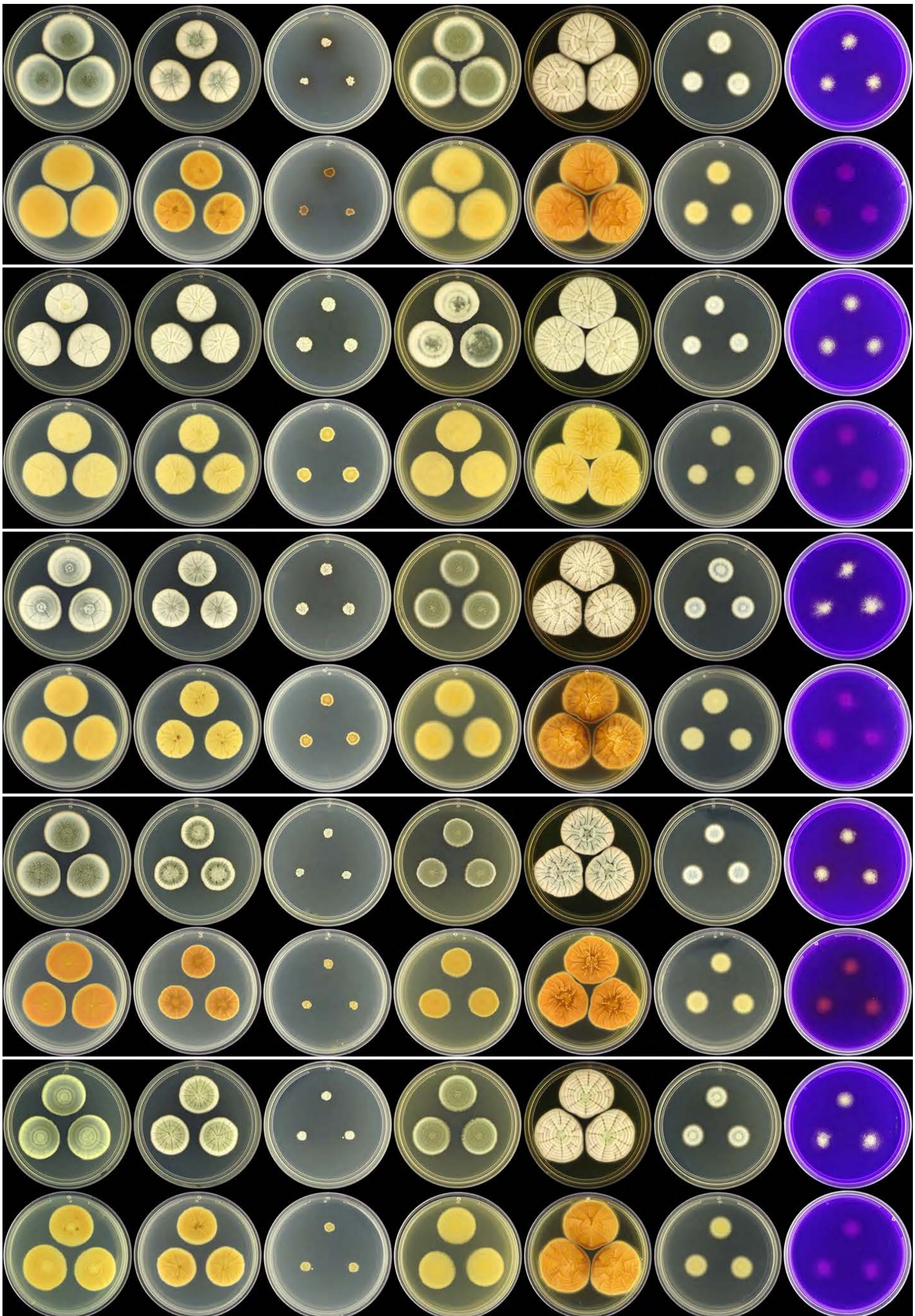

PLATE 71. *Penicillium fynbosense* strains representing different clades formed showing intraspecies variation. Colonies on CYA 25 °C, 30 °C, 37 °C, MEA, YES, G25N and CREA from left to right (top = obverse, bottom = reverse). a. CV30. b. CV475. c. CV899. d. CV931. e. CV969.

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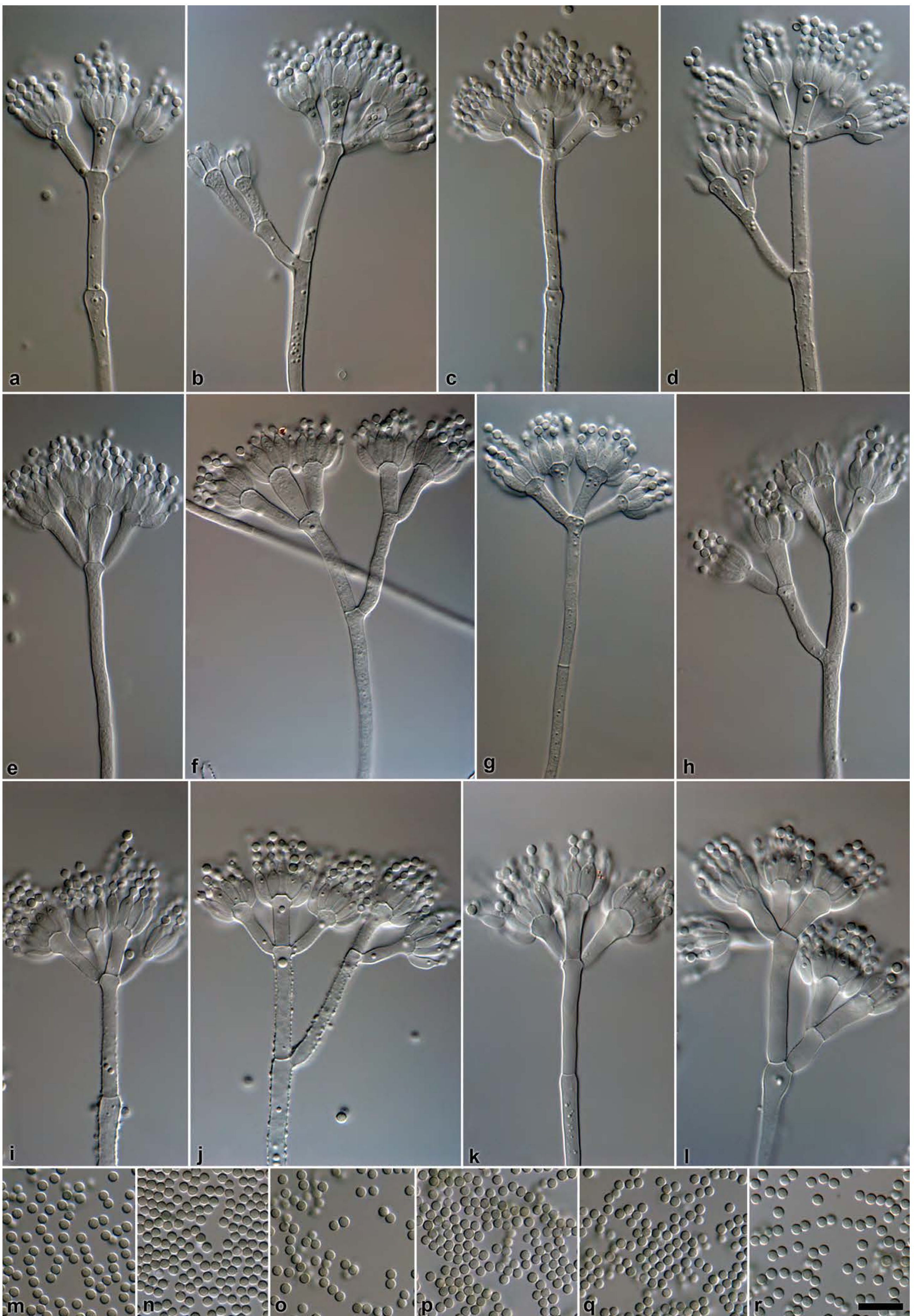

PLATE 72. *Penicillium fynbosense*. a–l. Conidiophores. m–r. Conidia. a, b. CV30. c, d. CV931. e, f. CV899. g, h. CV893. i, j. CV1704. k, l. CV475. m. CV30. n. CV931. o. CV899. p. CV893. q. CV1704. r. CV475 (— Scale bar in r = 10µm, applies to a–r).

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43. ***Penicillium novae-zeelandiae*** van Beyma

PLATES 73, 74, 83b

Antonie van Leeuwenhoek 6: 175. 1940.

EX-TYPE: CBS137.41 = NRRL2128
TYPE ISOLATED FROM: Apothecium of *Sclerotinia*, New Zeeland
SPECIMENS EXAMINED: CV117, CV129, CV1290, CV147, CV1560, CV1812, CV200, CV2029, CV2051, CV337, CV355, CV406, CV42, CV452, CV47, CV587, CV616, CV818, CV909, CV910, CV992
ISOLATED FROM: Soil, air, Mites and Bracts from *Protea repens* infructescences, Stellenbosch, Malmesbury and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 37–42 mm, low, very lightly radially sulcate, black sclerotia underneath colony produced inside media; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose, velutinous near margin; sporulation moderately dense, conidia *en masse* dull green (26E4) at centre, greyish turquoise (24D5) near margin; exudate clear, soluble pigment absent, reverse pigmentation brownish orange to yellow (5C5–5D7) to light yellow (4A5), fading into olive brown (4D7) near margin, black sclerotia easily visible.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 12–16 mm, sometimes up to 22 mm, some isolates only 5–6 mm, low, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture floccose with some velutinous conidiophores; sporulation moderately dense, conidia *en masse* dull to greyish green (26E4–26E5); exudate absent, soluble pigment brownish orange, reverse pigmentation brown (6E8) to brownish orange (5C5), mostly dominated by black sclerotia produced in media.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 38–46 mm, low, plane, black sclerotia sometimes produced inside media at point of inoculation; margins low, wide (3–4 mm), entire; mycelia white; texture floccose, with some velutinous; sporulation moderately dense, conidia *en masse* greyish to dull green (25C4–25E4); exudate absent, soluble pigment absent, reverse pigmentation yellowish white (2A2) at centre, fading into greyish yellow (2C3).

YES, 25 °C, 7d: Colonies 46–52 mm, low, randomly sulcate, black sclerotia produced inside media; margins low, wide (2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish to dark green (25E7–25F7); exudate absent, soluble pigment absent, reverse pigmentation pale to greyish yellow (4A3–4B5), although black sclerotia dominating reverse.

G25N, 25 °C, 7d: Colonies 18–25 mm, low, radially and concentrically sulcate; margins low, wide (2–3 mm), entire; mycelia white; texture floccose, with some velutinous; sporulation moderately dense, conidia *en masse* dull green (28F4) at centre, greyish green (26D5) near margin; exudate absent, soluble pigment very light yellow to brown sometimes produced, reverse pigmentation olive to dark brown (4F4–7F4) areas near centre, olive brown (4E4) to greenish white (28A2) elsewhere.

CREA, 25 °C, 7d: Colonies 18–30 mm, weak to moderate acid produced in some isolates.

Micromorphology — Conidiophores biverticillate, minor proportion terverticillate; stipes finely rough walled, 90–470 × 2.5–3.5 µm; branches when present 2, 15–50 × 2.5–3.5 µm; metulae 4–8, appressed to divergent, 38–85° [56±12°], 9.5–15 × 2–3.5 [12.2±1.3 × 2.7±0.3] µm, vesicle 2.5–5.5 [3.7±0.5) µm; phialides ampulliform, 6–10 per metula, 8–11 × 2–3.5 [9±0.7 × 2.7±0.3] µm; conidia smooth walled, spheroid to broadly ellipsoidal, 2–3 × 2–3 [2.6±0.1 × 2.6±0.1] µm, average width/length = 0.97±0.03, n = 64.

Notes — *Penicillium novae-zeelandiae* is characterized by strains that typically produce black sclerotia inside media, especially on CYA and YES. The species grows poorly at 30 °C. Conidiophores are rough walled and produce smooth walled conidia. The Fynbos strains resolved in two main clades, separate from the ex-type sequences (FIGURES 15, 16). However, morphologically and physiologically they are considered typical of *P. novae-zeelandiae*. It is also interesting to note that strains are consistently resolved in the same clades for all the genes studied. Acid production on CREA was also only observed in strains from one clade. This is, however, not considered sufficient to validate distinct species.

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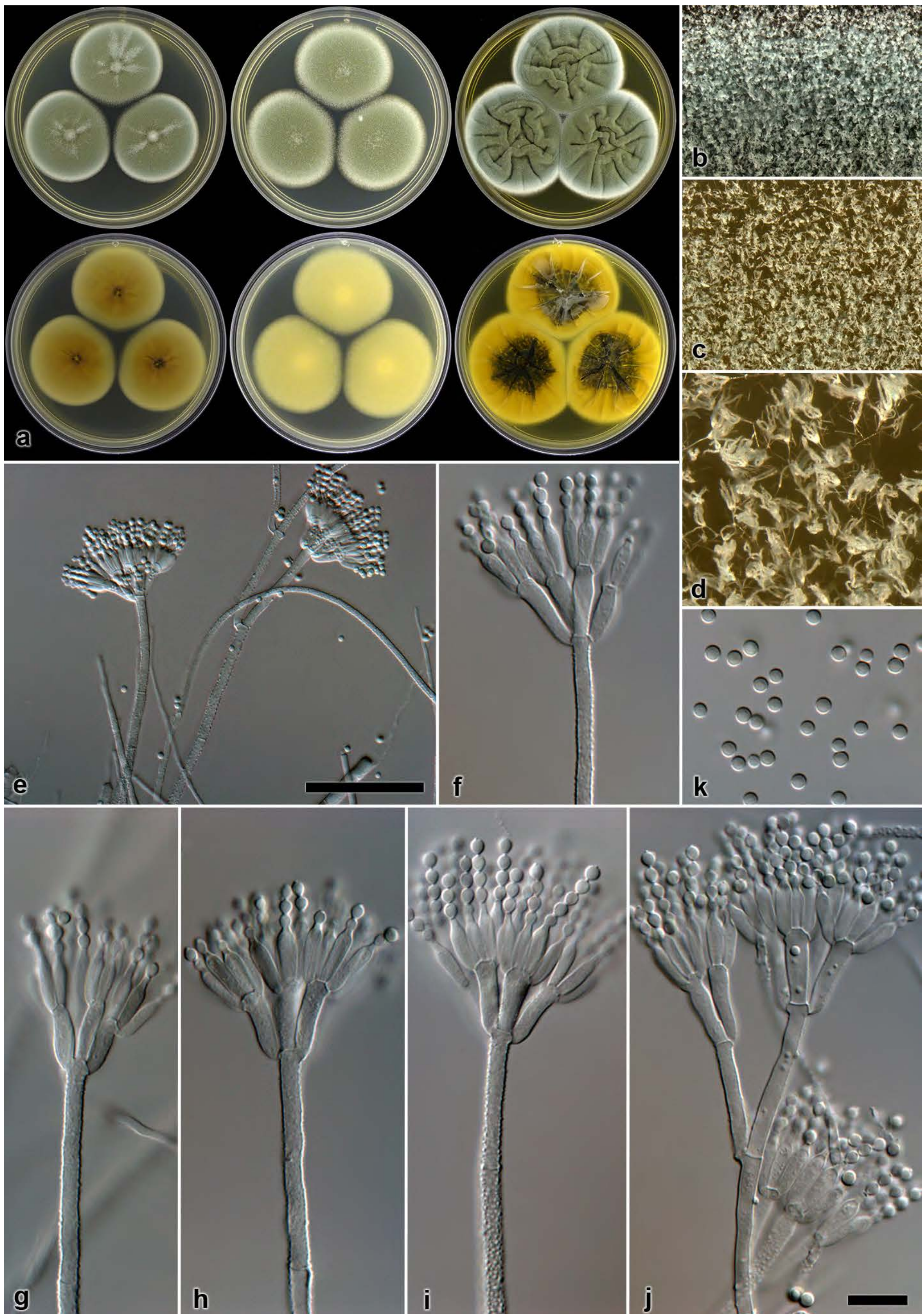

PLATE 73. *Penicillium novae-zeelandiae* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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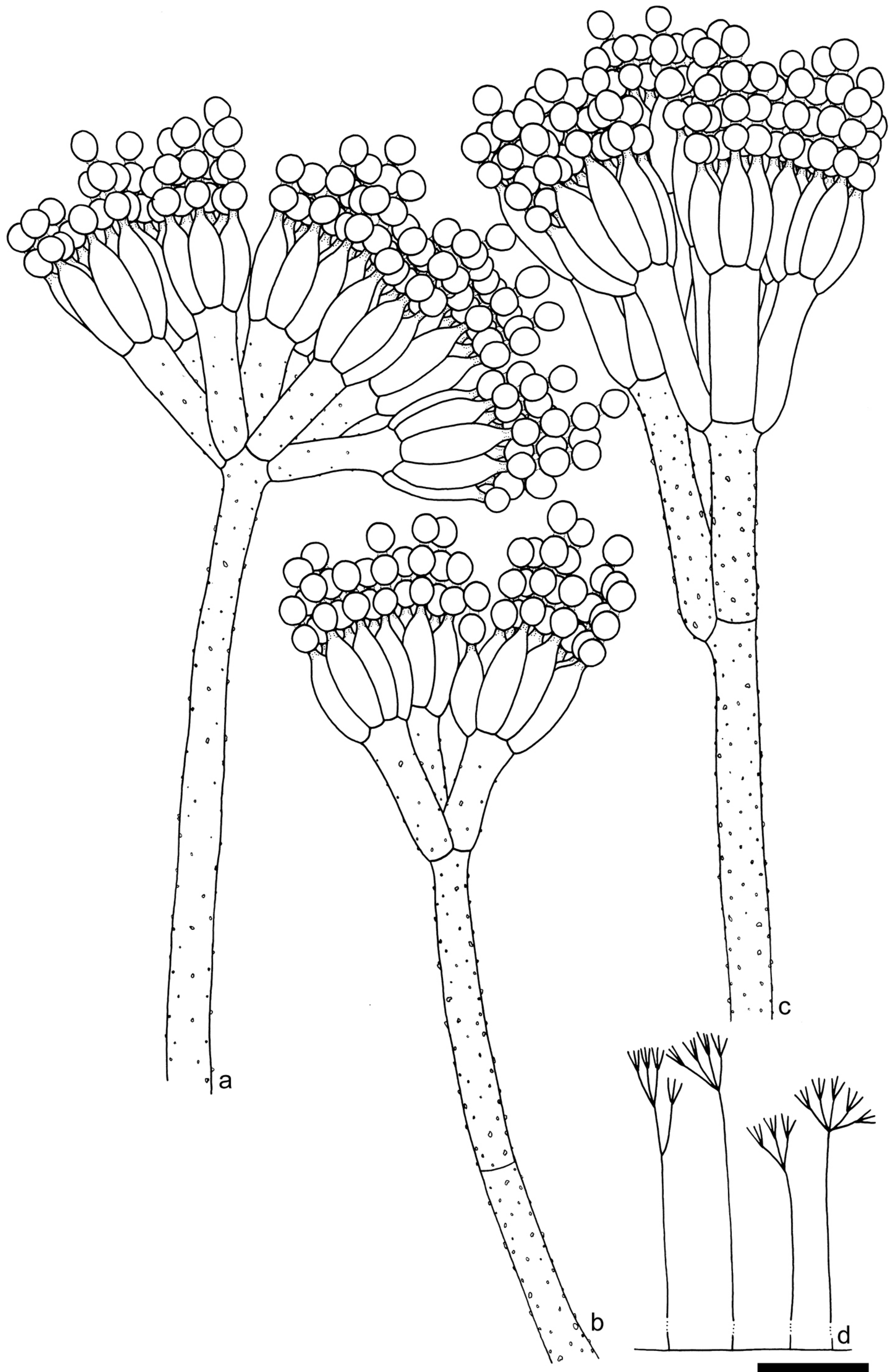

PLATE 74. Line drawing of *P. novae-zeelandiae*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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44. *Penicillium pseudoantarcticum* Visagie **prov. nom.** PLATES 75, 76, 83c

ETYMOLOGY: Reference to close resemblance to *P. antarcticum*
EX-TYPE: CV2189 = DTO183G7 = KAS4072 = DAOM241107
TYPE ISOLATED FROM: Mite from *Protea repens* infructescence, Struisbaai
SPECIMENS EXAMINED: CV2191
ISOLATED FROM: Mite from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 36–40 mm, moderately deep, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (25D5) at centre, greyish green (25C4) at margin; exudate absent, soluble pigment brownish orange, sometimes absent, reverse pigmentation greyish orange (5B5) at centre, elsewhere greyish yellow to brownish orange (4C5–5C5).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 20–25 mm, moderately deep, radially and concentrically sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture floccose; sporulation sparse to moderately dense at colony centre, conidia *en masse* greyish green (25B4–25C4); exudate absent, soluble pigment absent, reverse pigmentation golden (4C6) and greyish yellow (4B4) areas.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 36–40 mm, low, plane; margins wide (5 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (27E5–27E7); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (1A3) at centre, fading into greenish white (30A2).

YES, 25 °C, 7d: Colonies 48–50 mm, moderately deep, radially sulcate; margins low, narrow (2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (25D5–25E5–25E6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B5) centrally, pale to light yellow (3A3–3A4) margin.

G25N, 25 °C, 7d: Colonies 19–21 mm, low, very lightly concentrically sulcate; margins low, narrow, (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense near centre, conidia *en masse* dull green (26E4) to greyish green (26C3) near white margin; exudate absent, soluble pigment absent, reverse pigmentation greenish white (28A2–30A2).

CREA, 25 °C, 7d: Colonies 20–23 mm, acid produced in colony periphery.

Micromorphology — Conidiophores biverticillate, with subterminal stipes borne, similar branching seen in species of *Thysanophora*; stipes smooth to finely rough walled, 85–600 × 3–4 µm; branches 23–30 × 3–4 µm; metulae 4–6, appressed to divergent, 26–76° [49.9±10°], 11–15 × 3–4.5 [12.7±0.97 × 3.5±0.3] µm, vesicle 3.5–5 [4±0.37] µm; phialides ampulliform, 7–10 per metula, 8–10.5 × 2–4 [9.4±0.5 × 2.9±0.3] µm; conidia smooth walled, subspheroid, 2–3 × 2–3 [2.5±0.1 × 2.4±0.1] µm, average width/length = 0.95±0.03, n = 60.

Notes — *Penicillium pseudoantarcticum* typically produces fast growth on media. Conidiophores are biverticillate with stipes smooth to finely rough walled and produce smooth conidia. Sometimes conidiophores are borne subterminally. However, the subterminal conidiophore branch is not borne at an angle to the main stipe, rather the branch leading to the terminal conidiophore is borne at an angle, a character similar to conidiophores of *Thysanophora*. *Penicillium pseudoantarcticum* cannot be distinguished from *P. antarcticum* with morphology or extrolite data. However, phylogenetically it is distinct and resolved as a close relative with *P. atrovenetum* and *P. pseudoatrovenetum* (FIGURES 15, 16). Its faster growth rate on most media, especially CYA (36–40 mm) and MEA (36–40 mm), makes this species distinct from *P. atrovenetum* and *P. pseudoatrovenetum* (CYA 21–26 mm; MEA 21–24 mm). Also, *P. pseudoantarcticum* conidiophores and to some degree its colonies resemble that of *P. novae-zeelandiae*. However, faster growth at 30 °C (20–25 mm) and the lack of black sclerotia makes it distinct from *P. novae-zeelandiae* (12–16 mm).

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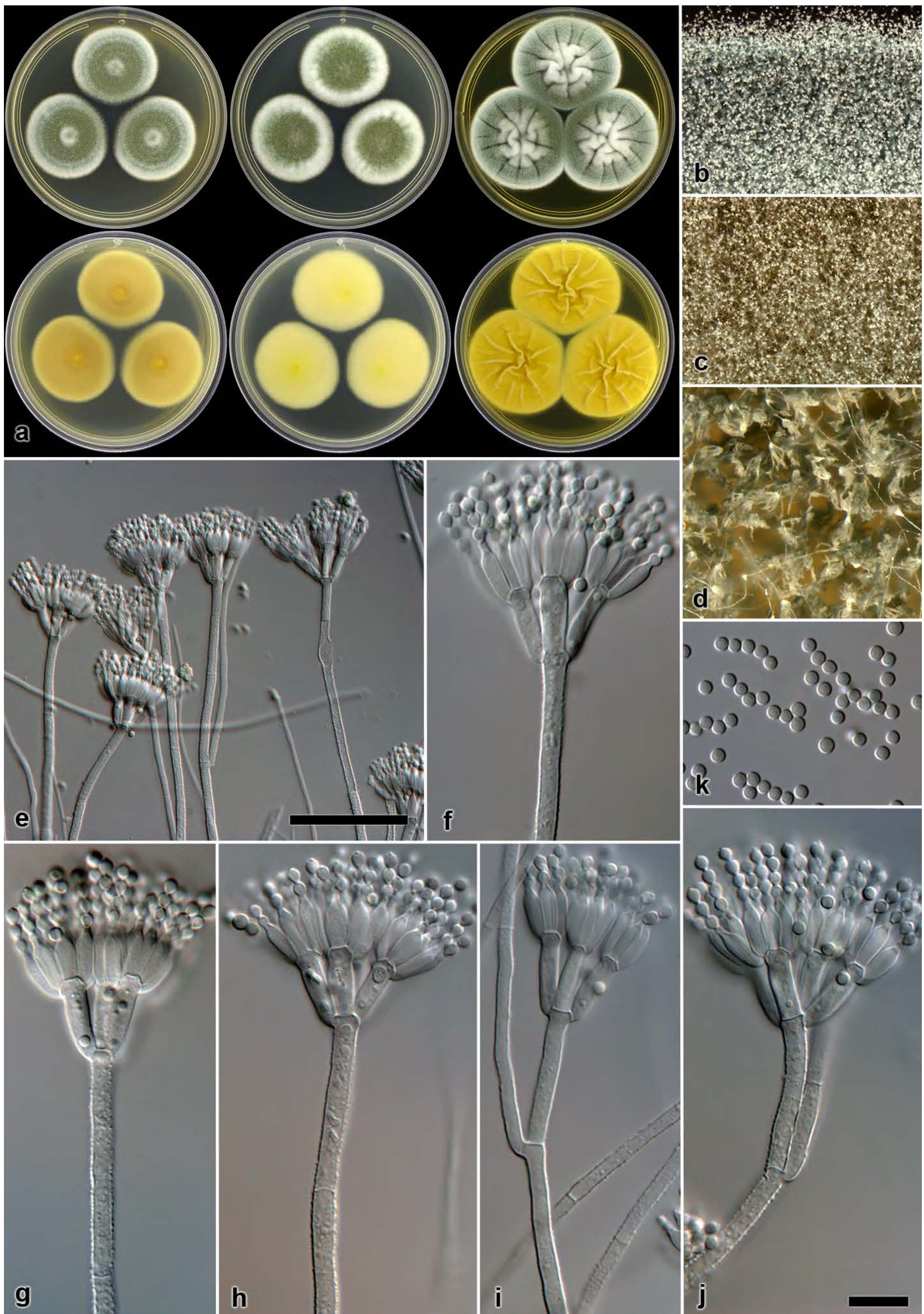

PLATE 75. *Penicillium pseudoantarcticum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 μm; — Scale bar in j = 10 μm, applies to f–k).

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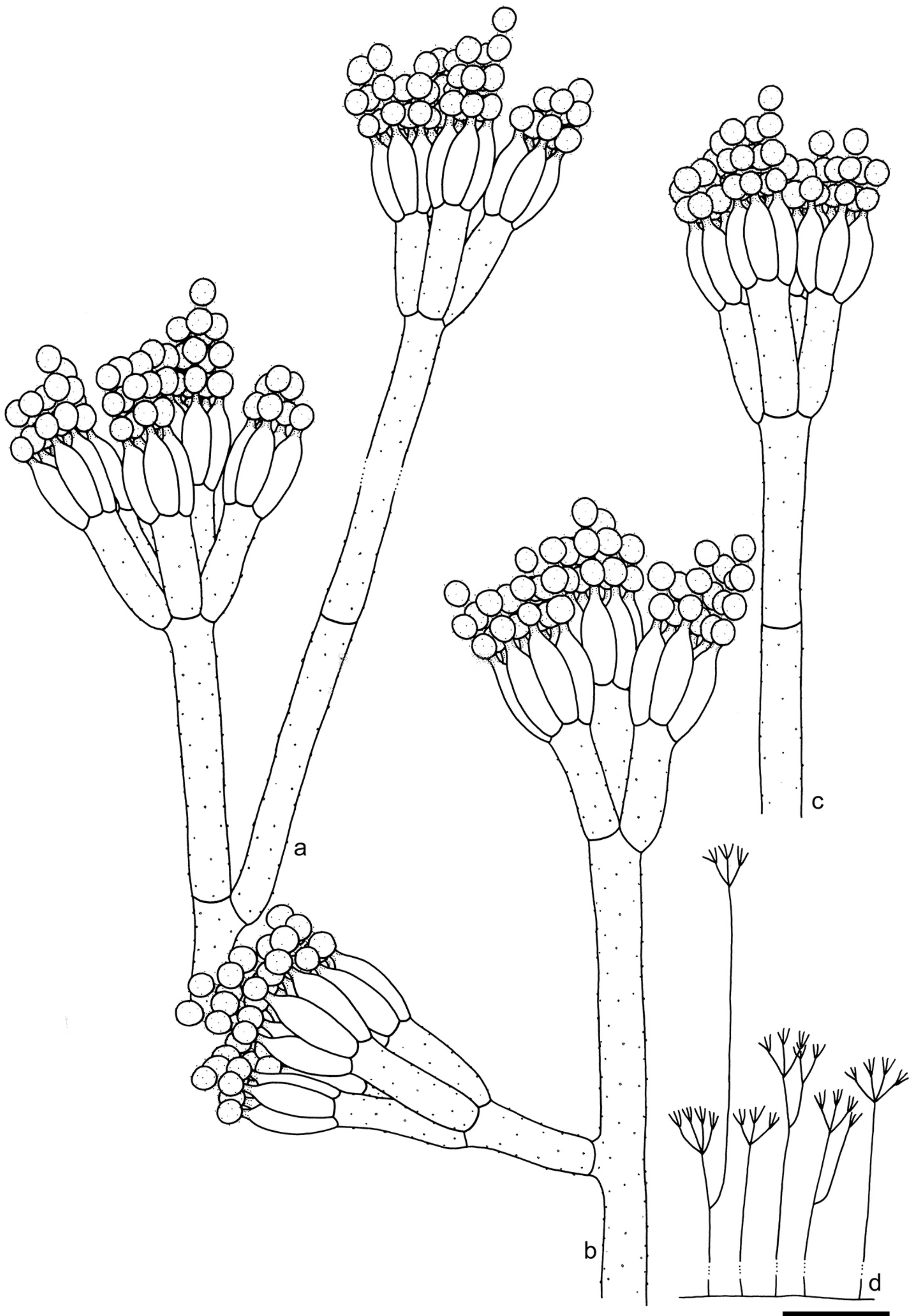

PLATE 76. Line drawing of *P. pseudoantarcticum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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45. ***Penicillium pseudoatrovenetum*** Visagie **prov. nom.** PLATES 77, 78, 83d

ETYMOLOGY: Reference to the species close resemblance to *P. atrovenetum*
EX-TYPE: CV1758 = DTO183D5 = KAS4033 = DAOM241106
TYPE ISOLATED FROM: Bract of *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 21–26 mm, low, radially sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25D5); exudate orange brown, soluble pigment orange brown, reverse pigmentation brown (5E5–5E7) at centre and margin, light brown (5D7) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 18–22 mm, low, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (25D5–25E5); exudate dark yellow, soluble pigment dark yellow, reverse pigmentation olive brown to brown (4E8–5E8) and dark yellow (4C8).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 21–24 mm, low, plane; margins low, very narrow (<1 mm), irregular; mycelia white; texture velutinous with some floccose mycelia present; sporulation moderately dense, conidia *en masse* greyish turquoise (24E4–24E6); exudate absent, soluble pigment yellow, reverse pigmentation greyish to olive yellow (2A6–2B6).

YES, 25 °C, 7d: Colonies 36–38 mm, low to moderately deep, radially and concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white near margin, yellowish centrally; texture floccose; sporulation sparse to moderately dense near margin, conidia *en masse* greyish turquoise (24B3–24C5); exudate yellow, soluble pigment absent, reverse pigmentation brownish yellow (5C7) in areas near centre, greyish yellow (4B5) elsewhere.

G25N, 25 °C, 7d: Colonies 19–21 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (27E4) at centre, greyish green (25D5) near margin; exudate absent, soluble pigment absent, reverse pigmentation light yellow (1A4) at centre, pale green to greyish green (29A3–29B3) elsewhere.

CREA, 25 °C, 7d: Colonies 21–23 mm, acid produced within colony limits.

Micromorphology — Conidiophores commonly biverticillate, minor proportion terverticillate; stipes very finely rough walled, 120–475 × 2.5–3.5 µm; branches when present 2, divergent, 20–65 × 2.5–3.5 µm; metulae 3–6, divergent, 42–79° [56±10°], 9.5–16 × 2.5–4 [12±1.4 × 3.1±0.3] µm, vesicle 3–4.5 [3.6±0.4) µm; phialides ampulliform, 5–7 per metula, 6.5–9 × 2.5–3.5 [7.9±0.6 × 2.9±0.2] µm; conidia spiny, spheroid, 2–3 × 2–3 [2.8±0.2 × 2.8±0.2] µm, average width/length = 0.97±0.02, n = 64.

Notes — *Penicillium pseudoatrovenetum* is distinguished by the copious amounts of dark yellow exudates and soluble pigments produced on CYA, MEA and YES. Conidiophores are mostly biverticillate, with only a minor proportion forming subterminal branches. This species is similar to *P. atrovenetum* based on morphology and extrolite data and cannot be distinguished using either of these criteria. However, multi-gene sequences resolves it as a distinct species and it is considered here as novel (FIGURES 15, 16). Its closest relatives, *P. pseudoantarcticum* and *P. antarcticum*, produces faster growth on most media, especially on CYA (21–26 mm) and MEA (21–24 mm).

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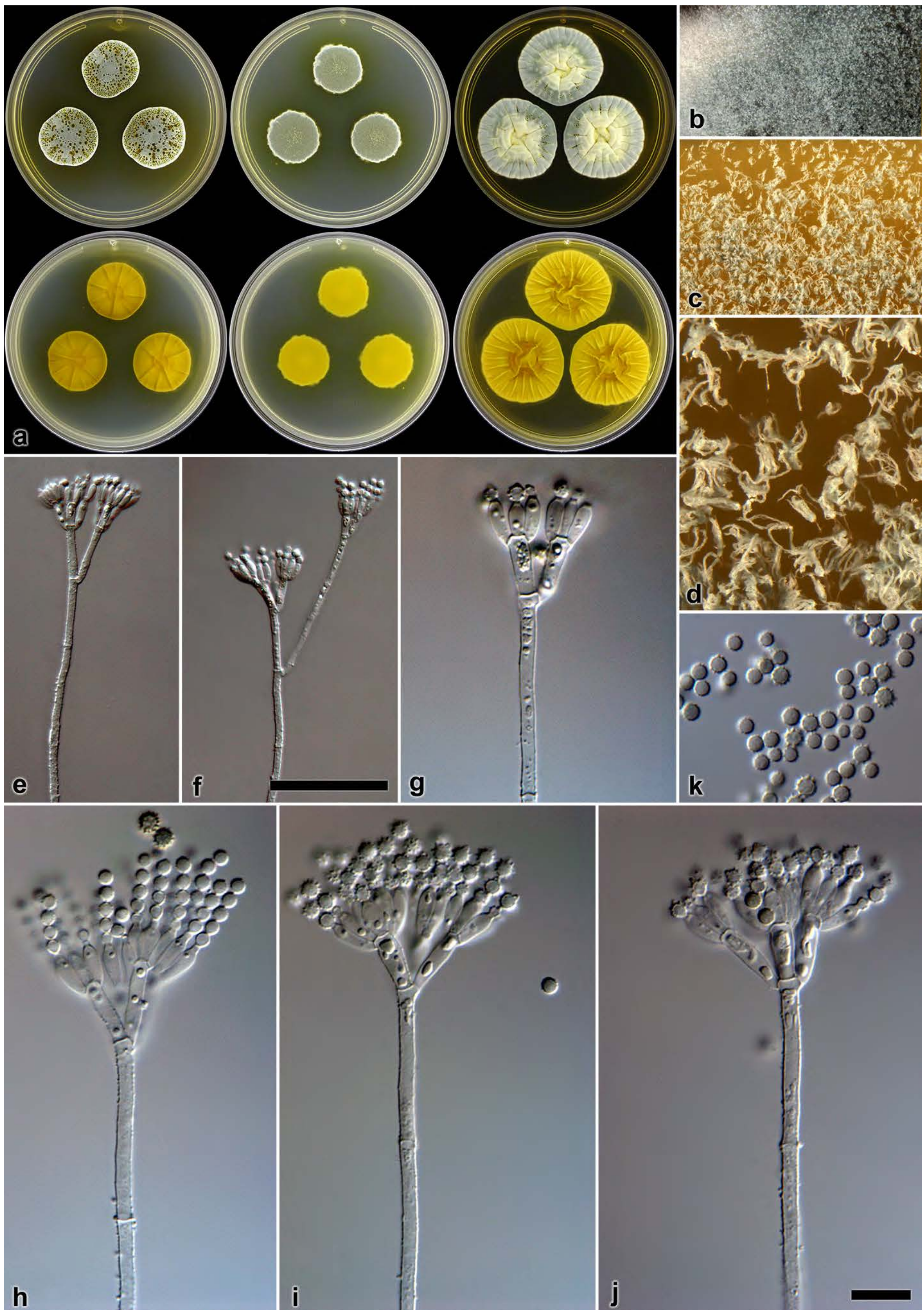

PLATE 77. *Penicillium pseudoatrovenetum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in f = 50 µm, applies to e, f; — Scale bar in j = 10 µm, applies to g–k).

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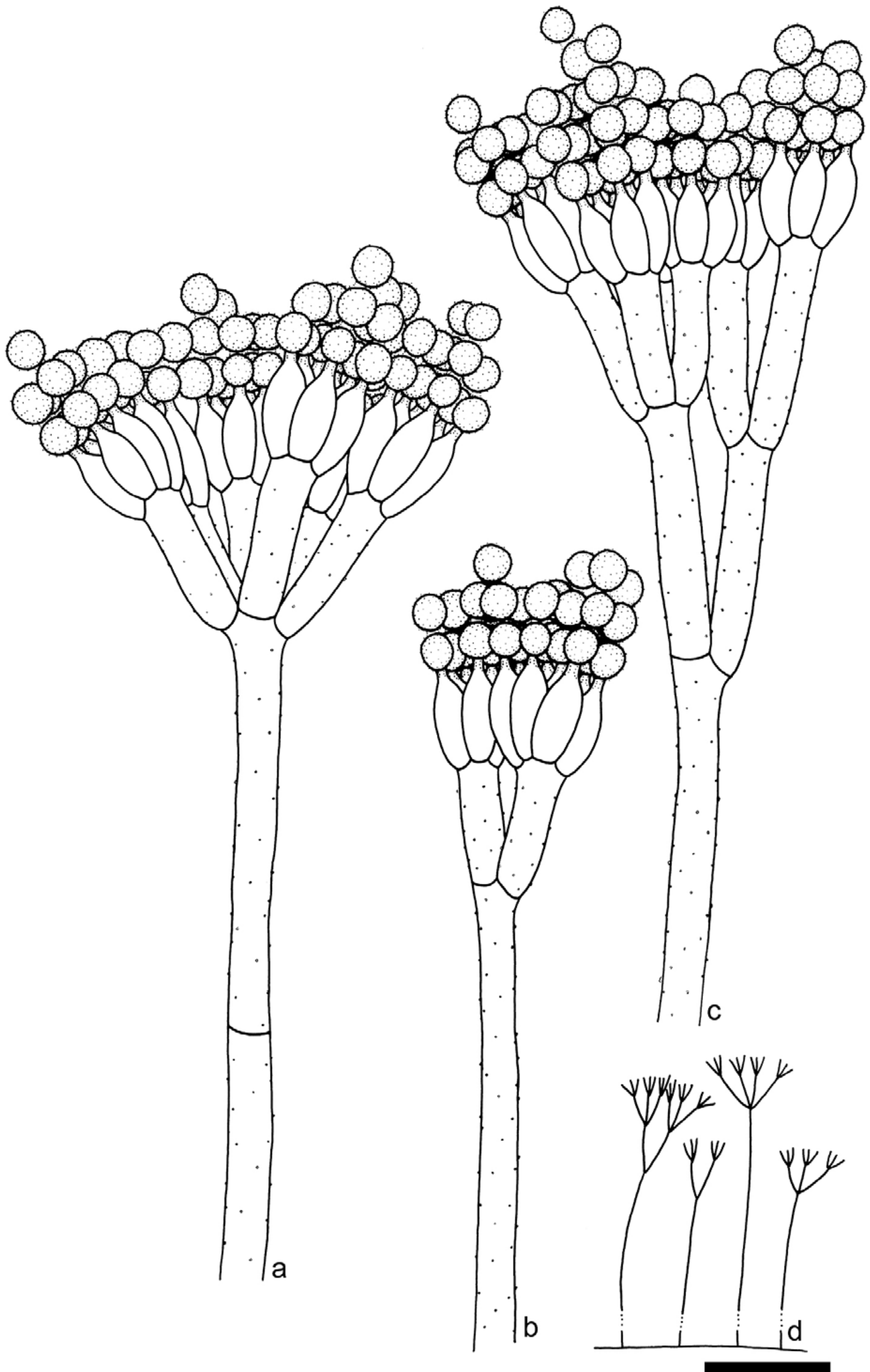

PLATE 78. Line drawing of *P. pseudoatrovenetum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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46. *Penicillium pseudocanescens* Visagie **prov. nom.** PLATES 79, 80, 83e

ETYMOLOGY: Reference to the species close resemblance to *P. canescens*

EX-TYPE: CV816 = DTO182A9 = KAS4179 = DAOM241111

TYPE ISOLATED FROM: Air, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 27–30 mm, moderately deep, radially and concentrically sulcate; margins low to moderately deep, narrow (1 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish to dull green (26B3–26D3); exudate clear abundant, soluble pigment very light brown visible when plate held against white background, reverse pigmentation brown (6E7) at centre, brown to yellowish brown (5C5–5D5) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 21–24 mm, moderately deep, radially sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture floccose; sporulation sparse to sometimes moderate, conidia *en masse* turquoise white (24A2); exudate yellowish, soluble pigment absent, reverse pigmentation light brown (6D6) and brownish orange (5C3–5C4) areas.

CYA, 37 °C, 7d: Colonies forming at point of inoculation, sometimes no germination, with dull green (29D3) color.

MEA, 25 °C, 7d: Colonies 18–21 mm, moderately deep, plane; margins low, narrow (1–2 mm), irregular; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (28D3–29D3); exudate absent, soluble pigment absent, reverse pigmentation light brown (6D6) at point of inoculation, greyish yellow (4B5–4C5) elsewhere.

YES, 25 °C, 7d: Colonies 38–41 mm, low, radially and concentrically sulcate; margins low, narrow, entire; mycelia white to almost beige color; texture floccose; sporulation sparse to moderately dense, conidia *en masse* pale green (26A3); exudate absent, soluble pigment brown, reverse pigmentation dark brown (6F8).

G25N, 25 °C, 7d: Colonies 16–20 mm, low to moderately deep, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation sparse, conidia *en masse* greyish green (25D5–25E5); exudate absent, soluble pigment brown, reverse pigmentation greyish yellow (4C4) at centre, greenish white to greenish grey (30A2–30B2) elsewhere.

CREA, 25 °C, 7d: Colonies 18–21 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, with a large number of subterminal branches that occur; Stipes smooth to very finely rough walled, 220–670 × 2.5–4 µm; branches 11–40 × 2.5–4 µm; metulae 3–6, divergent, 40–105° [77.8±15.5°], 8.5–17 × 2–3.5 [11.2±1.7 × 2.8±0.2] µm, vesicle 2.5–5 [3.6±0.5] µm; phialides ampulliform, 6–10 per metula, 5.5–8 × 2–3 [6.8±0.5 × 2.6±0.2] µm; conidia rough walled, spheroid, 2–2.5 × 2–2.5 [2.3±0.1 × 2.3±0.1] µm, average width/length = 0.97±0.02, n = 57.

Notes — *Penicillium pseudocanescens* typically produces floccose colonies on all media and shows restricted growth on MEA. Colony reverse pigmentation is mostly dark brown to brown. Conidiophores are divaricate biverticillate and have smooth to very finely rough walled stipes and produce rough walled conidia. *Penicillium canescens* and *P. radiatolobatum* are its closest relatives. However, *Penicillium canescens* (CBS340.48) produce rough walled stipes with smooth walled conidia. *Penicillium radiatolobatum* is distinguished based on faster growth rates on MEA and CYA at 30 °C and 37 °C, as well as yellow soluble pigments consistently produced on CYA, which is absent in this species.

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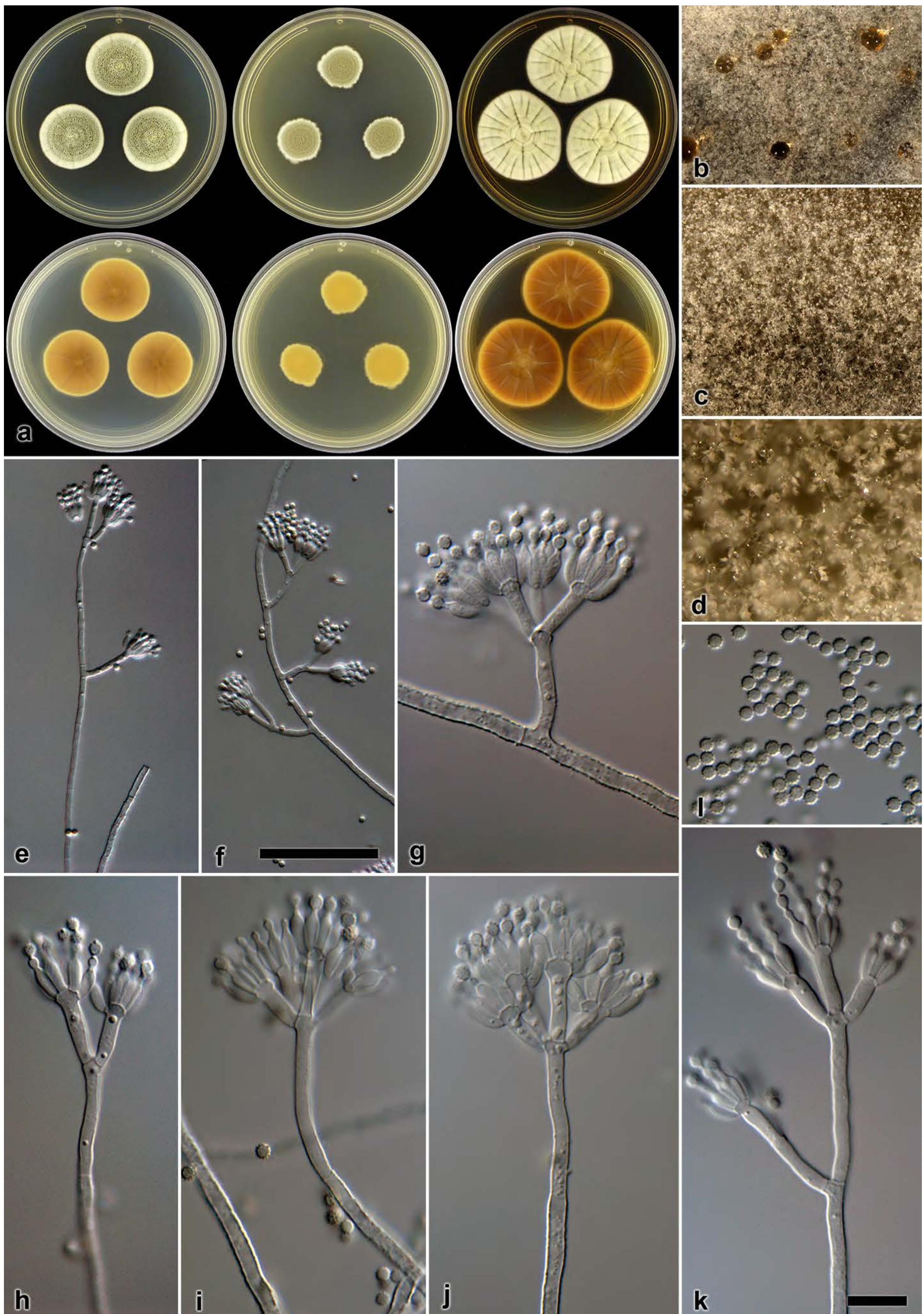

PLATE 79. *Penicillium pseudocanescens* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in f = 50 µm, applies to e, f; — Scale bar in k = 10 µm, applies to g–l).

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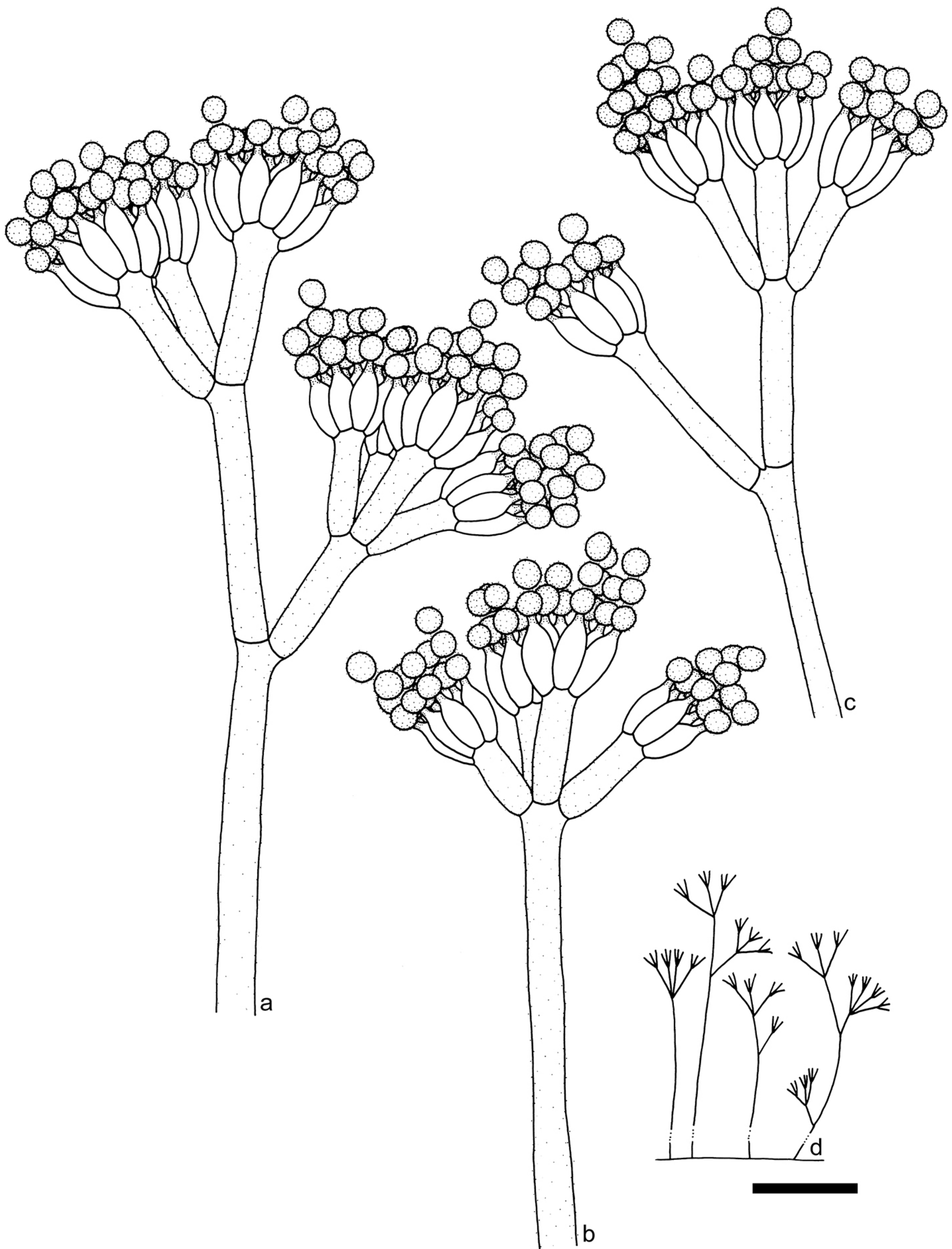

PLATE 80. Line drawing of *P. pseudocanescens*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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47. ***Penicillium radiatolobatum*** Lörinczi

PLATES 81, 82, 83f

Lucrarile Conferintei Nationale de Ştiinţe Solului, Bucuresti 10: 435. 1972.

EX-TYPE: CBS340.79
TYPE ISOLATED FROM: Soil, Romania
SPECIMENS EXAMINED: CV68, CV198
ISOLATED FROM: Soil, Bracts from *Protea repens* infructescences, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 27–31 mm, moderately deep, radially and concentrically sulcate; margins low to moderately deep, narrow (1 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish to dull green (25B4–25D4); exudate clear, soluble pigment yellow, reverse pigmentation greyish yellow (2B5–2C5).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 24–26 mm, moderately deep, radially sulcate; margins low, narrow (<1 mm), entire; mycelia white; texture floccose; sporulation sparse, conidia *en masse* greenish to turquoise white (24A2–25A2); exudate clear to yellowish, soluble pigment yellow, reverse pigmentation light yellow to yellow to greyish yellow (2A4–2A6–2B6).

CYA, 37 °C, 7d: Colonies 10–15 mm, having a peache to pink color; sporulation absent; soluble pigment yellowish, reverse pigmentation (4C6) at centre, light yellow (3A4) elsewhere.

MEA, 25 °C, 7d: Colonies 25–34 mm, moderately deep, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (25D3–25D4); exudate absent, soluble pigment absent, reverse pigmentation light yellow (4A4) at point of inoculation, greyish yellow (4B5–4C5) elsewhere, sometimes light brown (6D6) near centre.

YES, 25 °C, 7d: Colonies 40–45 mm, low to moderately deep, radially sulcate, colonies sometimes have a brownish to peach colour in mycelia; margins low, narrow (1 mm), entire; mycelia white; texture floccose; sporulation sparse to moderately dense areas, conidia *en masse* dull green (25E4) in dense areas, greyish green (25B4–25C4) elsewhere; exudate clear when produced, soluble pigment light brown, reverse pigmentation dark brown (7F8–8F8–9F8), fading into brown (7D7), with light yellow (4A4) margin, dark brown color sometimes lack.

G25N, 25 °C, 7d: Colonies 18–20 mm, low to moderately deep, radially and concentrically sulcate, having a greyish red to brownish orange colour in non-sporulating areas; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense at centre, conidia *en masse* greyish green (25D5–25E5); exudate absent, soluble pigment brown, reverse pigmentation greyish yellow (4B4) at centre and margin, reddish brown to olive brown (8E8–4E6) elsewhere.

CREA, 25 °C, 7d: Colonies 20–24 mm, weak acid produced in colony periphery.

Micromorphology — Conidiophores biverticillate, with a large number of subterminal branches that occur; stipes finely rough walled, 200–800 × 2.5–3.5 µm; Branches 9–53 × 2.5–3.5 µm; metulae 4–6, divergent, 55–100° [75±11°], 9–19 × 2.5–3.5 [11.6±1.4 × 2.9±0.3] µm, vesicle 3–5 [3.9±0.5] µm; phialides ampulliform, 8–13 per metula, 6–8.5 × 2–3.5 [7.1±0.5 × 2.6±0.2] µm; conidia rough walled, spheroid, 2–3 × 2–3 [2.4±0.17 × 2.4±0.19] µm, average width/length = 0.97±0.02, n = 53.

Notes — *Penicillium radiatolobatum* is characterized by strains that produce floccose colonies on all media and conidiophores that are typically divaricate biverticillate. Stipes are finely rough walled and it produces rough walled conidia. This character distinguishes it from *P. canescens*. Frisvad *et al*. (1990c) reduced *P. radiatolobatum* to synonymy with *P. canescens*. Although they are close relatives, *P. canescens* (CBS300.48) grows more restricted and does not produce the rough walled conidia.

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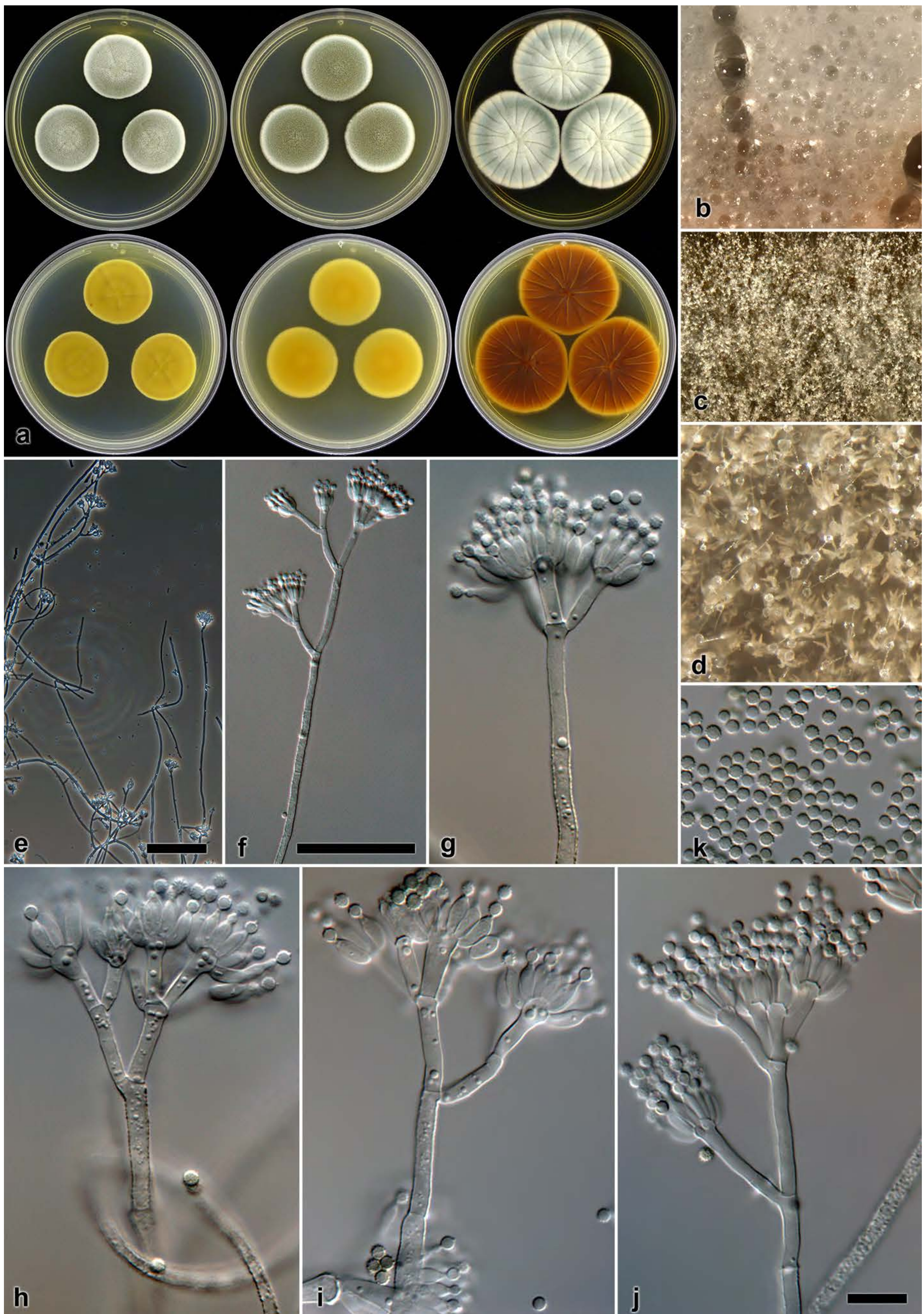

PLATE 81. *Penicillium radiatolobatum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in j = 10 µm, applies to g–k).

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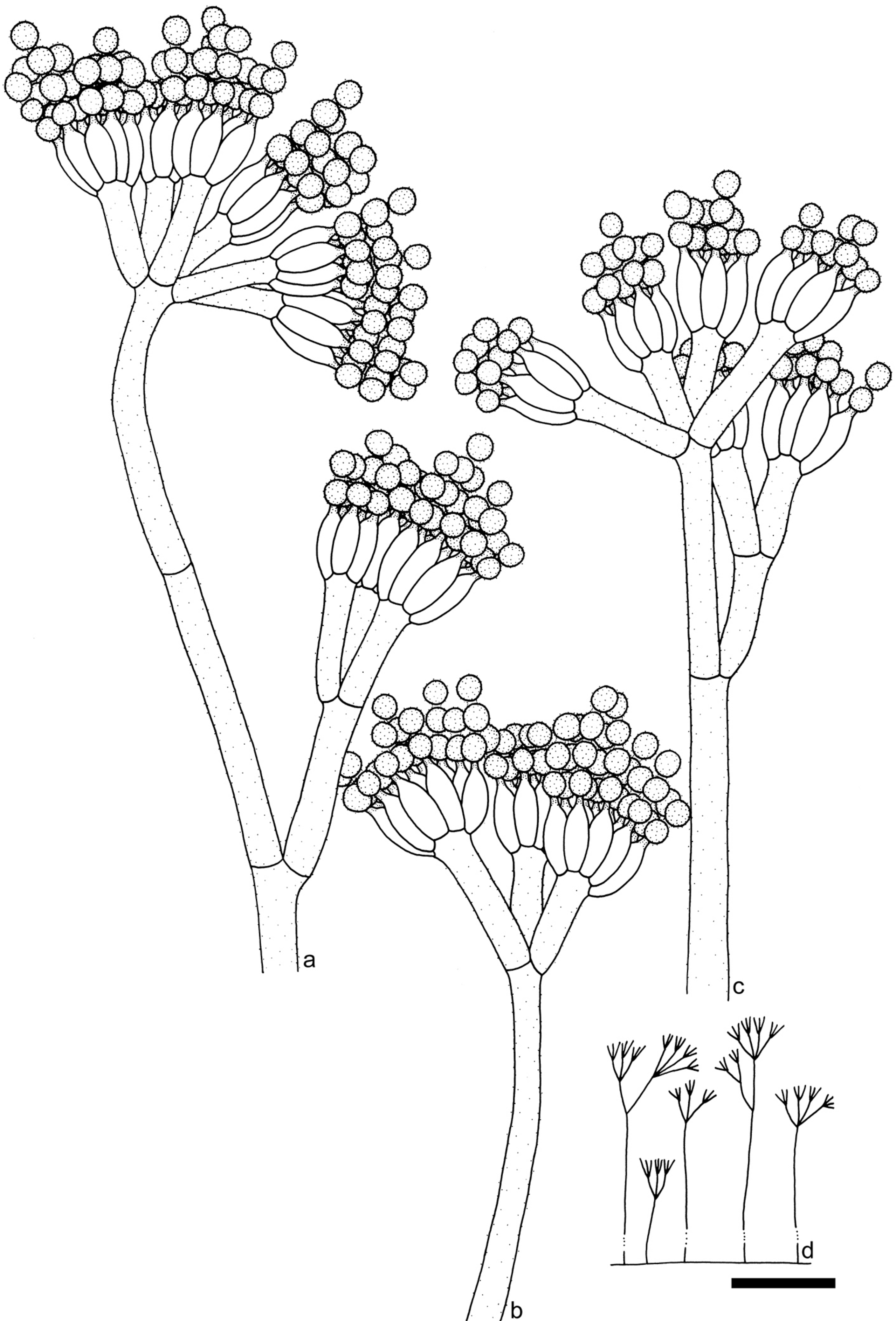

PLATE 82. Line drawing of *P. radiatolobatum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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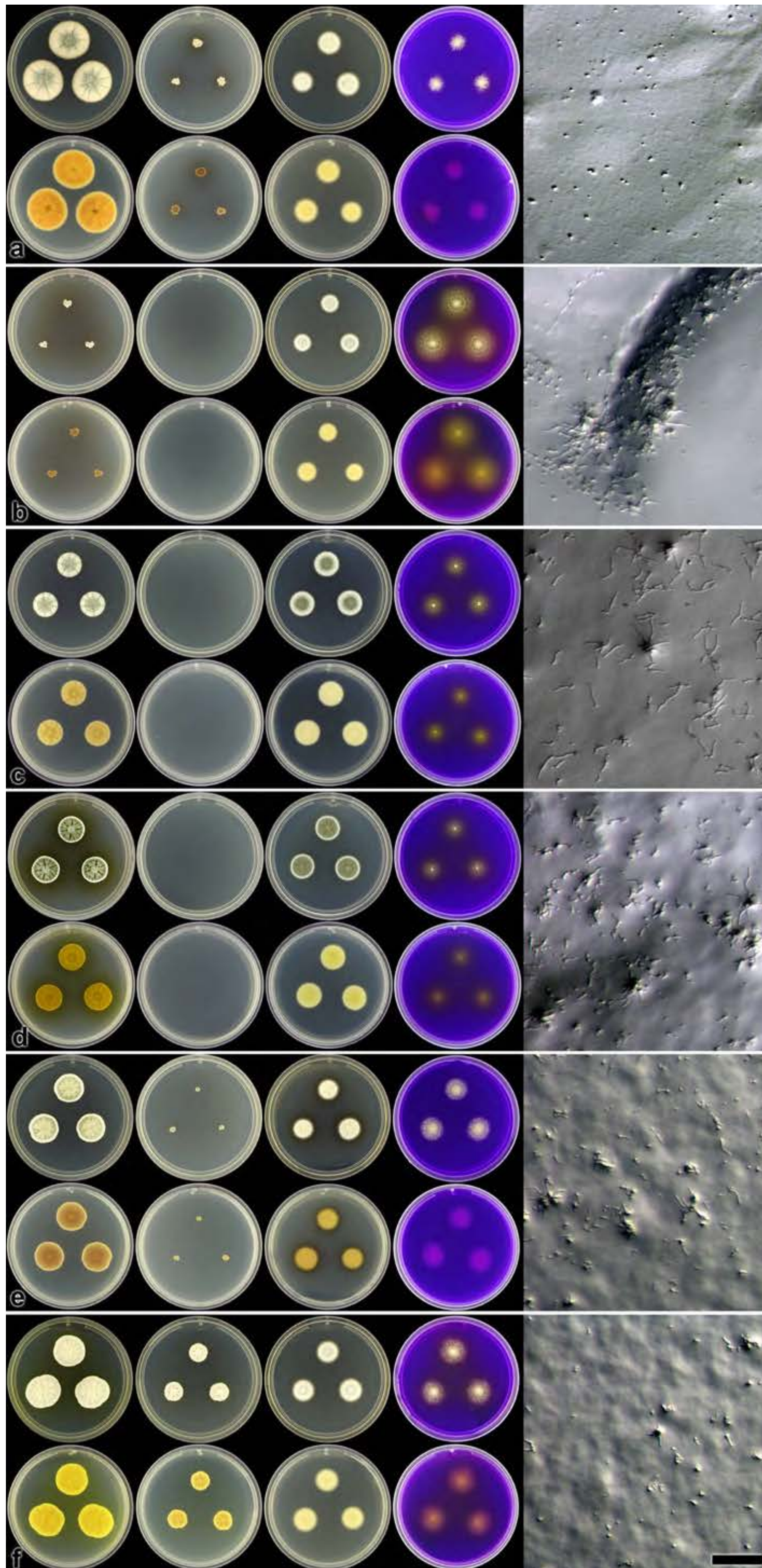

PLATE 83. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium fynbosense*. b. *P. novae-zeelandiae*. c. *P. pseudoantarcticum*. d. *P. pseudoatrovenetum*. e. *P. pseudocanescens*. f. *P. radiatolobatum*.

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The section *Torulomyces* (Delitsch) Stolk & Samson

Advances in *Penicillium* and *Aspergillus* systematics 169. 1985.

TAXONOMIC NOVELTIES: *Penicillium austricola* prov. nom.

SPECIES TREATED: *Penicillium parviverrucosum*-like

Delitsch (1943) introduced the genus *Torulomyces*, with *T. lagena* as generic type, for fungi that produce conidiophores with solitary swollen phialides borne on short stipes (Delitsch 1943, Barron 1967, Gams 1971, Stolk and Samson 1983, Ando *et al.* 1998, Seifert *et al*. 2011). Currently, MycoBank list eight species in the genus, all described from soil. Similar to other *Penicillium* species, conidia are linked together by disjunctors to form long chains (Gams 1971, Stolk & Samson 1983, Ando *et al.* 1998). Stolk & Samson (1983) went on to link *T. lagena* with its teleomorph, *Eupenicillium limoneum*. These observations suggested a close association with *Penicillium*. The genus *Monocillium* Saksena (1955) also produces conidiophores with solitary phialides and has often been confused with *Torulomyces*. *Torulomyces,* however, produces dry conidia compared to the slimy conidia of *Monocillium* (Seifert *et al*. 2011). In addition, *Monocillium indicum*, the generic type, has conidia connected by truncated ends in contrast to the disjunctors that connects the conidia of *T. lagena* (Domsch *et al*. 1980). The distinct nature of *Monocillium* and *Torulomyces* is supported by sequence data that resolves *M. indicum* in the Hypocreaceae (Sigler *et al*. 2010).

Based on the *E. limoneum* and *T. lagena* teleomorph-anamorph association, Stolk & Samson (1985) transferred *Torulomyces* to *Penicillium* and proposed a new sectional taxonomic scheme for *Penicillium* anamorphs, including a section *Torulomyces* to accommodate this transfer. Pitt & Hocking (1985) did not accept the transfer of *T. lagena* as a synonym of *Penicillium* and argued that it does not fit the generic concept of *Penicillium* by any modern author, because *T. lagena* produces a conidiophore with a solitary phialide, which does not resemble a penicillus. The transfer was also not accepted in the list of current names in use for the *Trichocomaceae* (Pitt & Samson 1993, Pitt *et al*. 2000). However, based on the phylogenetic position of *T. lagena*, Houbraken & Samson (2011) transferred the name to *P. lagena* and reduced *Torulomyces* as a synonym of *Penicillium*. In their reclassification, they included seven species in the section *Torulomyces*. New combinations for *Penicillium laeve* (= *T. laeve*)*, P. ovatum* (= *T. ovatus*)*, P. parviverrucosum* (= *T. parviverrucusos*) and *P. porphyreum* (= *T. brunneus*) from Ando *et al*. (1998) were proposed. Houbraken & Samson (2011) also included *P. cryptum* and *P. lassenii* in the section, although they do not produce monophialidic conidiophores. *Torulomyces macrosporus* was not transferred, since based on the Matsushima (1987) protologue, Ando *et al*. (1998) argued that it might belong in *Monocillium*. Also, *T. viscosus* Delitsch (1943) has no type material available and the description was not considered sufficient for determining the placement of the species (Stolk & Samson 1983, Houbraken & Samson 2011). As such, *T. viscosus* is not listed in the current names in use in the *Trichocomaceae* list (Pitt & Samson 1993, Pitt *et al*. 2000).

In this study, a number of strains resembling *Torulomyces* were isolated. Strains were mostly isolated at the Malmesbury and Struisbaai sites. *Torulomyces* is generally considered to be a soil borne fungus. Interestingly, however, the isolates studied here were not only obtained from soil, but also from the *Protea repens* infructescences and mites. Morphologically the strains represented two species, which was confirmed by the phylogenetic analysis (FIGURE 17). The two species seem to be geographically isolated with *P. parviverrucosum*-like mainly isolated from Malmesbury and *P. austricola* from Struisbaai, with the exception of a single strain (CV1851) of *P. parviverrucosum*-like*.*

Unfortunately, species comparisons between the two Fynbos species and those described by Ando *et al*. (1998) were not possible, as the original type material could not be obtained for these species. Comparisons were thus based on the descriptions of Ando *et al*. (1998). However, comparisons were found to be difficult since these species are morphologically very similar. They grow slowly on media and produce monophialidic conidiophores with similar dimensions. Ando *et al*. (1998) proposed the use of conidial shape, surface texture and size to be of taxonomic significance. The rough walled, globose conidia of the two Fynbos species suggest *P. lagena, P. brunneus* and *P. parviverrucosum* as morphologically similar species. However, *P. lagena* and *P. porphyreum* are phylogenetically distinct from the Fynbos strains. Based on the *P. parviverrucosum* description, Fynbos strains had similar conidial dimensions as well as producing the brown soluble pigment and similar growth rates reported by Ando *et al*. (1998). Since material for the previously described species could not be obtained, this species are considered here as a *P. parviverrucosum*-like species. *Penicillium austricola* is considered distinct from both these species based on its even slower growth rate, lighter colored conidial masses and the lack of colors in soluble pigments and reverse pigmentations.

Based on the findings, it is clear that this section needs revision. However, the lack of reference material from the Ando *et al*. (1998) study will remain an obstacle. Despite these difficulties, a

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number of *P. lagena* cultures deposited in the CBS collection were included in this study and data suggest some of these strains may represent new species (Houbraken & Samson 2011).

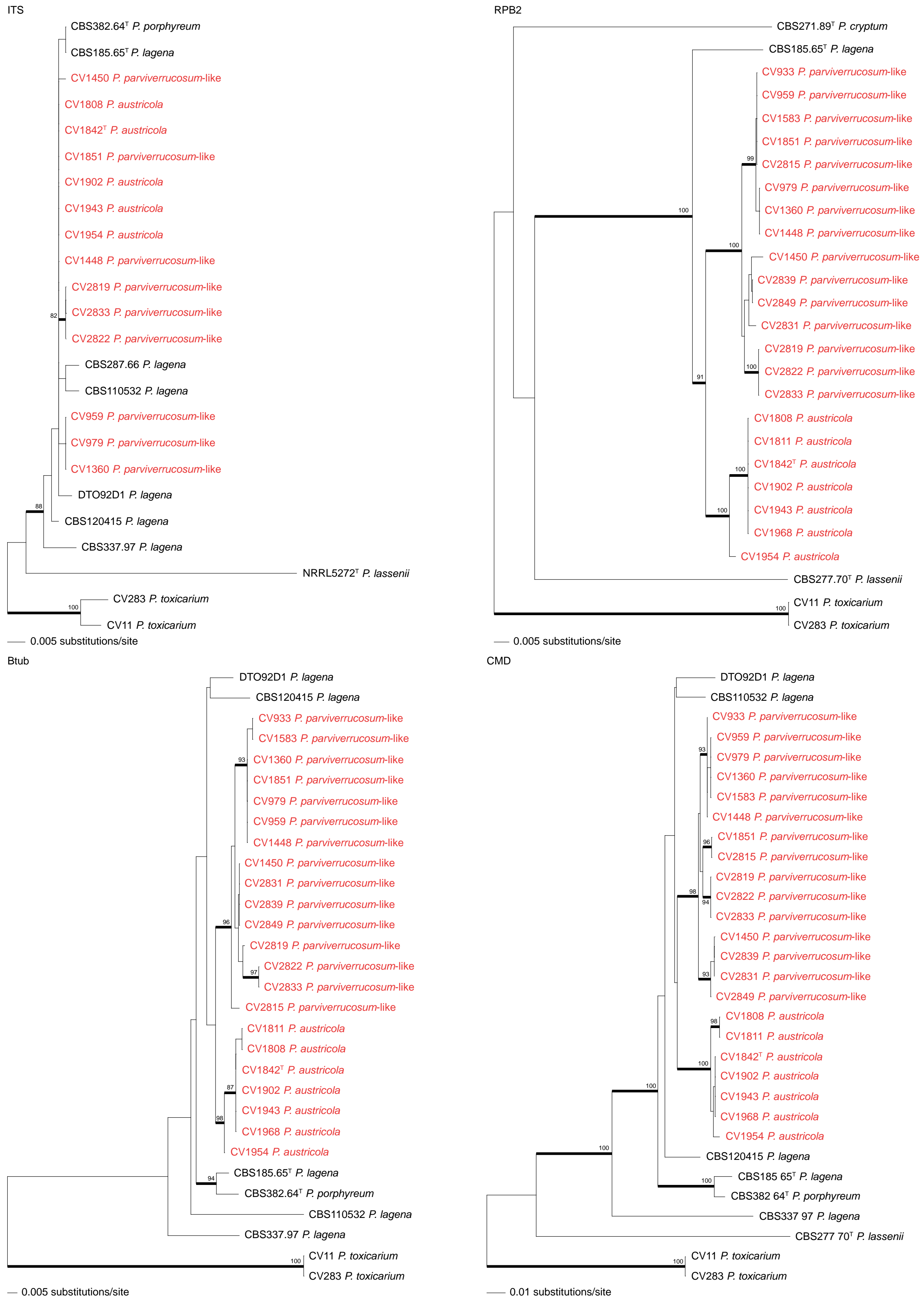

FIGURE 17: Phylogenetic trees based on ITS, RPB2, β-tubulin and Calmodulin, showing relationship of species in the section *Torulomyces*. *Penicillium toxicarium* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type). Colored names indicate strains isolated from Fynbos.

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48. *Penicillium austricola* Visagie **prov. nom.** PLATES 84, 86a

ETYMOLOGY: Latin, *austricola* = meaning resident from the south; named after this species that was isolated from the most southern point of Africa
EX-TYPE: CV1842
TYPE ISOLATED FROM: Mite, *Protea repens*, Struisbaai, South Africa
SPECIMENS EXAMINED: CV1808, CV1811, CV1902, CV1943, CV1954, CV1968
ISOLATED FROM: Mites and bracts from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 8–14 mm, deep, crateriform, forming cell-like compartments in colony; margins low, narrow, irregular; mycelia white; texture floccose to loosely funiculose; sporulation sparse, conidia *en masse* greyish green (25C4); exudate absent, soluble pigment absent, reverse pigmentation greyish green (25E4).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 7–12 mm, irregular colonies with a greenish grey (1C2) color; sporulation sparse; reverse pigmentation olive (3D4).

CYA, 37 °C, 7d: Microcolonies.

MEA, 25 °C, 7d: Colonies 12–13 mm, low, lightly sulcate; margins low, narrow, regular; mycelia white; texture floccose to loosely funiculose; sporulation sparse to almost moderately dense, conidia *en masse* greyish turquoise to dull green (24D4–25D4) near centre, becoming lighter near margin, with a light greyish green (25E6) ring near margin; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3C3–3C5).

YES, 25 °C, 7d: Colonies 12–15 mm, very deep, forming cell like compartments in colony; margins low, narrow, regular to irregular; mycelia white; texture floccose to loosely funiculose; sporulation sparse, conidia *en masse* dull green (25D4); exudate absent, soluble pigment absent, reverse pigmentation olive (2E5–2E7) to greyish orange (5B6).

G25N, 25 °C, 7d: Colonies 4–6 mm, consisting out of white mycelia; sporulation mostly absent; reverse pigmentation white.

CREA, 25 °C, 7d: Colonies 4–6 mm, no acid produced.

Micromorphology — Conidiophores monophialidic; stipes smooth walled, short 4–11 × 1–1.5 µm; phialides ampulliform, often swollen creating lobside or two bulges, 5–8 × 2–3 µm; conidia rough walled to almost spiny, spheroid, 1.5–2 × 1.5–2 [2.1±0.09 × 2.1±0.1] µm, average width/length ± stdev = 0.98±0.02, n = 22.

Notes — *Penicillium austricola* is characterized by restricted growth on all media. Conidiophores are monophialidic and produce spheroid, rough walled conidia. The new species is morphologically distinct from its closest relative *P. parviverrucosum*-like. The new species produces lighter, dull green conidia and lacks the brown reverse colors produced by *P. parviverrucosum*. The new species also displays consistent slower growth on CYA at 37 °C (microcolonies vs. 8–14 mm) and on average had larger conidia (2.1±0.09 × 2.1±0.1 vs. 1.8±0.1 × 1.8±0.08 µm).

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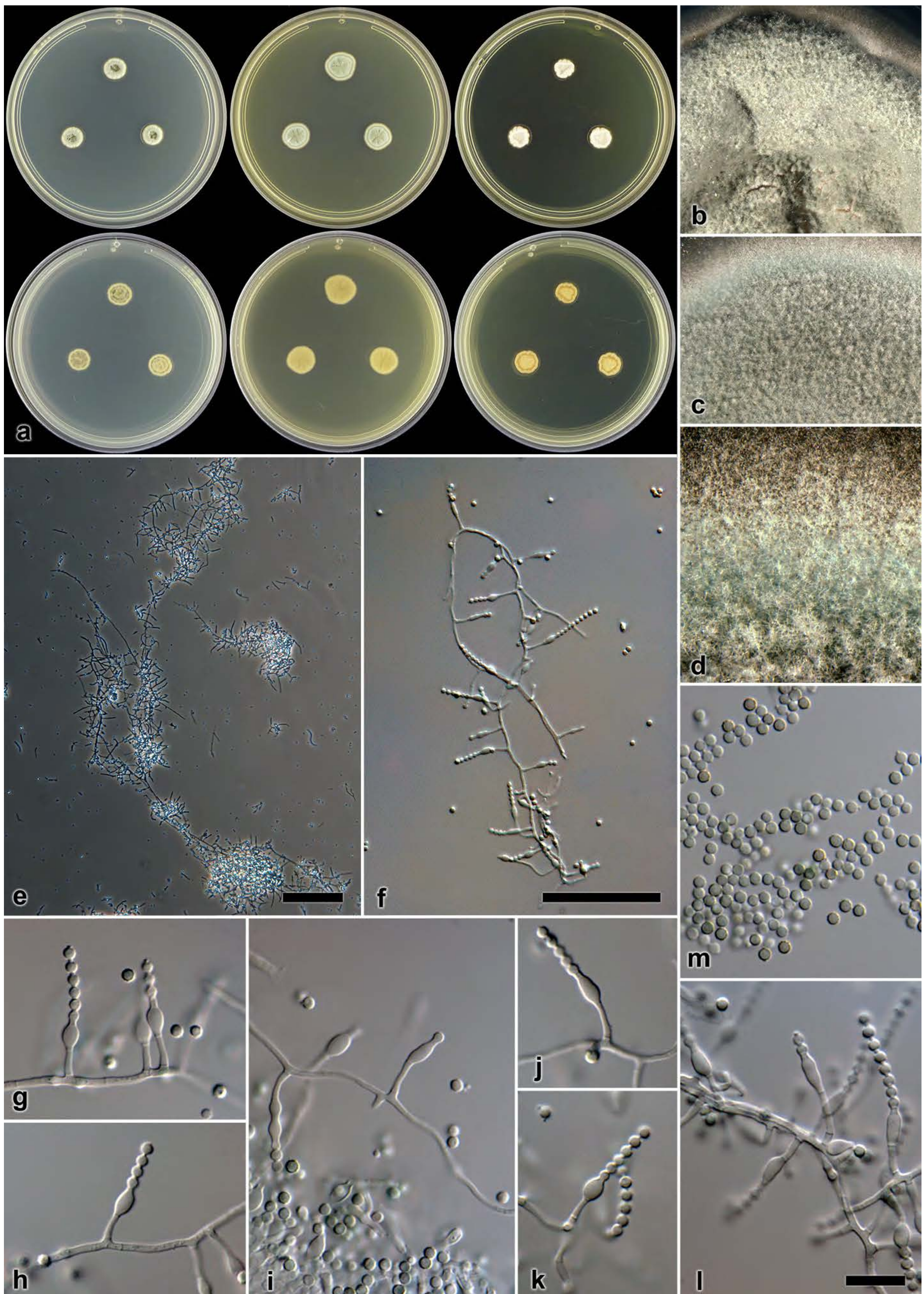

PLATE 84. *Penicillium austricola* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–l. Conidiophores. m. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in l = 10 µm, applies to g–m).

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49. *Penicillium parviverrucosum*-like (Ando & Pitt) Houbraken & Samson PLATES 85, 86b
Studies in Mycology 70: 48. 2011.

BASIONYM: *Torulomyces parviverrucuosus* Ando & Pitt (1998. Mycoscience 39: 317)
EX-TYPE: KY12720
TYPE ISOLATED FROM: Soil, Gap Park, Sydney, Australia
SPECIMENS EXAMINED: CV933, CV959, CV979, CV1360, CV1448, CV1450, CV1583, CV1851, CV2815, CV2819, CV2822, CV2831, CV2833, CV2839, CV2849.
ISOLATED FROM: Soil, Malmesbury; Mites and bracts from *Protea repens* infructescence, Malmesbury; Isolate CV1851 from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 8–10 mm, moderately deep, plane; margins low, very narrow, regular to irregular; mycelia white; texture floccose to somewhat funiculose; sporulation sparse, conidia *en masse* pale turquoise to greyish turquoise (24A3–24B3); exudate absent, soluble pigment brownish orange, reverse pigmentation dark brown (6F7) in some isolates, brown in others (6E6–6E7).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 8–14 mm, irregular colonies with turquoise grey to greyish turquoise (24B2–24B3) color; sporulation sparse; reverse pigmentation dark brown (5F8) to almost a blackish brown.

CYA, 37 °C, 7d: Colonies 7–10 mm, primary colony at centre surrounded by clear zone followed by secondary colony; sporulation sparse to moderate, conidia *en masse* pale turquoise to greyish turquoise (24A3–24B3); reverse pigmentation olive brown (4E5–4F5).

MEA, 25 °C, 7d: Colonies 11–14 mm, low to moderately deep, plane; margins low, narrow, regular; mycelia white; texture floccose, to funiculose; sporulation sparse, conidia *en masse* greyish turquoise (24C3) fading into a lighter (24B5) near margin; exudate minute droplets sometimes present, soluble pigment brown, reverse pigmentation yellowish brown (5E5) at centre, brownish orange (5C4) elsewhere, some isolates olive (3F3), pale yellow (4B3) at margin.

YES, 25 °C, 7d: Colonies 9–13 mm, moderately deep, crateriform; margins moderately deep, narrow, irregular; mycelia white; texture somewhat floccose, but mostly funiculose; sporulation sparse, conidia *en masse* greyish green (25B3–25C3) to greenish white (25A2); exudate absent, soluble pigment brownish orange, reverse pigmentation olive brown (4F5) at centre, greyish yellow (4C6–4D6) near margin.

G25N, 25 °C, 7d: Colonies 4–6 mm, consisting out of white mycelia; sporulation mostly absent; reverse pigmentation white.

CREA, 25 °C, 7d: Colonies 4–6 mm, no acid produced.

Micromorphology — Conidiophores monophialidic; stipes smooth walled, short 3.5–10 × 1–2 µm; phialides ampulliform, often swollen creating lobside or two bulges, 4–8 × 2–3 µm; conidia rough walled, spheroid, 1.5–2 × 1.5–2 [1.8±0.1 × 1.8±0.08] µm, average width/length ± stdev = 0.98±0.01, n = 35.

Notes — The true identity of this species is unclear, but is morphologically identical to the *P. parviverrucosum* description of Ando *et al*. (1998). The species produce colonies with brownish soluble pigments and monophialidic conidiophores with rough walled spheroid conidia, which makes it distinct from previously described species. Sequence data from the ex-type culture are considered necessary to confirm this species identity. Without it though, strains are identified here as *P. parviverrucosum*-like.

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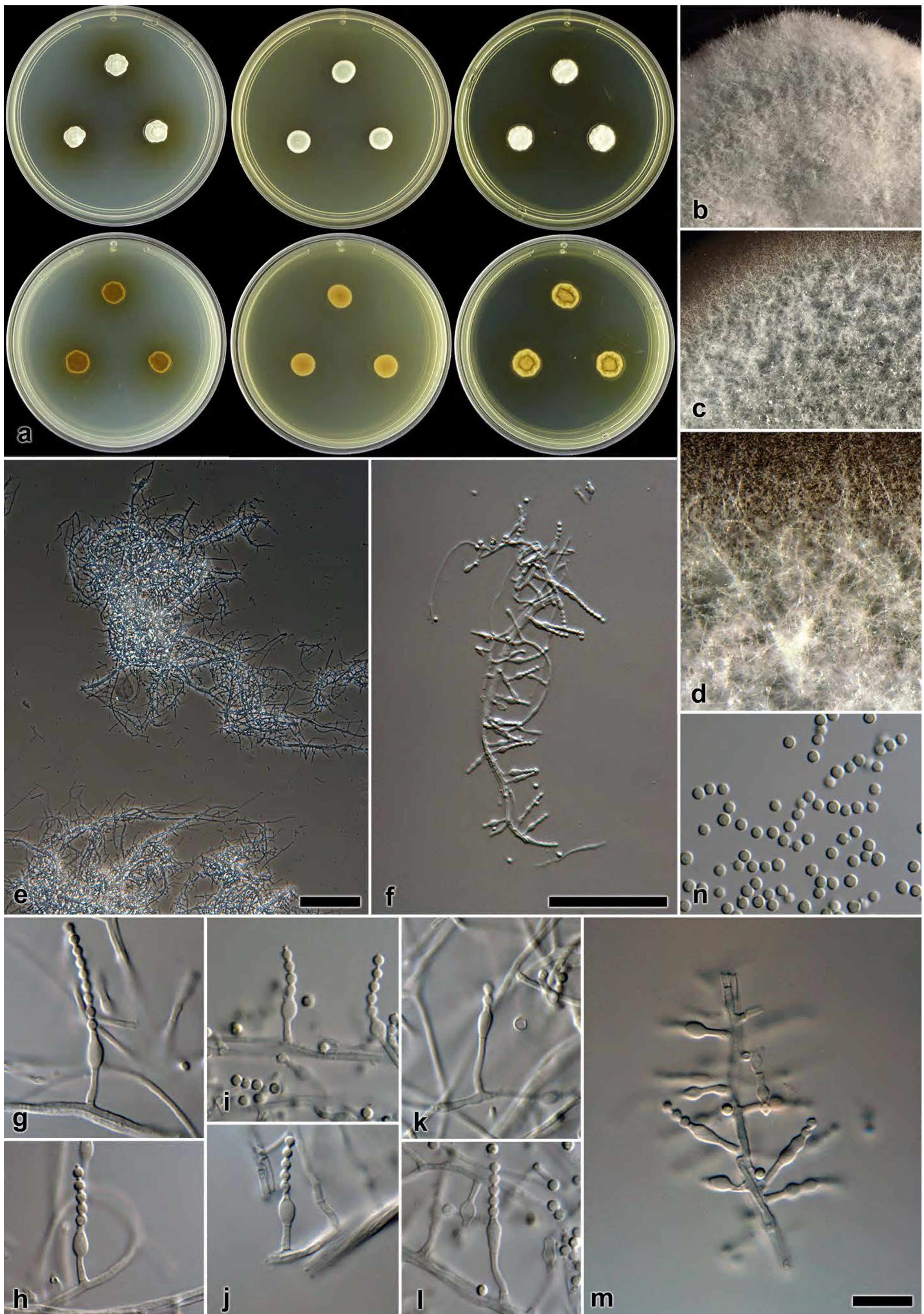

PLATE 85. *Penicillium parviverrucosum-like* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–m. Conidiophores. n. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in m = 10 µm, applies to g–n).

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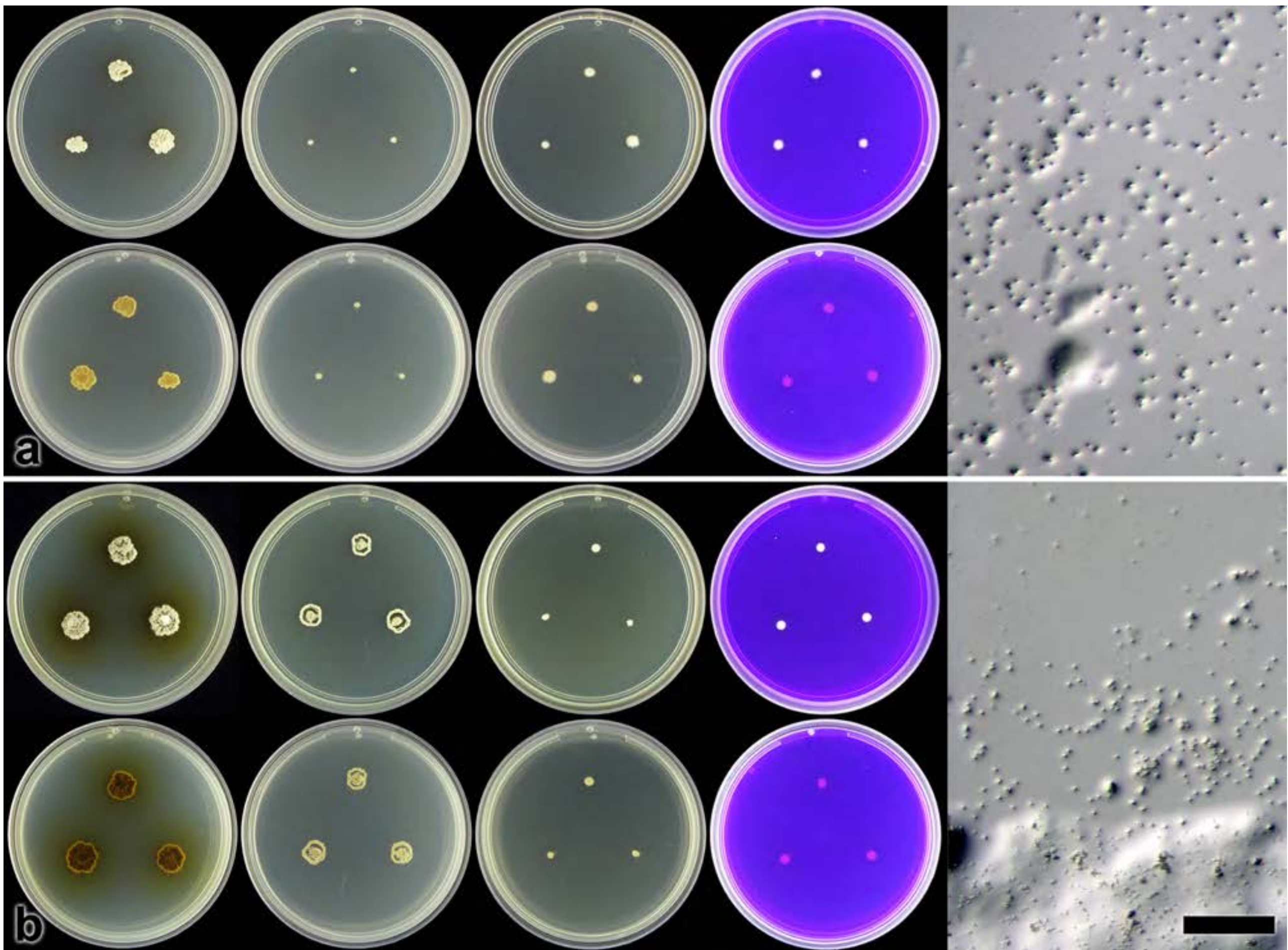

PLATE 86. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *P. austricola*. b. *P. parviverrucosum-like.*

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The section *Citrina* Houbraken & Samson

Studies in Mycology 70: 40. 2011.

TAXONOMIC NOVELTIES: *Penicillium sucrivorum* prov. nom.

SPECIES TREATED: *Penicillium cairnsense, P. citrinum, P. pancosmium, P. pasqualense, P. sanguifluum, P. sizovae, P. sumatrense, P. ubiquetum*

Houbraken *et al*. (2011b) introduced the section *Citrina* and provided an extensive review of the 39 species included, describing 17 as new. This section accommodates species that mostly produce symmetrical biverticillate conidiophores, with smooth walled stipes and small conidia. Exceptions are *P. roseopurpureum, P. galliacum* and *P. sanguifluum* that produce monoverticillate conidiophores, and *P. paxilli*, *P. terrigenum*, *P. manginii* and *P. atrofulvum* that produce finely rough to rough walled stipes (Houbraken *et al*. 2011b). Members of the section are commonly associated with soil and leaf litter (Pitt 1979, Houbraken *et al*. 2011b). This study isolated numerous section *Citrina* strains from mites and *Protea repens* infructescences, with only a minor proportion from soil samples.

The nine species isolated from Fynbos, all produced conidiophores with smooth walled stipes. Eight of the species conformed to descriptions of the Houbraken *et al*. (2011b) study, with ITS, β-tubulin and Calmodulin phylogenies that confirmed the identifications. Species were identified as *Penicillium cairnsense, P. citrinum, P. pancosmium, P. pasqualense, P. sanguifluum, P. sizovae, P. sumatrense* and *P. ubiquetum.*

Strain CV1840 did not conform to the descriptions of other section *Citrina* species and consistently resolved as distinct in the phylogenetic analysis (FIGURES 18, 19). It was resolved in a clade closely related to *P. aurantiacobrunneum*, *P. myczynskii* and *P. neomiczynskii*. Houbraken *et al*. (2011b) distinguished *P. aurantiacobrunneum* from the other two species based on its pinkish-violet Ehrlich reaction while *P. neomiczynskii* had a CYAS:CYA growth ratio of 1.1–1.2, compared to *P. miczynskii* that had a ratio lower than 1. The new Fynbos species are easily differentiated from these species by its faster growth rate on YES (45–50 mm) and CYA at 30 °C (12–15 mm), compared to *P. aurantiacobrunneum* (31–35 mm; germination–3 mm), *P. miczynskii* (26–33 mm; no growth) and *P. neomiczynskii* (25–31 mm; no growth). Colony differences were confirmed by the multigene phylogenies, which resolved *P. sucrivorum* separate from these three species (FIGURES 18, 19).

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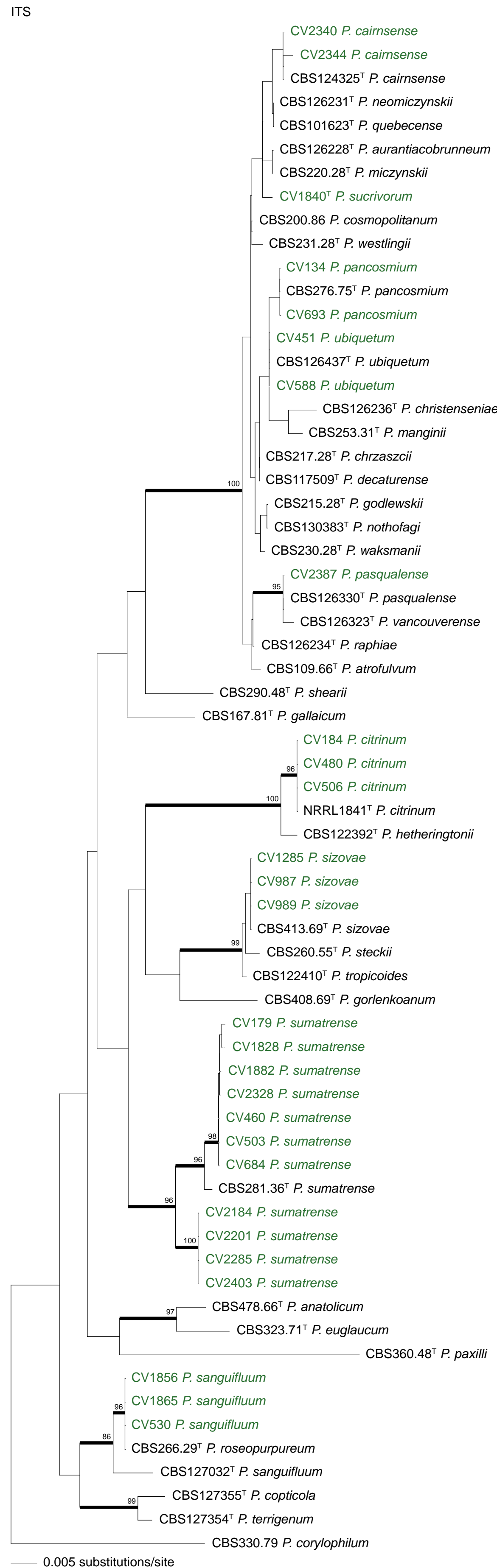

FIGURE 18: Phylogenetic tree based on ITS, showing relationship of species in the section *Citrina*. *Penicillium corylophilum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type). Colored names indicate strains isolated from Fynbos.

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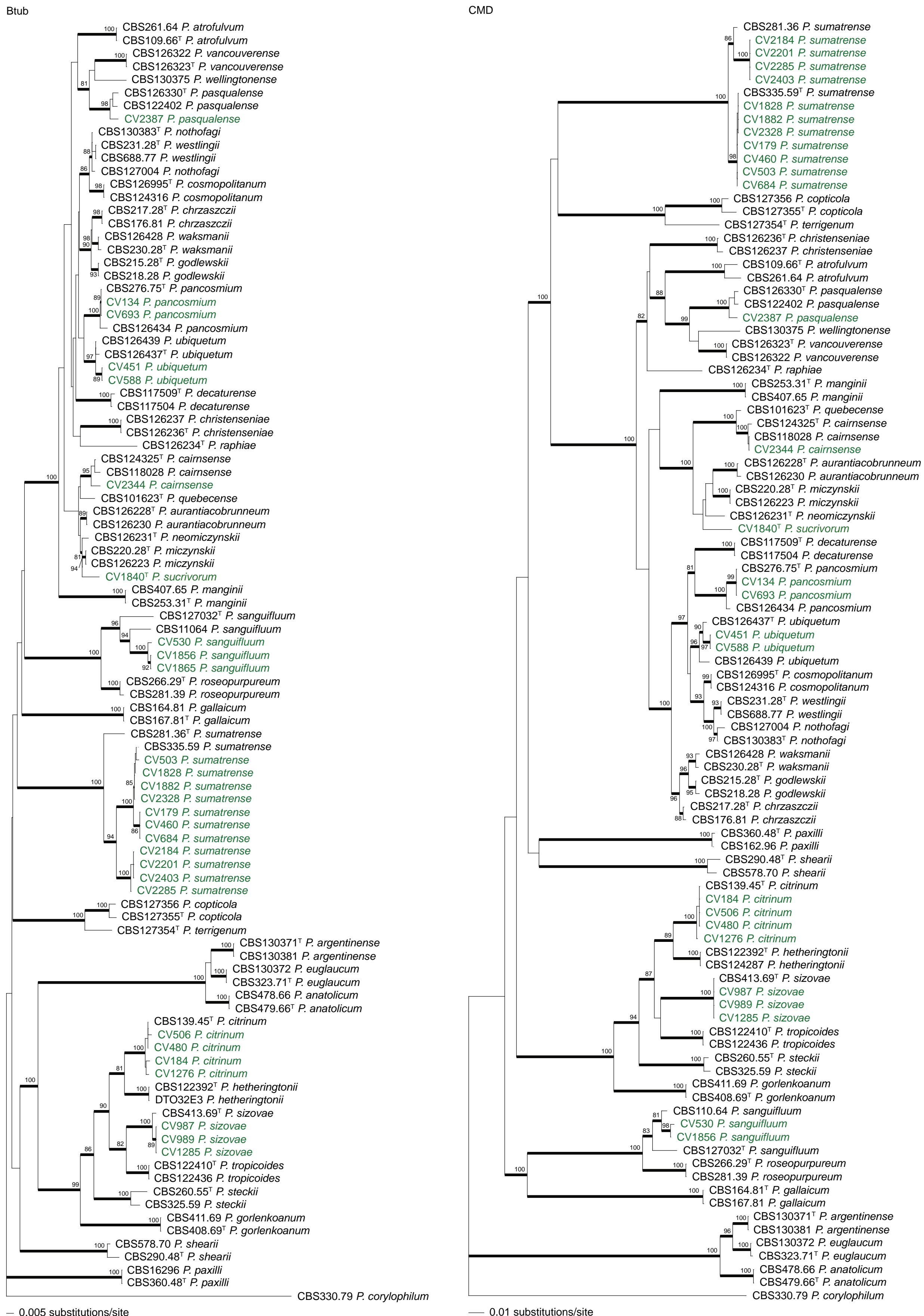

FIGURE 19: Phylogenetic trees based on β-tubulin and Calmodulin, showing relationship of species in the section *Citrina*. *Penicillium corylophilum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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50. *Penicillium cairnsense* Houbraken, Frisvad & Samson PLATES 87, 88, 105a

Studies in Mycology 70: 83. 2011.

EX-TYPE: CBS124325 = DTO30E6 = IBT29042
TYPE ISOLATED FROM: Soil, Atherton Tableland, Australia
SPECIMENS EXAMINED: CV2340, CV2343, CV2344.
ISOLATED FROM: *Protea repens* infructescens, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 41–44 mm, low, plane, dark brown sclerotia produced; margins low, wide (3–4 mm), entire; mycelia white; texture velutinous, with some floccose mycelia present; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25D5) at centre, greyish turquoise (24C4) near margin; exudate brownish orange to red, soluble pigment brownish orange areas in between colonies, reverse pigmentation dark brown (6F6) at centre, brownish orange (5C6) and greyish yellow (1B6) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 22–25 mm, moderately deep, lightly radially and concentrically sulcate, craterform; margins low to subsurface, narrow (1–2 mm), somewhat irregular; mycelia white; texture velutinous; sporulation moderate, conidia *en masse* similar to CYA at 25 °C; exudate brownish orange to red, soluble pigment brownish red, reverse pigmentation dark brown (6B6) at centre, greyish yellow (4B4–4C4) elsewhere.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 41–44 mm, low, plane, dark brown to brown sclerotia produced; margins low, wide (3–4 mm), entire; mycelia white; texture velutinous, floccose mycelia present; sporulation moderately dense, conidia *en masse* dull to greyish green (26D3–26D4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A3) at centre, fading to greyish yellow (2B3).

YES, 25 °C, 7d: Colonies 48–51 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation similar to CYA at 25 °C.

G25N, 25 °C, 7d: Colonies 17–20 mm, low, radially sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, condia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation pale yellow (2A3) at centre, greyish yellow (2B3) elsewhere.

CREA, 25 °C, 7d: Colonies 24–27 mm, no acid production.

Micromorphology — Conidiophores mostly biverticillate with minor proportion terverticillate; stipes smooth walled, 180–450 × 2.5–3.5 µm; branches when present only 2, 11–40 × 2.5–3.5 [23±6.2] µm; metulae 4–8, 44–93° [65.2±11.7°], 9–15.5 × 2–4 [11.7±1.5 × 3.2±0.4] µm, vesicle 3–5 [3.7±0.4] µm; phialides ampulliform, 5–8 per metula, 6–8.5 × 2.5–3.5 [7.2±0.5 × 3±0.27] µm; conidia finely rough walled, subspheroid to spheroid, 2–2.5 × 2–2.5 [2.4±0.1 × 2.3±0.1] µm, average width/length = 0.94±0.03, n= 95; sclerotia 100–300 × 80–250 [198.9±45.4 × 165.3±36.1] µm.

Notes — *Penicillium cairnsense* typically produces dark brown to red reverse pigmentation at colony centre on CYA and YES. Dark brown sclerotia were consistently produced on most media, with a red to brown exudate observed on CYA at 25 °C. Morphologically and phylogenetically (FIGURES 18, 19), strains match the description of Houbraken *et al*. (2011b), except for faster growth displayed on CYA at 30 °C. Its closest related species is *P. quebecense*. However, Houbraken *et al*. (2011b) reported a CYAS:CYA ratio lower than 1 for the latter species.

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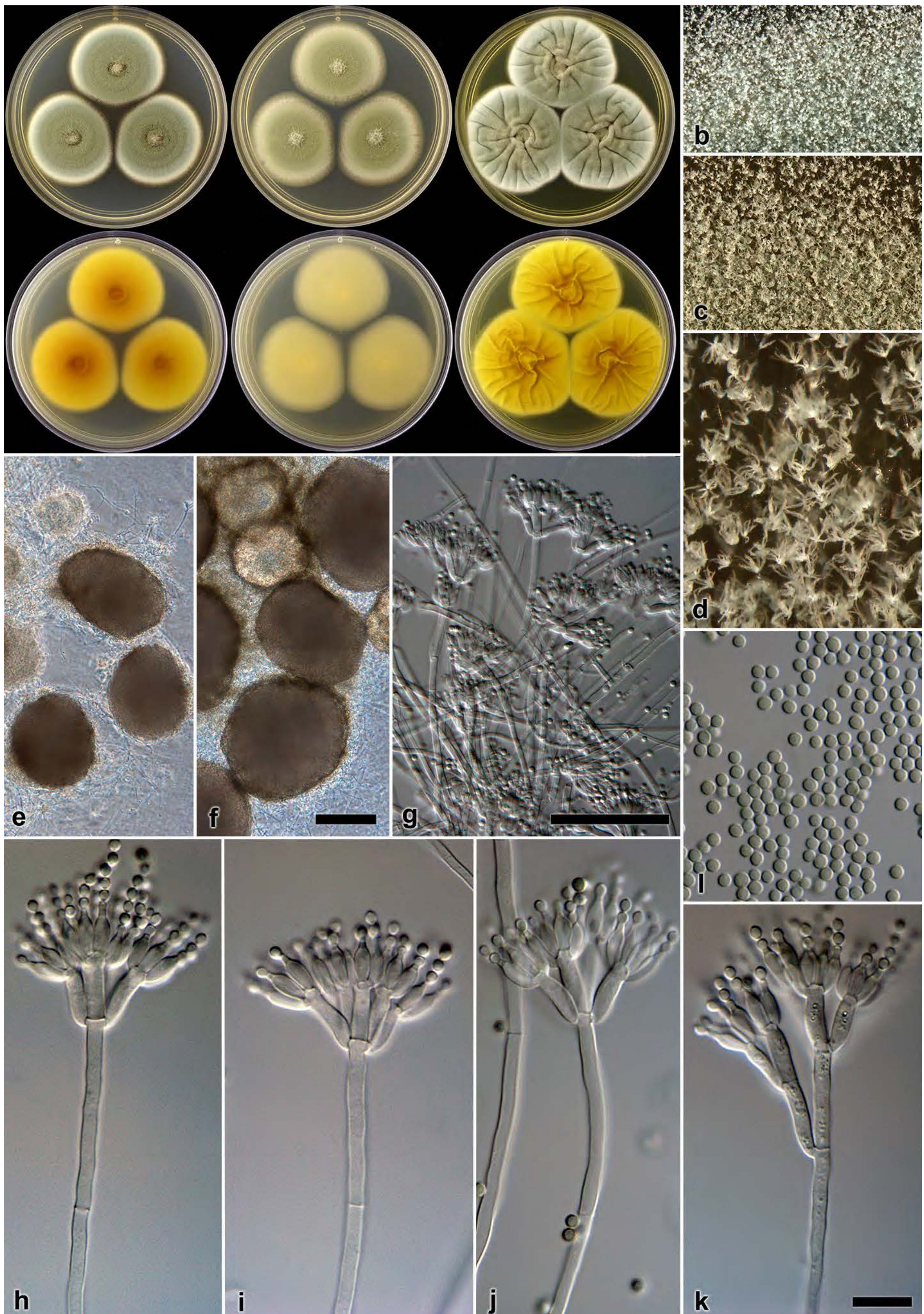

PLATE 87. *Penicillium cairnsense.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e. Sclerotia on CYA. f. Sclerotia on MEA. g–k. Conidiophores. l. Conidia (— Scale bar in f = 100 µm, applies to e, f; — Scale bar in g = 50 µm; — Scale bar in k = 10 µm, applies to f–k).

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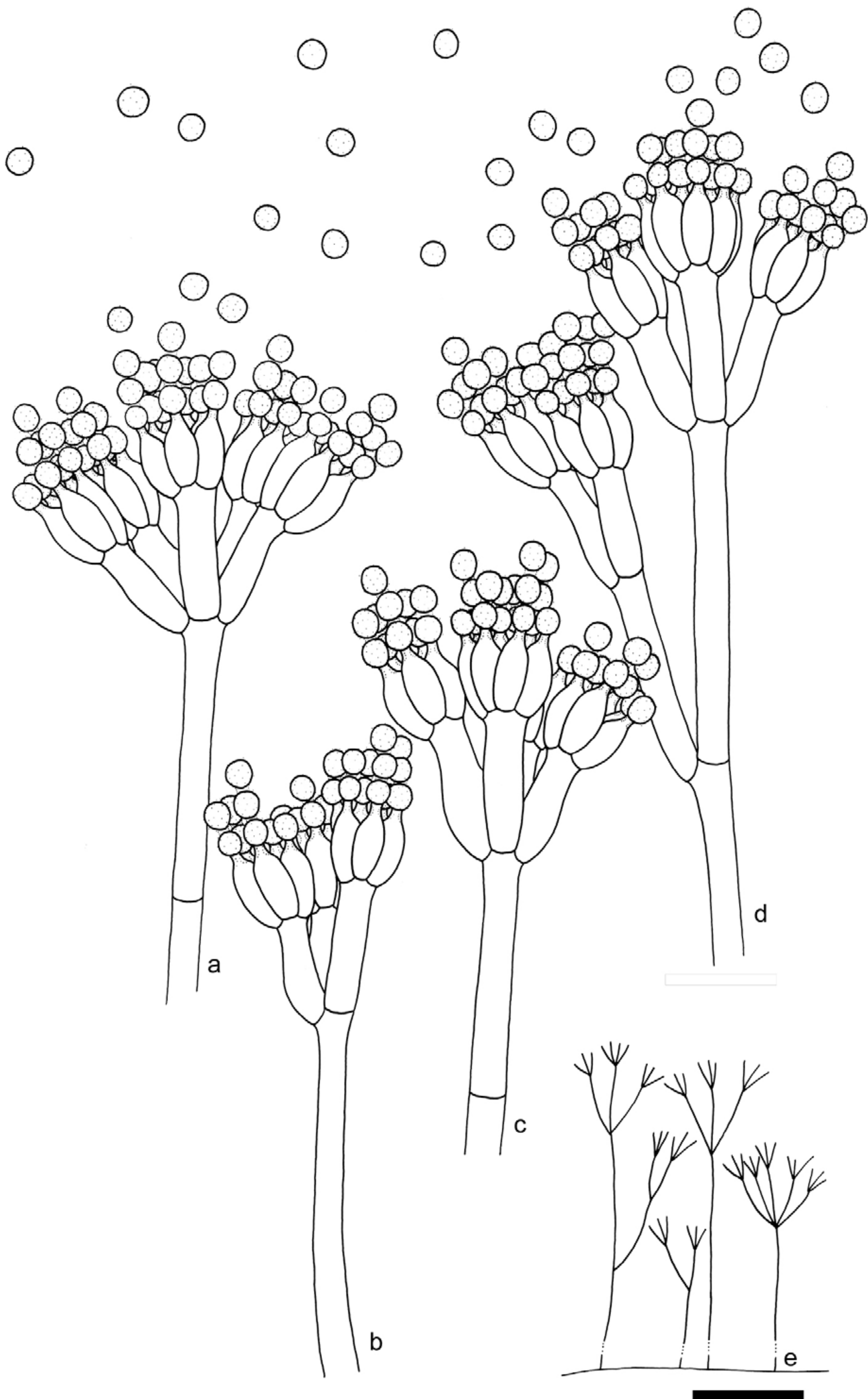

PLATE 88. Line drawing of *P. cairnsense*. a–d. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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51. *Penicillium citrinum* Thom PLATES 89, 90, 105b

The Bureau of Animal Industry: US department of Agriculture 118: 61. 1910.

EX-TYPE: IMI92196ii = CBS139.45 = NRRL1841 = NRRL1842
TYPE ISOLATED FROM: Unrecorded source
SPECIMENS EXAMINED: CV56, CV181, CV184, CV356, CV480, CV506, CV1276, CV2334.
ISOLATED FROM: Air sample, Stellenbosch; Mites and bracts of *Protea repens* infructescences, Stellenbosch, Malmesbury, Struisbaai; Soil, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 29–32 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25E4–25E7); exudate yellow, soluble pigment yellow, reverse pigmentation greyish yellow (2B5–3B5) at centre, yellowish white to light yellow (1A2–1A5).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies similar to CYA at 25 °C except for colonies 28–34 mm, exudate more abundant.

CYA, 37 °C, 7d: Colonies 5–10 mm, forming random ridges and furrows, having a yellowish grey color; margins moderately deep, narrow, irregular; mycelia white; texture velutinous; sporulation sparse, conidia *en masse* greenish grey to greyish green (27C2–27C3); exudate absent, soluble pigment absent, reverse pigmentation greyish orange (5B3).

MEA, 25 °C, 7d: Colonies 15–19 mm, low, plane; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous, floccose mycelia present at centre; sporulation dense, conidia *en masse* dark green (25F7–25F8–26F8–26F8); exudate absent, soluble pigment absent, reverse pigmentation olive to olive yellow (2D5–2D6), yellowish orange (4B7) areas sometimes present.

YES, 25 °C, 7d: Colonies 30–43 mm, low to moderately deep, radially and concentrically sulcate; margins low, narrow (1–2 mm), somewhat irregular; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation orange to brownish orange (6B8–6C8), in some isolates yellow (2A6).

G25N, 25 °C, 7d: Colonies 13–17 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation yellow (3A7) at centre, fading to greenish grey to greyish green to yellowish green (30B2–30B7).

CREA, 25 °C, 7d: Colonies 23–26 mm, very moderate acid production underneath colonies.

Micromorphology — Conidiophores mostly biverticillate, terverticilalte and subterminal branching not uncommon, especially in fresh isolates; stipes smooth, 175–325 × 2.5–3.5 µm; branches when present only 2, divergent, 15–38 × 2.5–3.5 [27.6±6.5] µm; Metulae 3–5, divergent, 30–80° [57.7±11.1°], 11.5–17 × 2.5–3.5 [13.6±1.3 × 2.9±0.3] µm, vesicle 3.5–5 [4.1±0.4] µm; phialides ampulliform, 8–12 per metula, 6.5–9 × 2–3.5 [7.6±0.57 × 2.7±0.23] µm; conidia very finely rough walled, spheroidal to subspheroidal, 2–3 × 2–3 [2.4±0.12 × 2.3±0.11] µm, average width/length = 0.96±0.03, n= 71.

Notes — *Penicillium citrinum* characteristically grows at 37 °C and produce yellow exudates on CYA and YES which is unique for species in the section *Citrina*. These characters, therefore, distinguishes *P. citrinum* from its closest relatives, *P. hetheringtonii, P. sizovae* and *P. tropicoides* (FIGURES 18, 19). This study found the conidia to be very finely roughened, although Houbraken *et al.* (2011b) reported them to be smooth walled.

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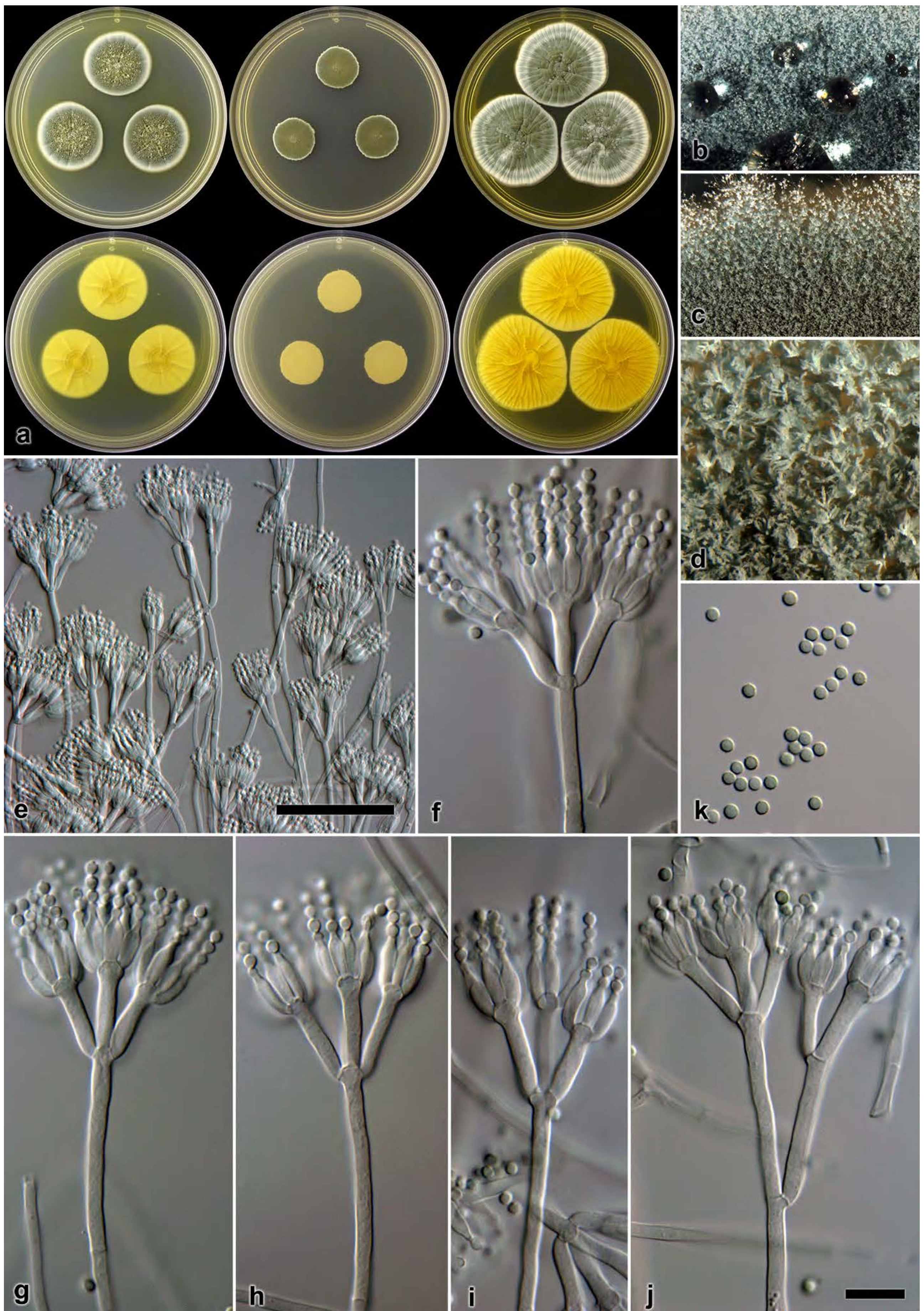

PLATE 89. *Penicillium citrinum.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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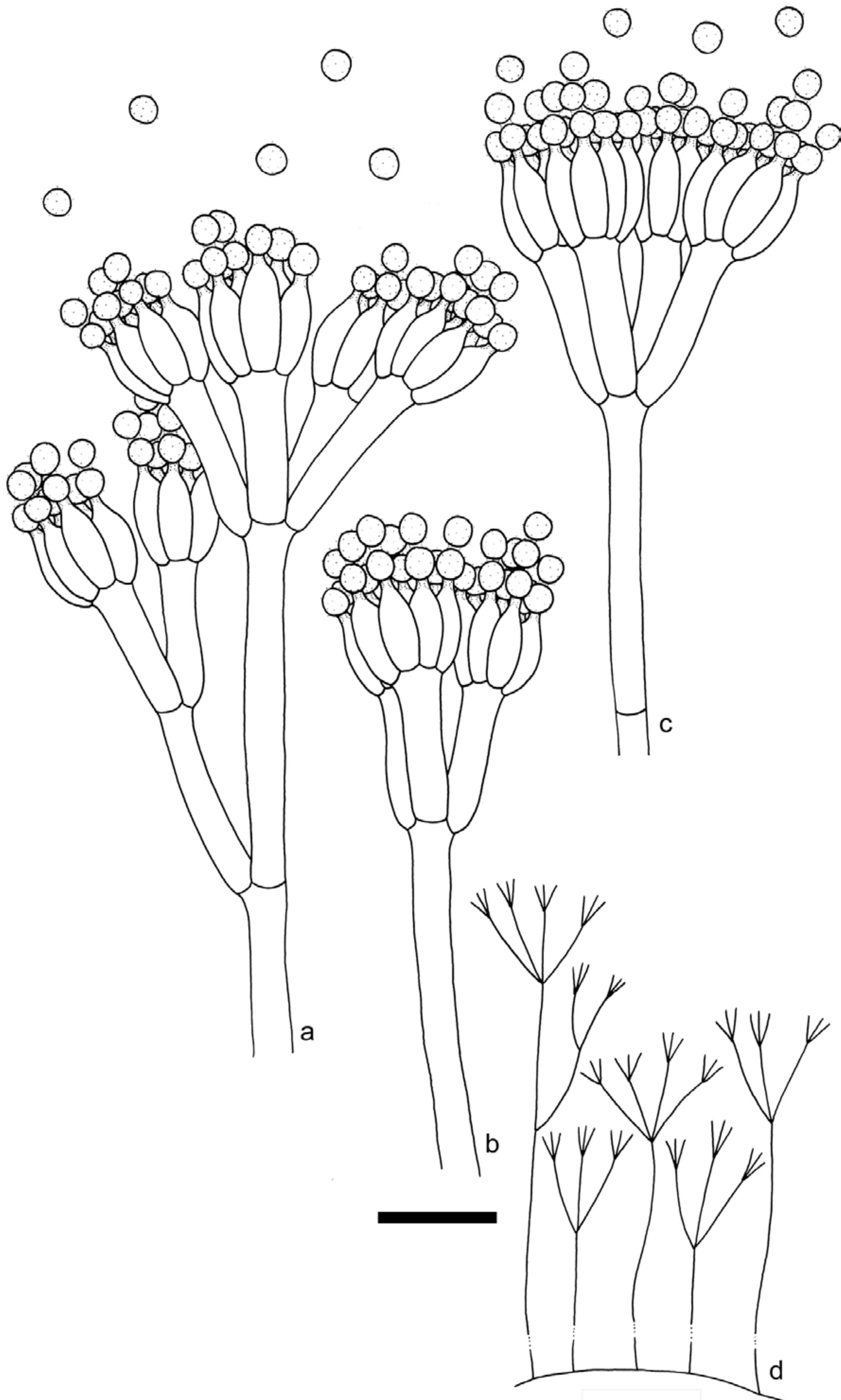

PLATE 90. Line drawing of *P. citrinum*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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52. *Penicillium pancosmium* Houbraken, Frisvad & Samson PLATES 91, 92, 105c

Studies in Mycology 70: 108. 2011.

EX-TYPE: CBS276.75 = DTO31B4 = DAOM147467 = IBT29991
TYPE ISOLATED FROM: *Armillaria mellea* on hardwood log, Meach Lake, Gatineau Park, Quebec, Canada
SPECIMENS EXAMINED: CV134, CV693.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescences, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 27–31 mm, low to moderately deep, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish turquoise (24E4–24E5–24D6); exudate absent, sometimes yellowish to clear, soluble pigment mostly absent, but sometimes yellow, reverse pigmentation yellow (2A6) centre, greyish yellow (2B4–3B6), with yellowish white (2A6) margin.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 12–14 mm, moderately deep, craterform; margin low, narrow (1 mm), entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* light to greyish turquoise (24A4–24D4); exudate absent, soluble pigment absent, reverse pigmentation blond (4C4) at centre, elsewhere greyish yellow (4B3) fading into yellowish white (4A2) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 22–26 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense to dense, conidia *en masse* greyish turquoise (24E5) fading into light turquoise (24D5–24D6) near margin; exudate absent, soluble pigment absent, sometimes yellow, reverse pigmentation pale yellow (3A4) at centre, fading into greyish yellow (4C4), and then olive (3D4) near the yellowish white (3A2) margin.

YES, 25 °C, 7d: Colonies 32–35 mm, sometimes up to 45 mm, moderately deep, sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, sometimes yellow, reverse pigmentation light yellow (3A4–3A5) at centre yellow (2B8–3B8) near the pale yellow (3A3) margin.

G25N, 25 °C, 7d: Colonies 14–15 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, sometimes yellow, reverse pigmentation yellow (3A7) at centre, fading to greenish grey to greyish green to yellowish green (30B2–30B7), sometimes more yellowish when soluble pigment present.

CREA, 25 °C, 7d: Colonies 19–22 mm, no acid production.

Micromorphology — Conidiophores biverticillate, with minor proportion having subterminal branches formed; stipes smooth walled, 200–575 × 2.5–3.5 µm; branches when present only 2, 14–42 × 2.5–3.5 [29.8±7.8] µm; metulae 6–8, divergent, 56–87° [73±7.9°], 9–19 × 3–4 [11.5±1.8 × 3.2±0.25] µm, vesicle 3–5 [3.8±0.4] µm; phialides ampulliform, metula, 8–12 per metula, 6–8 × 2.5–3 [7.1±0.39 × 2.7±0.15] µm; conidia smooth walled, spheroid to broadly ellipsoidal 2–2.5 × 2–2.5 [2.3±0.13 × 2.2±0.1] µm, average width/length = 0.95±0.03, n= 69.

Notes — *Penicillium pancosmium* is characterized by moderate growth on most media, with slow growth observed at higher temperautres. Its close relative, *P. ubiquetum*, was also isolated in this study. Houbraken *et al*. (2011b) considered the orange to red reverse pigmentation on CYA and YES produced by *P. ubiquetum*, compared to the yellow coloration in *P. pancosmium*, as diagnostic for this species. This pigmentation was also observed in this study. Also, *P. ubiquetum* produces darker conidia on CYA, than *P. pancosmium*. Conidiophores for both species were found to be very divergent, compared to other species in the section.

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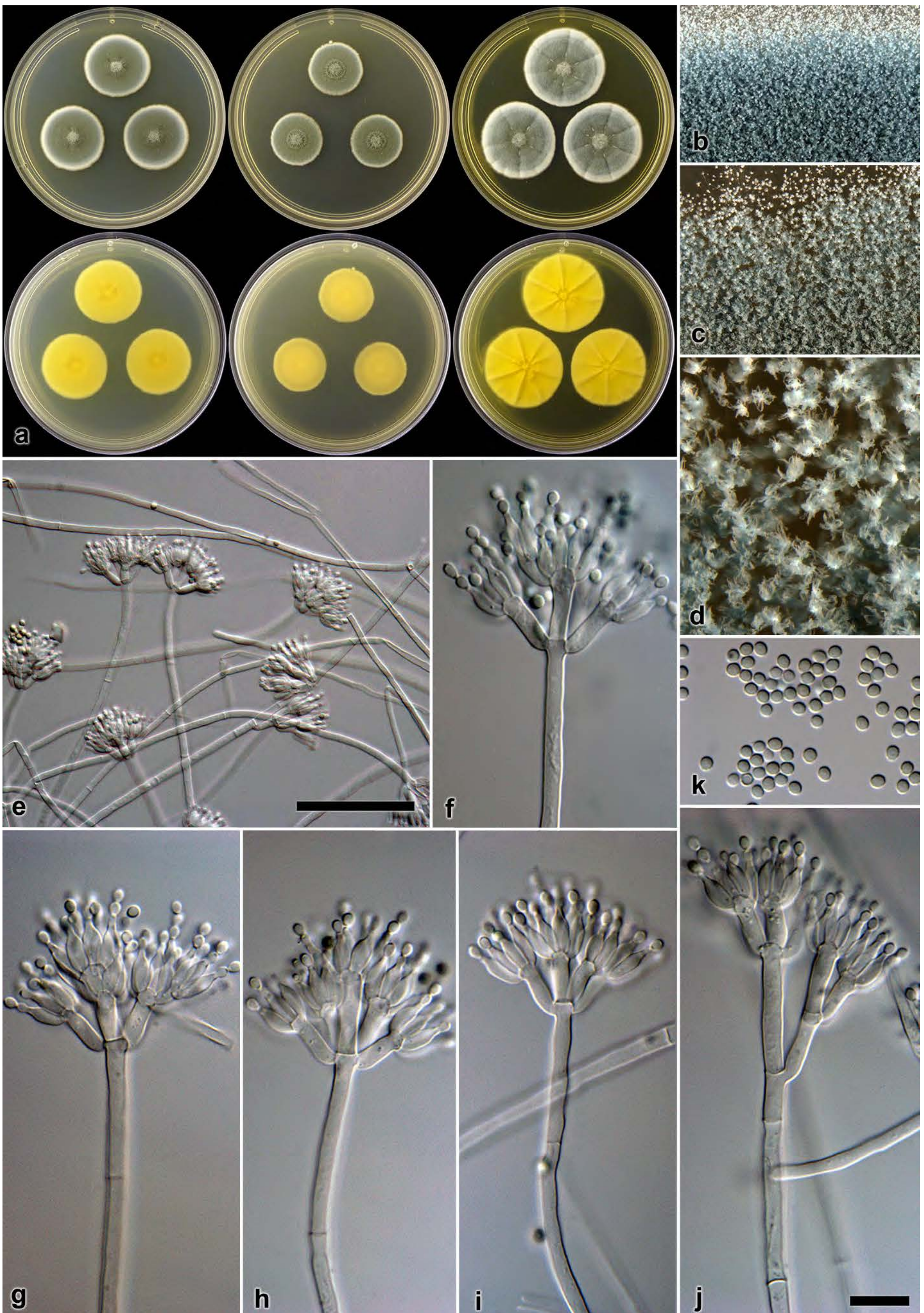

PLATE 91. *Penicillium pancosmium.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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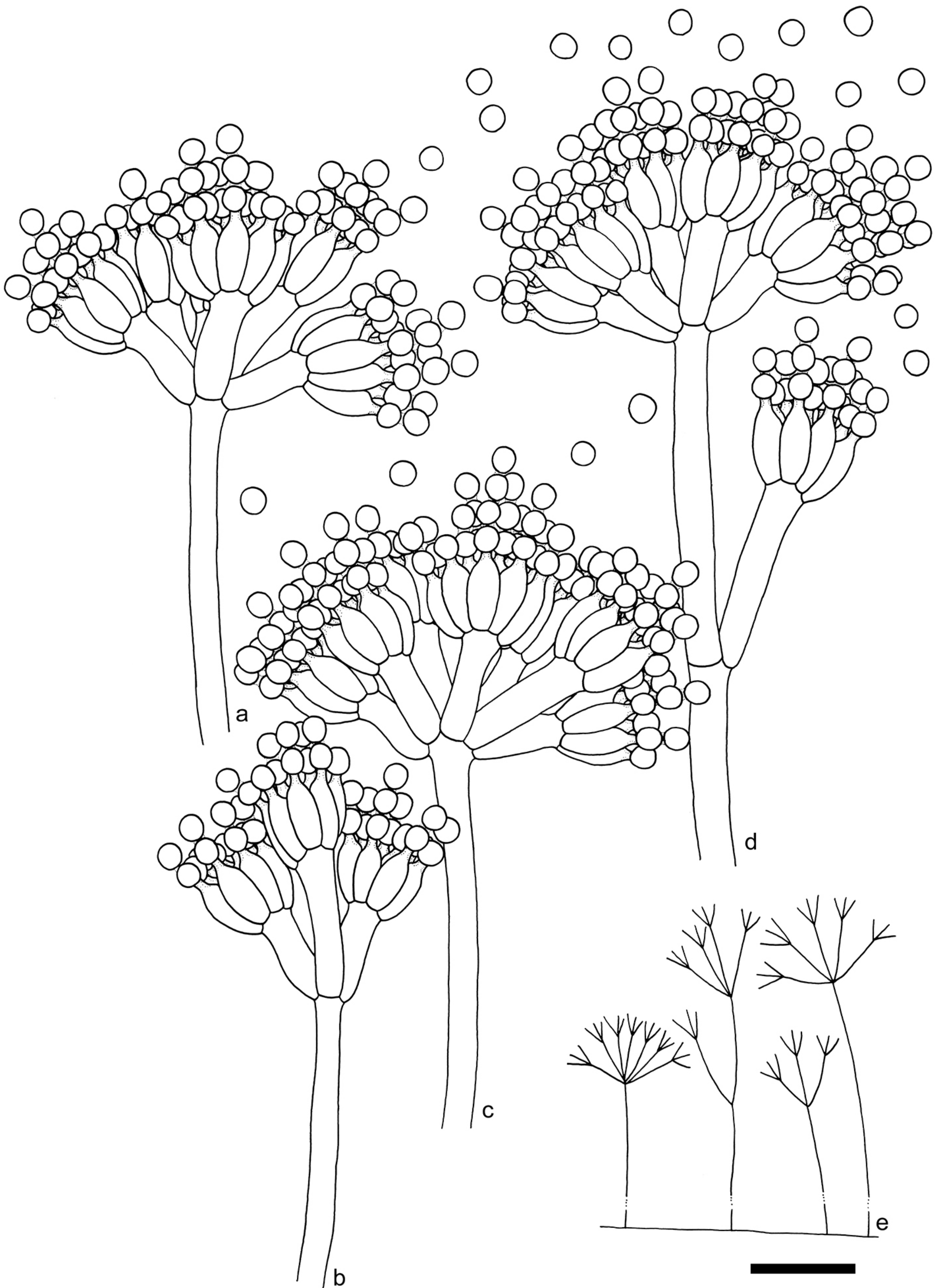

PLATE 92. Line drawing of *P. pancosmium*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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53. ***Penicillium pasqualense*** Houbraken, Frisvad & Samson

PLATES 93, 94, 105d

Studies in Mycology 70: 108. 2011.

EX-TYPE: CBS126330 = DTO80D5 = IBT14235
TYPE ISOLATED FROM: Soil, Easter Island, Chile
SPECIMENS EXAMINED: CV2387.
ISOLATED FROM: *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 24–27 mm, low, radially sulcate, dark brown sclerotia produced which is covered by overlaying colony; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous with some floccose mycelia present; sporulation moderately dens, conidia *en masse* dull to greyish green (25D4–25D6–25E6–25E4) and a lighter greyish green (25B3) areas, exudate clear to yellowish, soluble pigment absent, reverse pigmentation olive brown (4F6) at centre, olive to olive brown (3F3–4F3) and greyish yellow to olive brown (4C3–4D3) elsewhere.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 18–21 mm, low, radially sulcate, slightly raised at centre; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous; texture velutinous with some floccose mycelia present; sporulation moderately dense, condia *en masse* greyish turquoise (24D4–24E4) near centre, turquoise white (24A2) fading to greyish turquoise (24D5) at margin; exudate clear, soluble pigment absent, reverse pigmentation similar to CYA at 25 °C.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 27–30 mm, low, plane, cream and dark brown sclerotia produced which is covered by overlaying colony; margins low to subsurface, narrow (1–2 mm), entire; mycelia white; texture velutinous with some floccose mycelia present; sporulation moderately dense, conidia *en masse* dull green (26D4–26E4); exudate absent, soluble pigment absent, reverse pigmentation greyish orange (5B6–5C6) and greyish green (1C3) areas.

YES, 25 °C, 7d: Colonies 27–29 mm, low, radially sulcate, raised centrally; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous with some floccose mycelia present; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation yellowish brown (5E5) fading to greyish yellow (4B3) margin.

G25N, 25 °C, 7d: Colonies 13–16 mm, low, lightly radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation olive brown (4D6) at centre, pale yellow (4A3) and greyish yellow elsewhere (1B3) elsewhere.

CREA, 25 °C, 7d: Colonies 18–20 mm, no acid produced.

Micromorphology — Conidiophores biverticillate, although terverticillate very common; stipes smooth walled, 170–500 × 2.5–3.5 µm; branches when present 2, 15–33 × 2.5–3.5 [21.9±4.1] µm; metulae 2–5, divergent, 24–73° [52.6±9.7°], 11–23 × 2.5–3 [15.9±2.6 × 3.1±0.28] µm, vesicle 3–5 [4±0.45] µm; phialides ampulliform, 4–11 per metula, 7–11.5(–13) × 2.5–4 [9.1±1 × 3.3±0.29] µm; conidia rough walled, spheroid, some subspheroid, 2.5–3.5 × 2.5–3.5 [2.8±0.15 × 2.8±0.16] µm, average width/length = 0.97±0.02, n= 73; sclerotia 80–175 × 70–140 [113.5±25.5 × 93.9±15.9] µm.

Notes — *Penicillium pasqualense* typically produces brown reverse pigmentation on most media, as well as dark brown sclerotia on CYA and MEA, similar to *P. cairnsense*. The faster growth rates on all media of *P. cairnsense*, easily distinguishes it from *P. pasqualense*. Sclerotia on CYA are produced closely together, almost embedded in media. This character was not observed in any other species examined in this study. *Penicillium pasqualense* is closely related to *P. vancouverense* and *P. wellingtonense* (FIGURES 18, 19). In comparison with *P. pasqualense*, *P. wellingtonense* grows much more restricted on CYA (10–15 mm) and MEA (8–13 mm), whereas *P. vancouverense* produces yellow mycelia and lacks sclerotia production (Houbraken *et al*. 2011b).

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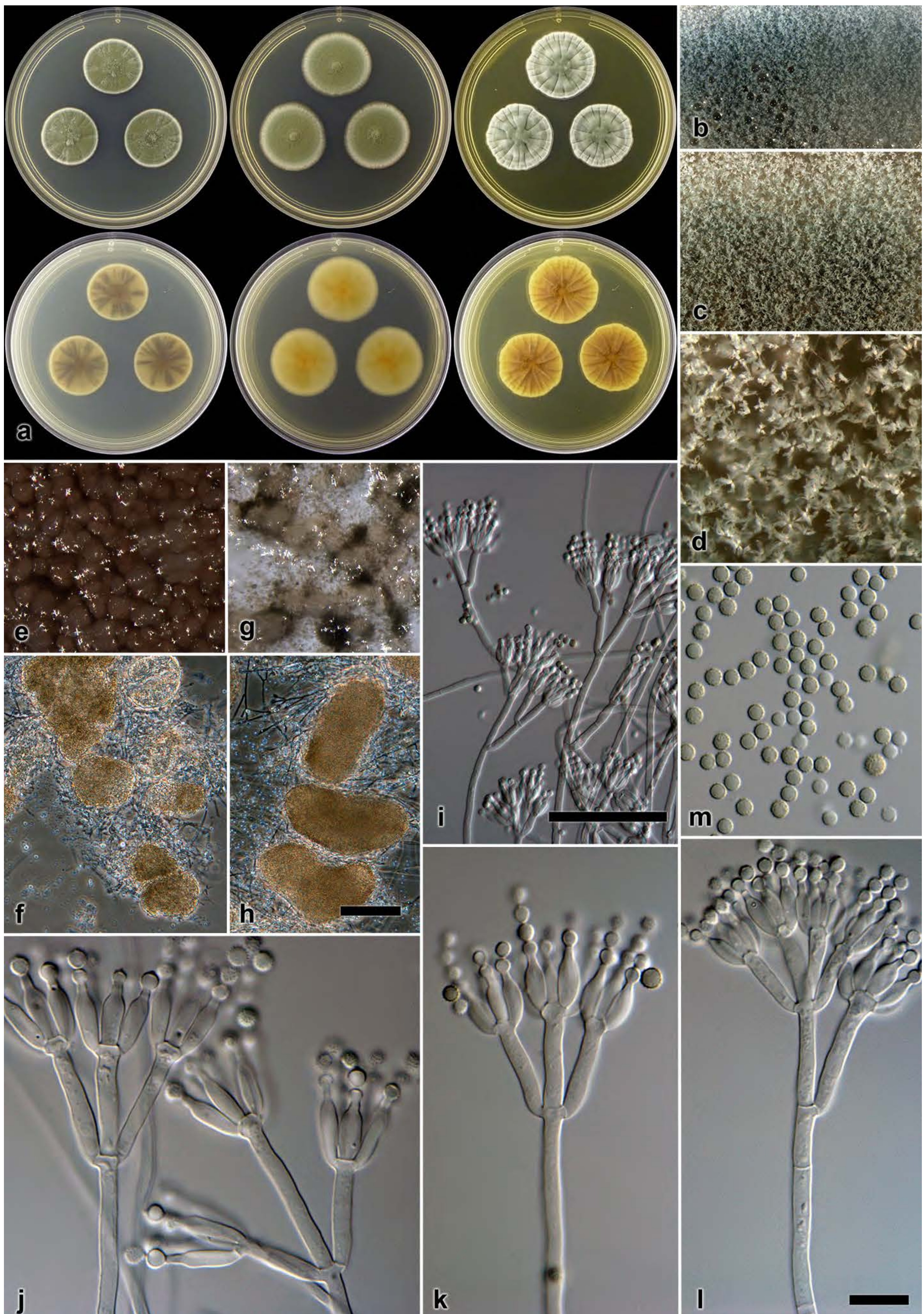

PLATE 93. *Penicillium pasqualense.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e, f. Sclerotia on CYA. g, h. Sclerotia on MEA. i–l. Conidiophores. m. Conidia (— Scale bar in h = 100 µm, applies to e–h; — Scale bar in i = 50 µm; — Scale bar in l = 10 µm, applies to j–m).

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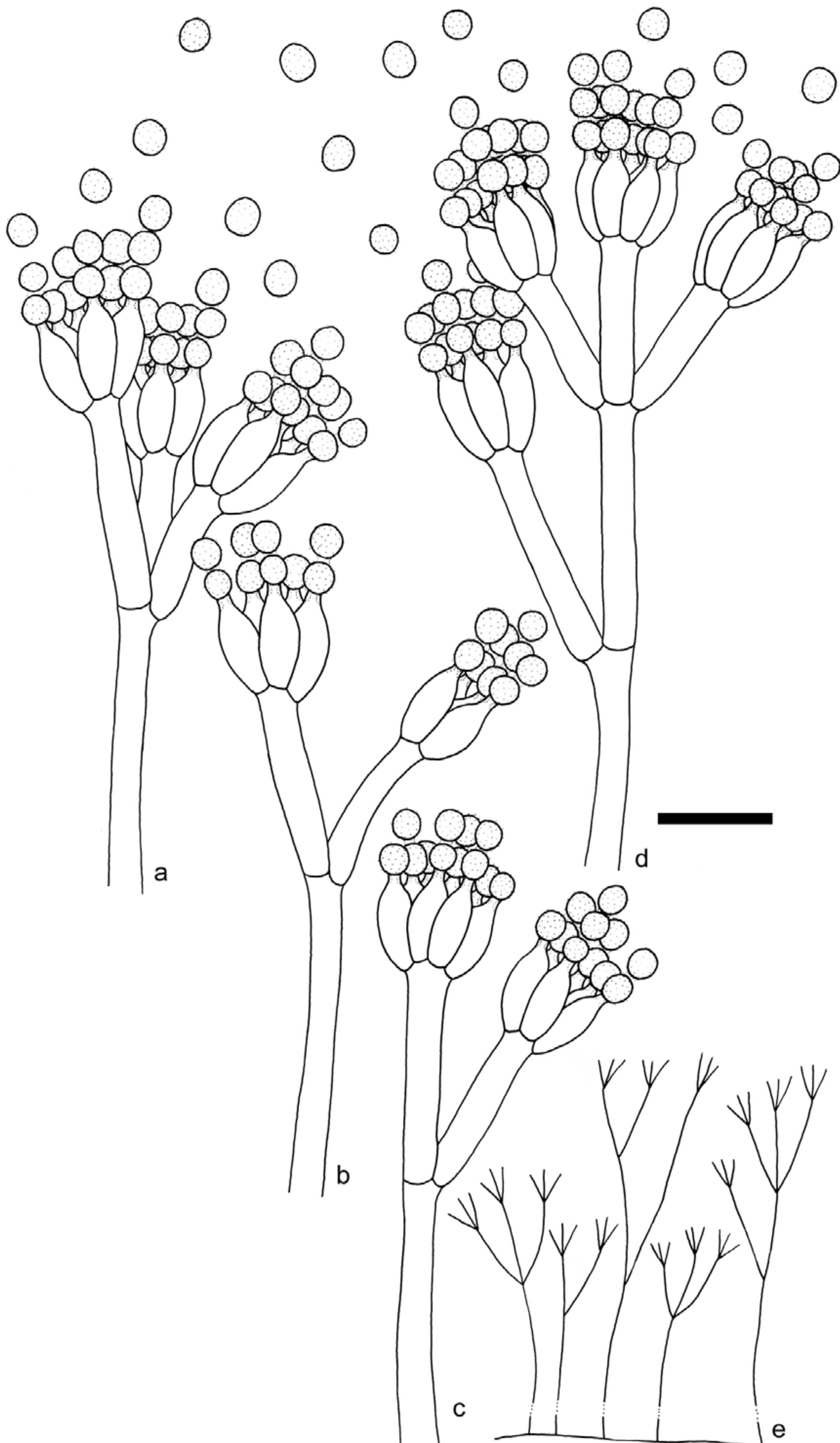

PLATE 94. Line drawing of *P. pasqualense*. a–d. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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54. *Penicillium sanguifluum* (Sopp) Biourge PLATES 95, 96, 105e

La Cellule 33: 105. 1923.

BASIONYM: *Citromyces sanguifluus* Sopp (1912. Videnskappsselskapets skrifter Christiana 11: 115)
EX-TYPE: CBS127032 = DTO20B7 = IBT29041
TYPE ISOLATED FROM: Soil, Calahonda, Costa del Sol, Spain
SPECIMENS EXAMINED: CV530, CV551, CV1655, CV1856, CV1865, CV2197.
ISOLATED FROM: Soil, Malmesbury, Stellenbosch and Struisbaai; Mites and bracts from *Protea repens* infructescences, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 22–25 mm, low, radially and concentrically sulcate, radially sulcate, sunken in at centre; margins low, narrow (1 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (25C5–25D5–26C5–26D5); exudate orange brown at colony centre, soluble pigment brown, reverse pigmentation brown (7E7) near centre, becoming brownish orange (6C6) near margins, less pronounced in some isolates.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 17–20 mm, low, radially sulcate, craterform; margins low, narrow (1 mm), entire; mycelia white; texture floccose; sporulation sparse to some moderately dense areas, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation similar to CYA at 25 °C.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 18–21 mm, low, plane; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (25D3) near centre fading into greyish green (25D7) nearer margin; exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (3B5–3B6).

YES, 25 °C, 7d: Colonies 29–33 mm, moderately deep, radially sulcate, raised centrally, having an almost greyish yellow color in non-sporulating regions; margins low, narrow (1 mm), irregular; mycelia white; texture floccose; sporulation sparse to moderately dense in some areas, conidia *en masse* greyish green dull to greyish green (25E4–25E5) and lighter greyish green (25B3) areas; exudate absent, soluble pigment brownish, reverse pigmentation brown (6E7–7E7) at centre, fading into greyish orange (5B6) near margin, only greyish orange in some isolates.

G25N, 25 °C, 7d: Colonies 11–14 mm, moderately deep, lightly radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (25C4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (1A3–2A3).

CREA, 25 °C, 7d: Colonies 16–18 mm, no acid production.

Micromorphology — Conidiophores mostly monoverticillate with short stipes, some can be interpreted as biverticillate; stipes smooth walled, 13–75 × 2–2.5 µm; metulae/branches 2, divergent, 10–30 × 2–2.5 [18.2±4.3 × 2.1±0.16] µm, vesicle 3–5 [3.7±0.43] µm; phialides ampulliform, 6–12 per metula, 5–7.5 × 2–3 [6.2±0.55 × 2.5±0.19] µm; conidia finely rough walled, spheroid to subspheroid, 2–2.5 × 2–2.5 [2.2±0.1 × 2.1±0.11] µm, average width/length = 0.96±0.03, n= 82.

Notes — *Penicillium sanguifluum* is characterized by slow growth on most media and produce reddish to brown soluble pigments and reverse pigmentation on most media. However, its most striking feature is the monoverticillate conidiophores produced on very short stipes, which is not often observed in species from this section. This species is very similar to *P. roseopurpureum*. Houbraken *et al*. (2011b) distinguished between these two species based on *P. sanguifluum* that are able to grow at 30 °C, as well as species that grow faster on CYA at 25 °C.

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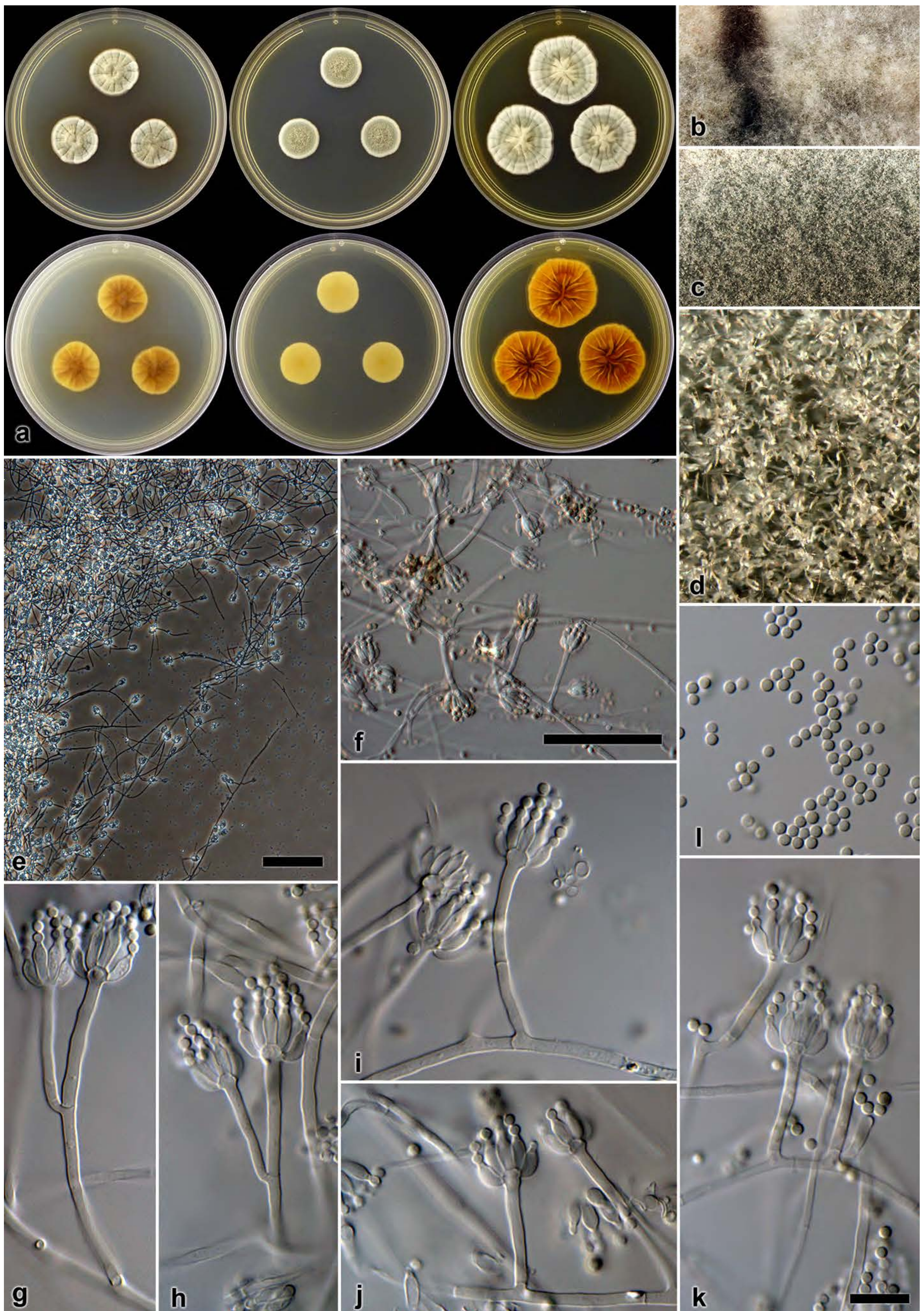

PLATE 95. *Penicillium sanguifluum*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 100 µm; — Scale bar in f = 50 µm; — Scale bar in k = 10 µm, applies to g–l).

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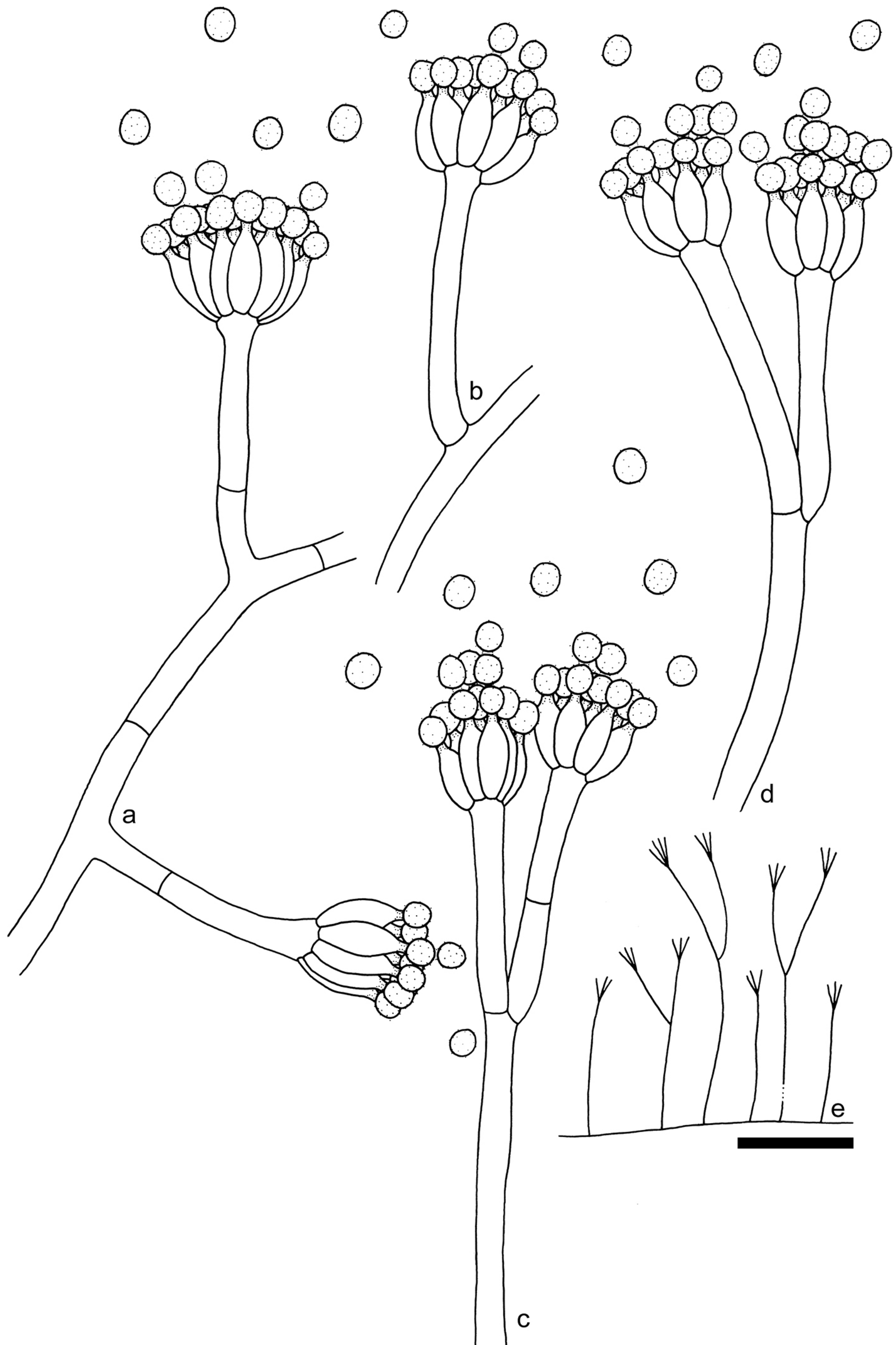

PLATE 96. Line drawing of *P. sanguifluum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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55. ***Penicillium sizovae*** Baghdadi

PLATES 97, 98, 105f

Novosti Sistematiki Nizshikh Rastenii 5: 103. 1968.

EX-TYPE: CBS413.69 = DTO23A7 = FRR518= IMI140344
TYPE ISOLATED FROM: Soil, Syria
SPECIMENS EXAMINED: CV987, CV989, CV1285, CV1287, CV1288.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescences, Malmesbury

Macromorphology — CYA, 25 °C, 7d: Colonies 29–33 mm, low, radially sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green (25E6) and dull green (27E3–27E4), with lighter greyish green (25B4) regions present; exudate clear, soluble pigment absent, reverse pigmentation dull yellow (3B3–3B4), pale yellow (3A3) at margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies similar to CYA at 25 °C except for colonies 30–32 mm, exudate more abundant.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 31–35 mm, low, plane; margins low, wide (3 mm), entire; mycelia white; texture mostly velutinous, floccose mycelia present near centre; sporulation moderately dense to dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation light yellow (2A5) at centre, greyish yellow (2C3–2C5) elsewhere, pale yellow (2A3) at margin.

YES, 25 °C, 7d: Colonies 41–44 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture mostly velutinous, but floccose present at colony centre; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation greyish green (30B3) at centre, greenish grey (30B2) elsewhere.

G25N, 25 °C, 7d: Colonies 13–16 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* similar to CYA; exudate absent, soluble pigment absent, reverse pigmentation yellow (3A7) at centre, fading to greenish grey to greyish green to yellowish green (30B2–30B7).

CREA, 25 °C, 7d: Colonies 22–24 mm, moderate acid production.

Micromorphology — Conidiophores mostly biverticillate, terverticillate and subterminal branching not uncommon, especially in fresh isolates; stipes smooth, 135–300 × 2.5–3.5 µm; branches when present only 2, divergent, 16–43 × 2.5–3.5 [29.3±6.79] µm; metulae 3–5, divergent, 35–95° [61.5±12.4°], 10.5–16.5 × 2.5–3.5 [13.6±1.67 × 2.9±0.23] µm, vesicle 3.5–5 [4.1±0.42] µm; phialides ampulliform, 8–12 per metula, 6.5–9.5 × 2–3 [7.8±0.55 × 2.7±0.2] µm; conidia very finely rough walled, spheroid to subspheroid, 2–2.5 × 2–2.5 [2.3±0.08 × 2.2±0.11] µm, average width/length = 0.96±0.03, n= 82.

Notes — *Penicillium sizovae* characteristically produce fast growth on CYA (25 °C and 30 °C), MEA and YES. One of its close relatives, *P. citrinum*, was also isolated from Fynbos. *Penicillium citrinum* typically produces slower growth on MEA (15–19 mm), and yellow exudates and soluble pigments on CYA, compared to fast growth of *P. sizovae* (MEA = 31–35 mm). Also, *P. sizovae* does not produce the yellow pigments observed for *P. citrinum*. *Penicillium sizovae* can be distinguished from the other two closely related species based on its faster growth on MEA (31–35 mm), compared to *P. hetheringtonii* (17–23 mm) and *P. tropicoides* (18–23 mm) (Houbraken *et al.* 2011b).

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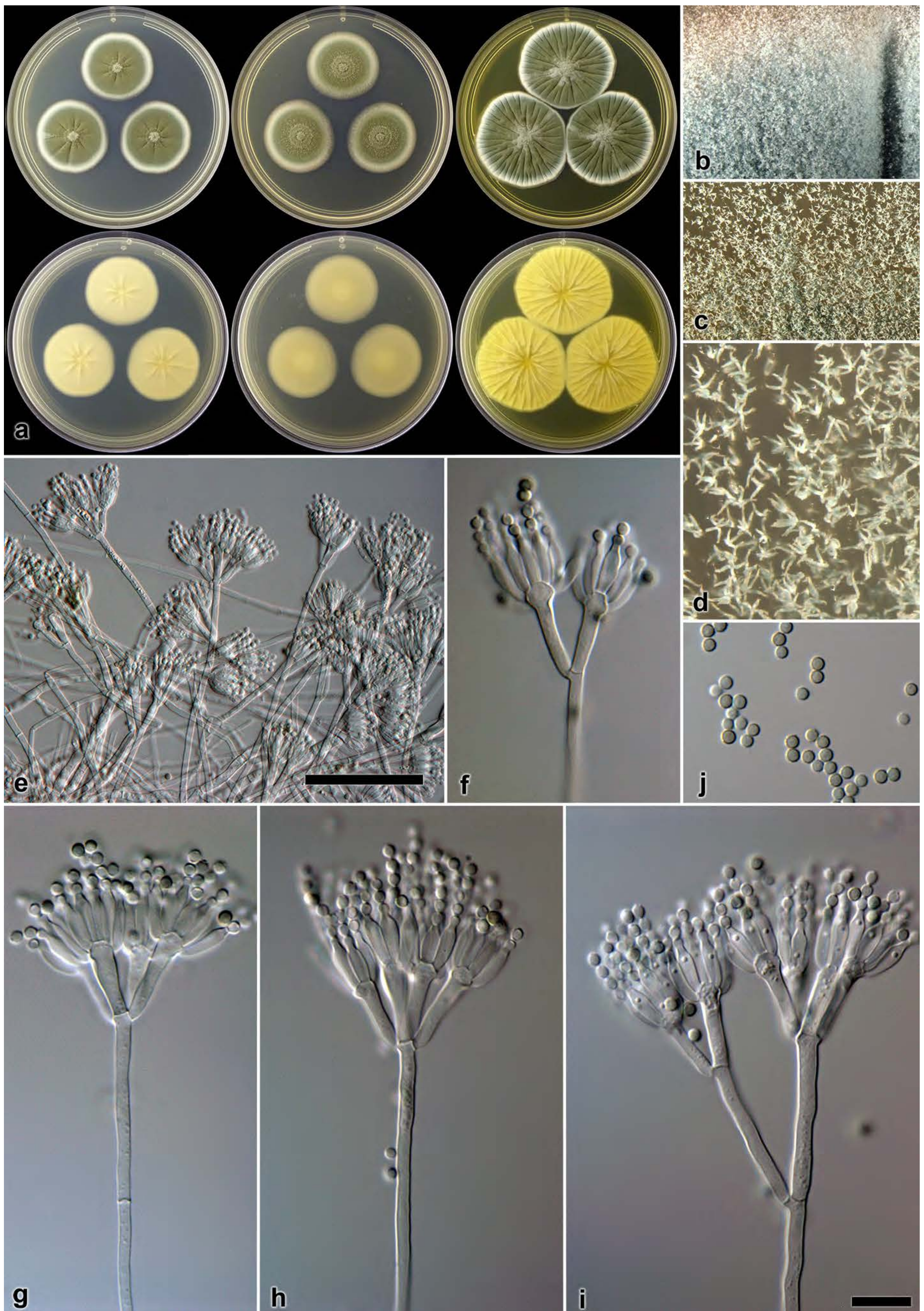

PLATE 97. *Penicillium sizovae.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–i. Conidiophores. j. Conidia (— Scale bar in e = 50 µm; — Scale bar in i = 10 µm, applies to f–j).

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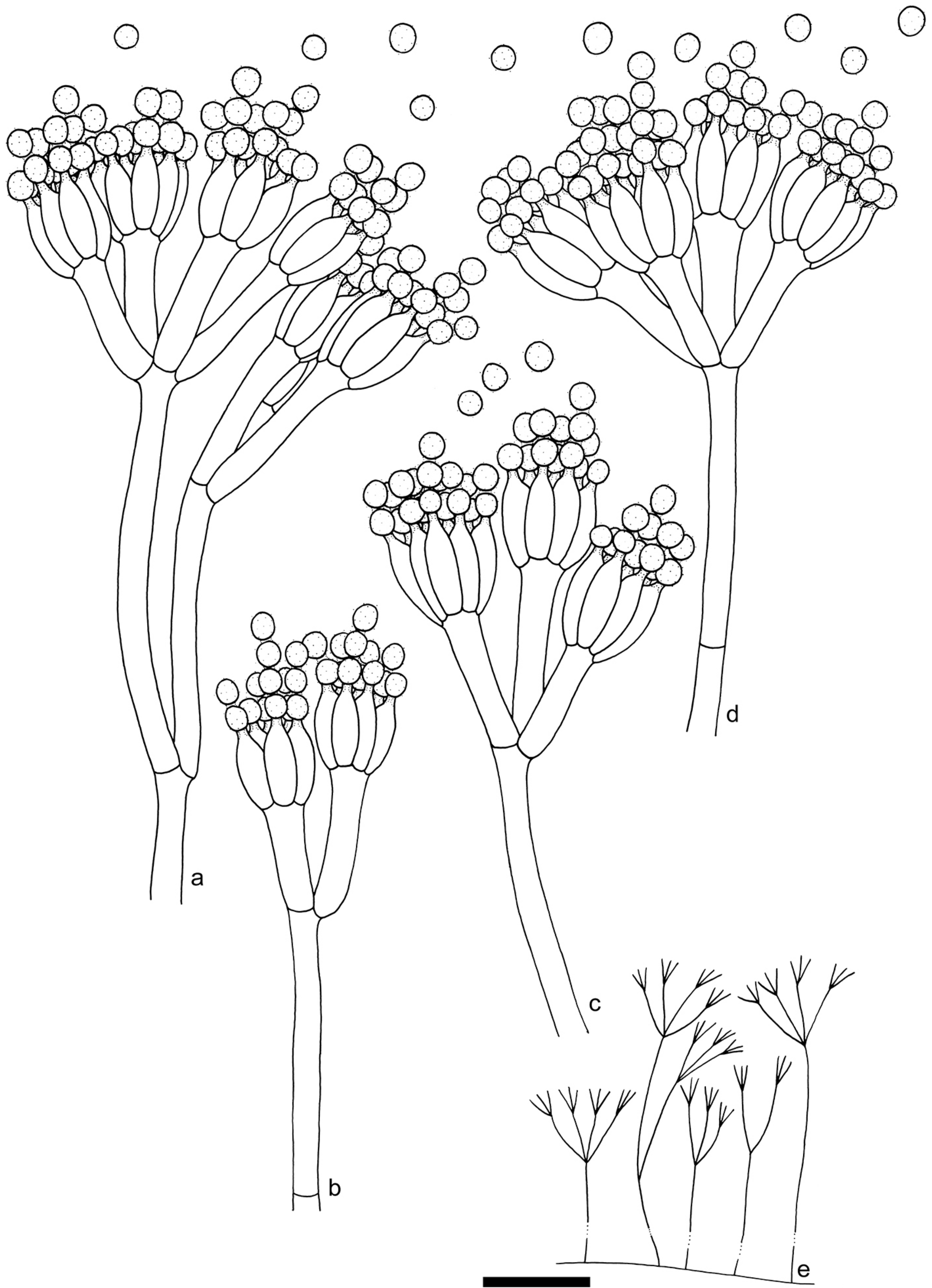

PLATE 98. Line drawing of *P. sizovae*. a–d. Conidiophores (— Scale bar = 10 μm). e. Conidiophore branching (— Scale bar = 50 μm).

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56. *Penicillium sucrivorum* Visagie prov. nom. PLATES 99, 100, 105g

ETYMOLOGY: Latin, *sucrivorum* = meaning sugar-eating; named after the fast growth observed on the sugar rich YES medium
EX-TYPE: CV1840 = DTO183E5 = KAS4046 = DAOM241042
TYPE ISOLATED FROM: Mite, *Protea repens* infructescences, Struisbaai.
ADDITIONAL SPECIMENS EXAMINED: This species is based on a single strain.

Macromorphology — CYA, 25 °C, 7d: Colonies 27–31 mm, moderately deep, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderate, conidia *en masse* greyish green (25D3–25D5) and greyish turquoise (24B3); exudate absent, soluble pigment yellow, reverse pigmentation yellow to greyish yellow (2A7–2A8–2B8–2B7).

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 12–15 mm, low, radially sulcate; margins low, narrow (1–2 mm), irregular; mycelia white; texture velutinous and floccose; sporulation sparse, conidia *en masse* greyish turquoise to greyish green (24C4–25C4); exudate absent, soluble pigment yellow, reverse pigmentation brownish orange (5C6) near centre, fading into light yellow (3A5) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 28–31 mm, low, plane; margins low to subsurface, narrow (1 mm), entire; mycelia white; texture velutinous, with floccose mycelia present; sporulation moderately dense to dense, conidia *en masse* greyish green (25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation greyish green to greenish grey (1C2–1C3–1B2).

YES, 25 °C, 7d: Colonies 45–50 mm, low, radially sulcate, centrally sunken in, having an almost brownish khaki colour; margins low, narrow (1 mm), somewhat irregular; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish green (25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation yellow (2A7–3A8).

G25N, 25 °C, 7d: Colonies 16–20 mm, moderately deep, lightly concentrically sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment light yellow present, reverse pigmentation greenish yellow (1A7) at centre, fading into pale yellow (1A3) margin.

CREA, 25 °C, 7d: Colonies 20–22 mm, no acid production.

Micromorphology — Conidiophores biverticillate, subterminal branching not common; stipes smooth, 160–700 × 2.5–3.5 µm; branches when present only 2, 15–32 × 2.5–3.5 [21.4±6.5] µm (only four branches measured); metulae 2–6, divergent, 48–91° [70±9.7°], 10.5–18 × 2.5–4 [13.5±1.4 × 3.3±0.26], vesicle 3–6 [4±0.51] µm; phialides ampulliform, 6–10 per metula, 7–10 × 2.5–3.5 [8.4±0.57 × 3±0.2] µm; conidia finely rough walled, broadly ellipsoidal to spheroid 2–2.5 × 2–2.5 [2.6±0.1 × 2.3±0.1] µm, average width/length = 0.88±0.04, n= 89.

Notes — This species typically produces biverticillate conidiophores with smooth walled stipes and finely rough walled spheroid conidia. Colonies typically produce yellow colors, especially soluble pigments on CYA and MEA. It is phylogenetically placed in a clade with *P. aurantiacobrunneum*, *P. neomiczynskii* and *P. miczynskii*. However, *P. aurantiacobrunneum* at most produce colonies that have a 3 mm diameter on CYA at 30 °C. *Penicillium miczynskii* and *P. neomiczynskii* do not grow at 30 °C, compared to the new Fynbos species that displays growth of 12–15 mm on CYA at 30 °C. *Penicillium sucrivorum* also displays faster growth on YES (45–50 mm), compared to *P. aurantiacobrunneum* (31–35 mm) *P. miczynskii* (26–33 mm) and *P. neomiczynskii* (25–31 mm). Conidiophores mostly share similar dimensions and characteristics and morphological distinctions are very difficult. However, ITS, β-tubulin and Calmodulin sequence data confirm *P. sucrivorum* as a distinct species (FIGURES 18, 19).

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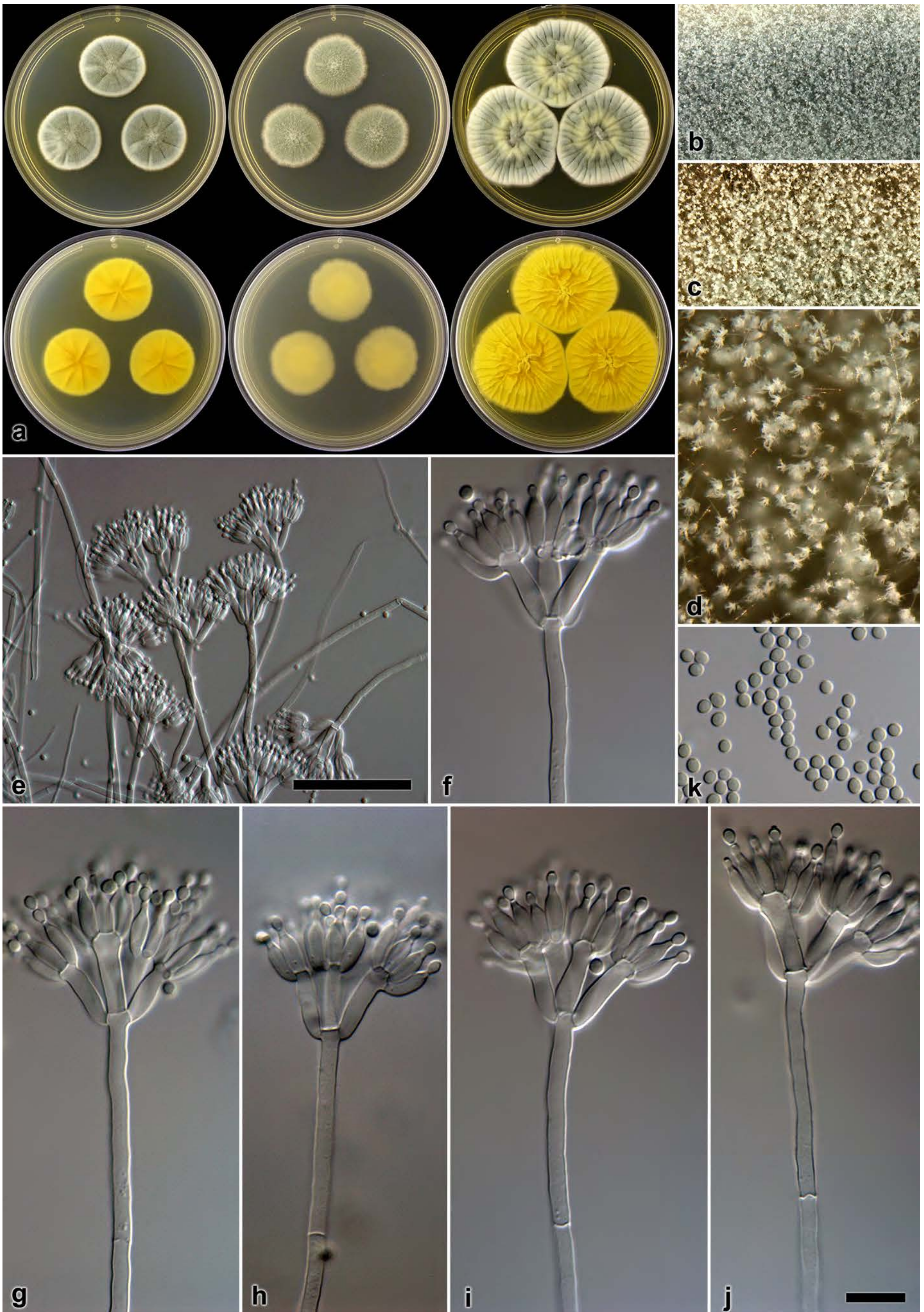

PLATE 99. *Penicillium sucrivorum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 µm; — Scale bar in j = 10 µm, applies to f–k).

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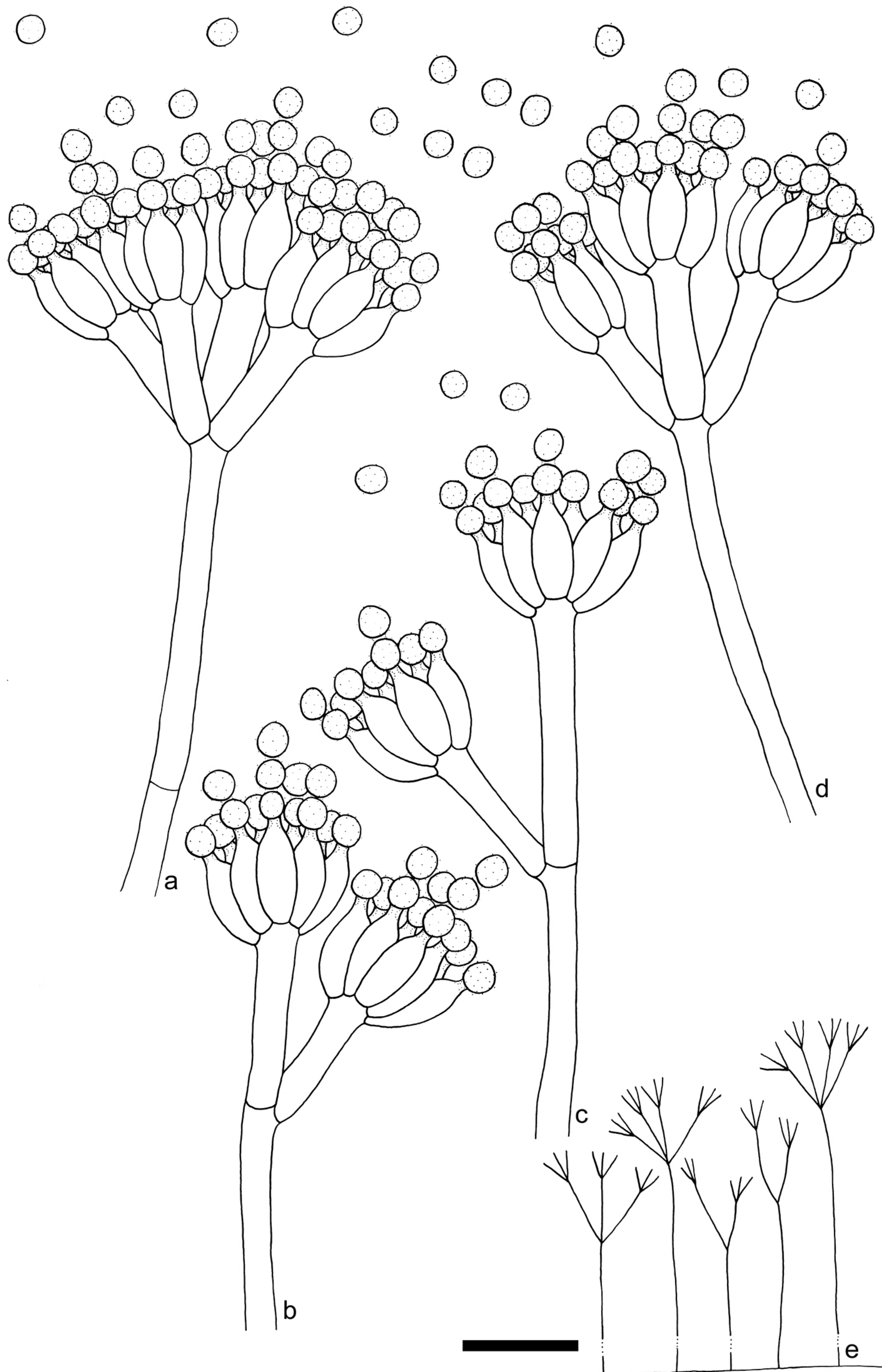

PLATE 100. Line drawing of *P. sucrivorum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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57. *Penicillium sumatrense* von Szilvinyi PLATES 101, 102, 105h

Archiv für Hydrobiologie 6: 533. 1936.

EX-TYPE: CBS281.36 = DTO22F1 = NRRL779 = FRR 779 = IBT4978 = IBT29658
TYPE ISOLATED FROM: Soil, Toba Heath, Sumatra, Indonesia
SPECIMENS EXAMINED: CV503, CV179, CV460, CV684, CV1828, CV1882, CV2201, CV2285, CV2328, CV2403.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescences, Stellenbosch and Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 32–45 mm, low to moderately deep, lightly sulcate, with some strains plane; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish green (25E5–25E7), near margin a lighter greyish green (25B4); exudate clear to yellowish, abundant in some isolates, soluble pigment absent, reverse pigmentation brownish orange to brownish yellow (5C6–5C7) near centre, becoming greyish yellow (4B4) fading into yellowish white (4A2) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: All features similar to CYA at 25 °C except for colonies 24–35 mm, and showing more pronounced sulcation in some isolates.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 22–30 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation similar to CYA at 25 °C.

YES, 25 °C, 7d: Colonies 48–55 mm, low, radially sulcate; margins low, narrow (1–2 mm), entire to somewhat irregular; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate sometimes absent, sometimes clear, soluble pigment absent, reverse pigmentation similar to CYA at 25 °C.

G25N, 25 °C, 7d: Colonies 20–22 mm, low, lightly sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation dull to greyish yellow (3B4–3B5) at centre, fading into pale yellow (2A3) margin.

CREA, 25 °C, 7d: Colonies 18–20 mm, no acid production.

Micromorphology — Conidiophores mostly biverticillate, terverticillate and subterminal branching not uncommon; stipes smooth, 220–500 × 2.5–3 µm; branches when present only 2, divergent, 15–35 × 2.5–3 [25.6±4.89] µm; metulae 3–6, divergent, 30–75° [52±9.3°], 10.5–17 × 2.5–3.5 [13.3±1.4 × 3.1±0.28] µm, vesicle 2.5–4.5 [3.6±0.4] µm; phialides ampulliform, 5–12 per metula, 7–9 × 2.5–3 [8±0.49 × 2.8±0.18] µm; conidia smooth walled, spheroid, broadly ellipsoidal to subspheroid, 2–2.5 × 2–2.5 [2.3±0.13 × 2.1±0.17] µm, average width/length = 0.89±0.04, n= 118.

Notes — *Penicillium sumatrense* produce fast growth on CYA (25 & 37 °C) and YES. Its growth rates are similar to that of *P. sizovae*, except for *P. sumatrense* that grows more restricted on MEA. Houbraken *et al*. (2011) reported sequence variation in *P. sumatrense* strains, although no meaningful differences were observed for phenotypes or extrolite patterns. This sequence variation was also observed for the Fynbos strains (FIGURES 18, 19). However, morphogroups were observed for Fynbos strains that correlated with clades formed for *P. sumatrense*. The morphological differences were considered minor, with slight differences observed for growth rates and exudate production on CYA and YES. Also, when all the strains from the Houbraken *et al*. (2011b) study were included in our analysis, these differences became less apparent.

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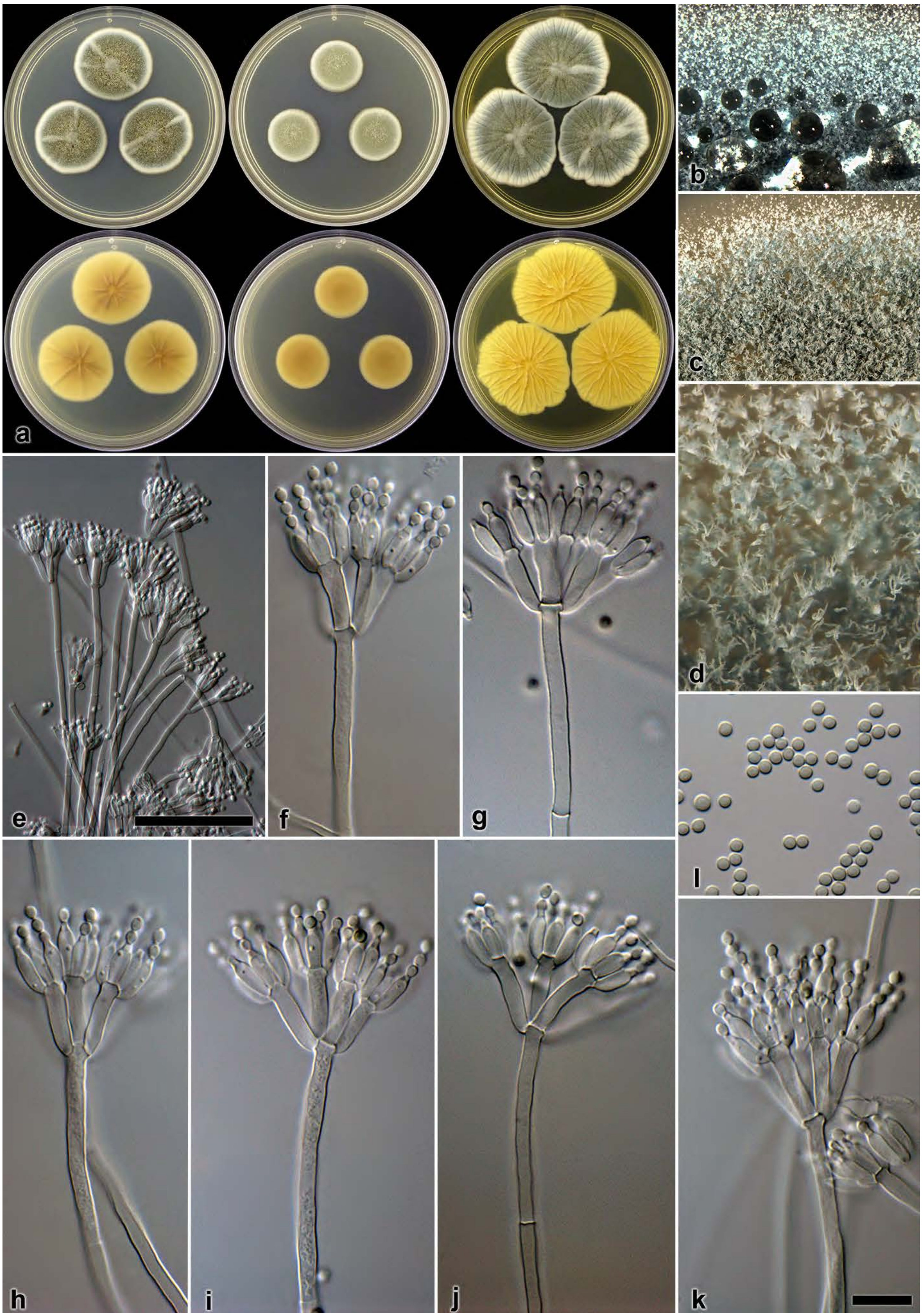

PLATE 101. *Penicillium sumatrense.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–k. Conidiophores. l. Conidia (— Scale bar in e = 50 µm; — Scale bar in k = 10 µm, applies to f–l).

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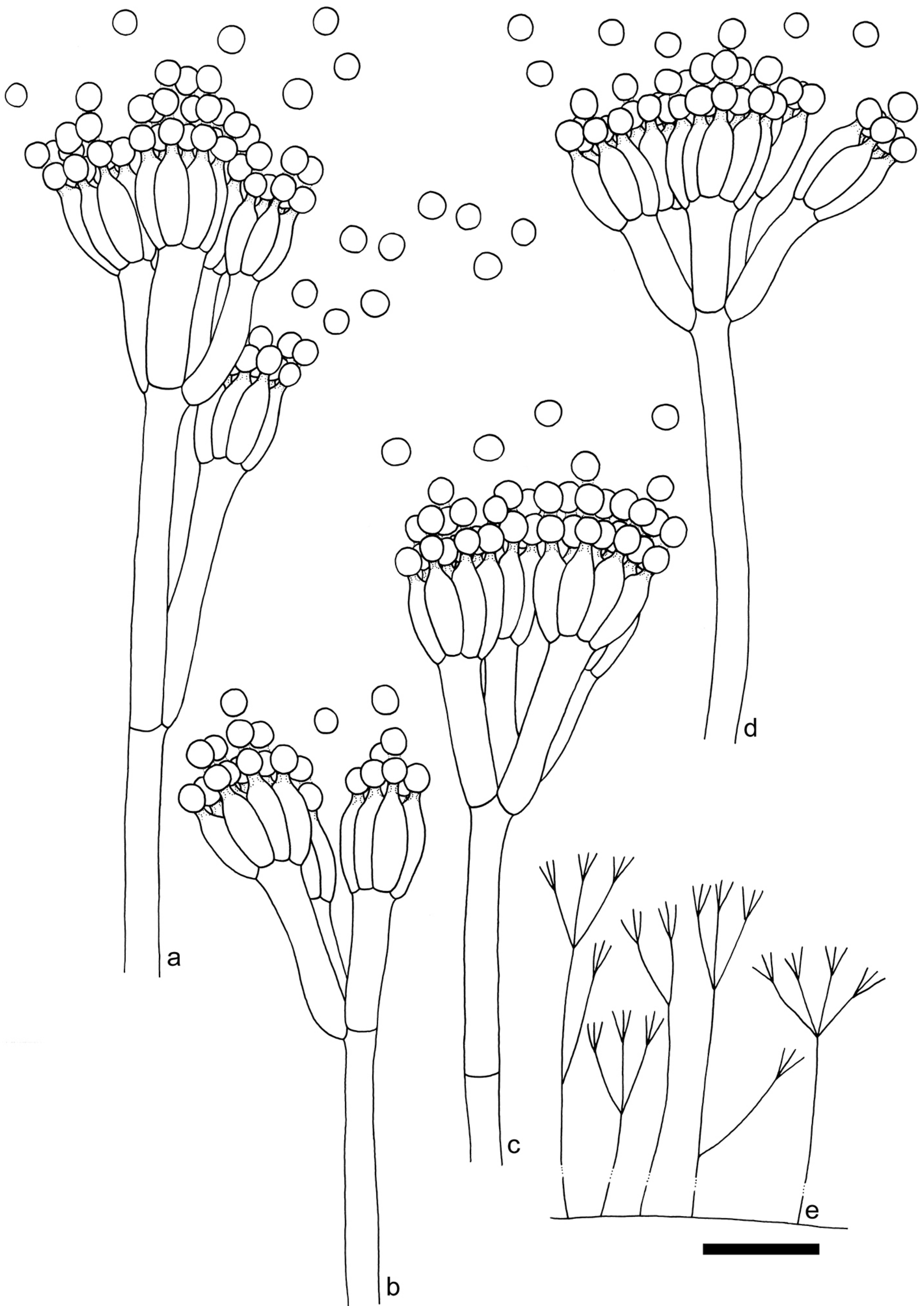

PLATE 102. Line drawing of *P. sumatrense*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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58. *Penicillium ubiquetum* Houbraken, Frisvad & Samson PLATES 103, 104, 105i

Studies in Mycology 70: 127. 2011.

EX-TYPE: CBS126437 = DTO78B5 = IBT22226
TYPE ISOLATED FROM: Soil, Wilson Botanical Garden, Costa Rica
SPECIMENS EXAMINED: CV451, CV588.
ISOLATED FROM: Mites from *Protea repens* infructescences, Stellenbosch

Macromorphology — CYA, 25 °C, 7d: Colonies 25–30 mm, low to moderately deep, lightly sulcate; margins low, narrow, regular; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia *en masse* greyish turquoise (24F5–24E5–24E6); exudate minute clear to yellowish droplets, soluble pigment absent, reverse pigmentation orange (5A6–5A7) and dull yellow (3B3–3B4) areas.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 6–9 mm, randomly furrowed, consisting of white mycelia, with moderately dense sporulation, conidia *en masse* greyish turquoise (24D4–24D5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (2C4–3C4).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 17–22 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense to dense, conidia *en masse* greyish turquoise (24E5–24E6); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A4) at centre, fading into greyish yellow (3B5–4C4) to lighter greyish yellow (3C3) near the yellowish white (3A2) margin.

YES, 25 °C, 7d: Colonies 33–36 mm, moderately deep, radially sulcate; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense to dense, conidia *en masse* similar to CYA at 25 °C; exudate absent, soluble pigment absent, reverse pigmentation orange (6A8–6B8) near centre, dull yellow (3B3–3B4) elsewhere.

G25N, 25 °C, 7d: Colonies 14–16 mm, low, radially and concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull to greyish green (25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation yellow (3A7) at centre, greyish yellow (3B6) elsewhere.

CREA, 25 °C, 7d: Colonies 18–23 mm, very moderate acid production only in area beneath colonies.

Micromorphology — Conidiophores biverticillate, with minor subterminal branches present; stipes smooth walled, 195–430 × 2.5–3.5 µm; branches when present only 2, 18–50 × 2.5–3.5 [28.9±3.8] µm; metulae 3–7, divergent, 45–100° [70±10.2°], 9–20 × 2.5–4 [14.5±2.7 × 3.3±0.35] µm, vesicle 3–5 [4±0.5] µm; phialides ampulliform, 7–12 per metula, 7–11.5 × 2.5–4 [9±1.2 × 3.1±0.3] µm; conidia finely rough walled, spheroid to broadly ellipsoidal, 2–3 × 2–3 [2.5±0.2 × 2.4±0.2] µm, average width/length = 0.96±0.03, n= 73.

Notes — This species characteristically produce orange to reddish coloration on CYA and YES. Its closest relative is *P. pancosmium*. However, *P. pancosmium* produces yellow colors in CYA and YES, compared to the orange to red observed in *P. ubiquetum*. Also, between the Fynbos isolates, *P. ubiquetum* grew more restricted than *P. pancosmium* (CYA 30°C 12–14 mm; MEA 22–26 mm), although this is only a minor difference.

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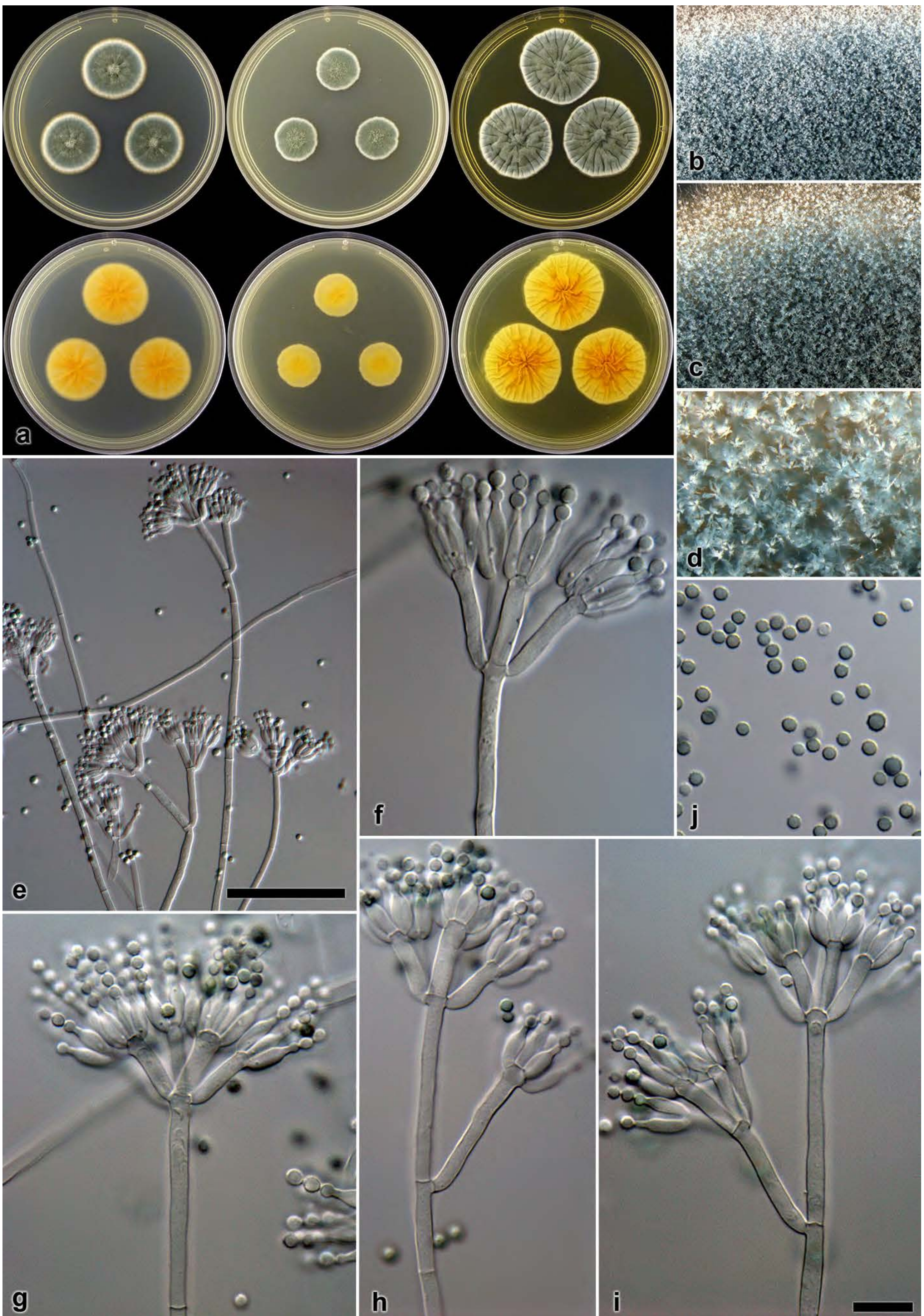

PLATE 103. *Penicillium ubiquetum.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–i. Conidiophores. j. Conidia (— Scale bar in e = 50 µm; — Scale bar in i = 10 µm, applies to f–j).

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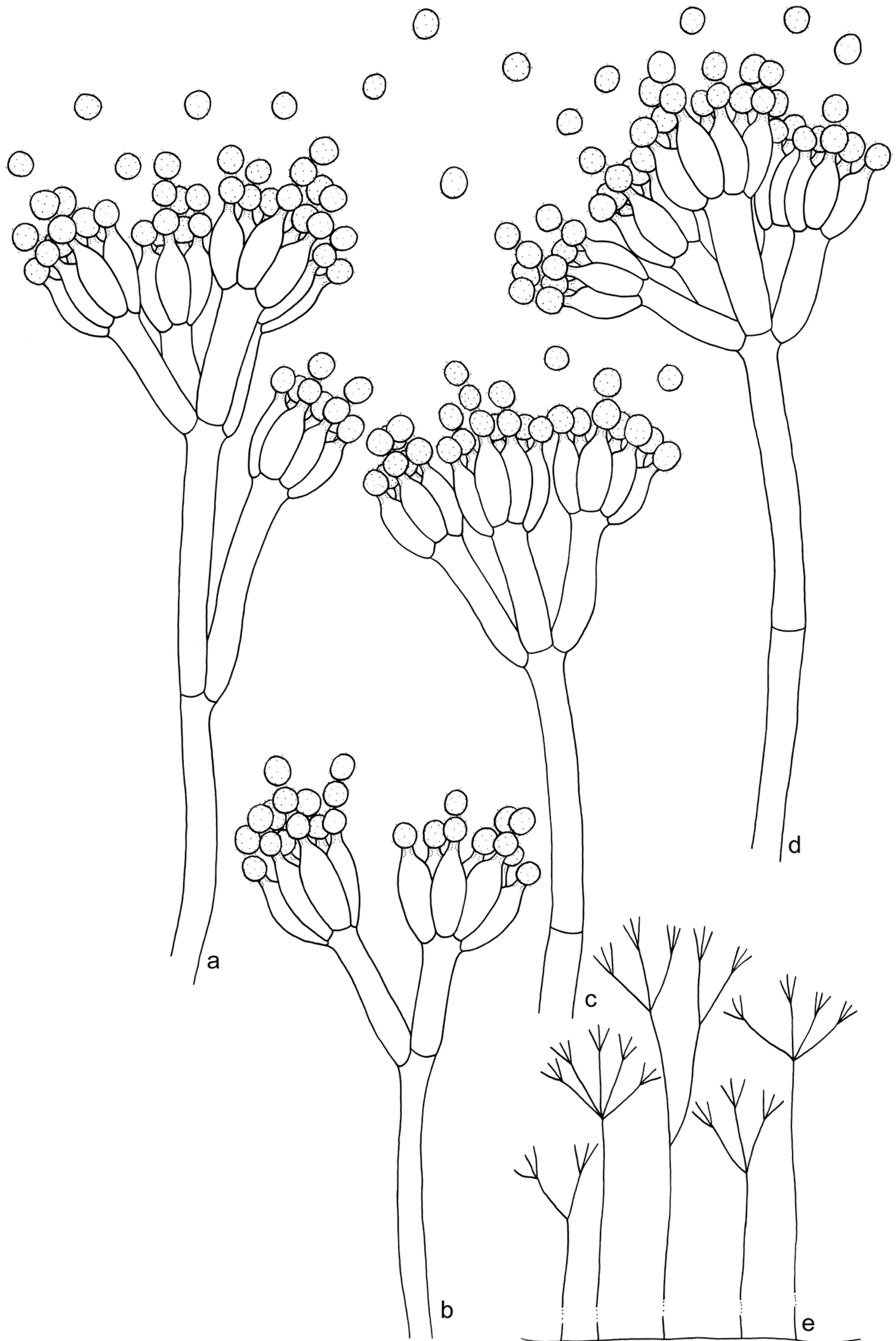

PLATE 104. Line drawing of *P. ubiquetum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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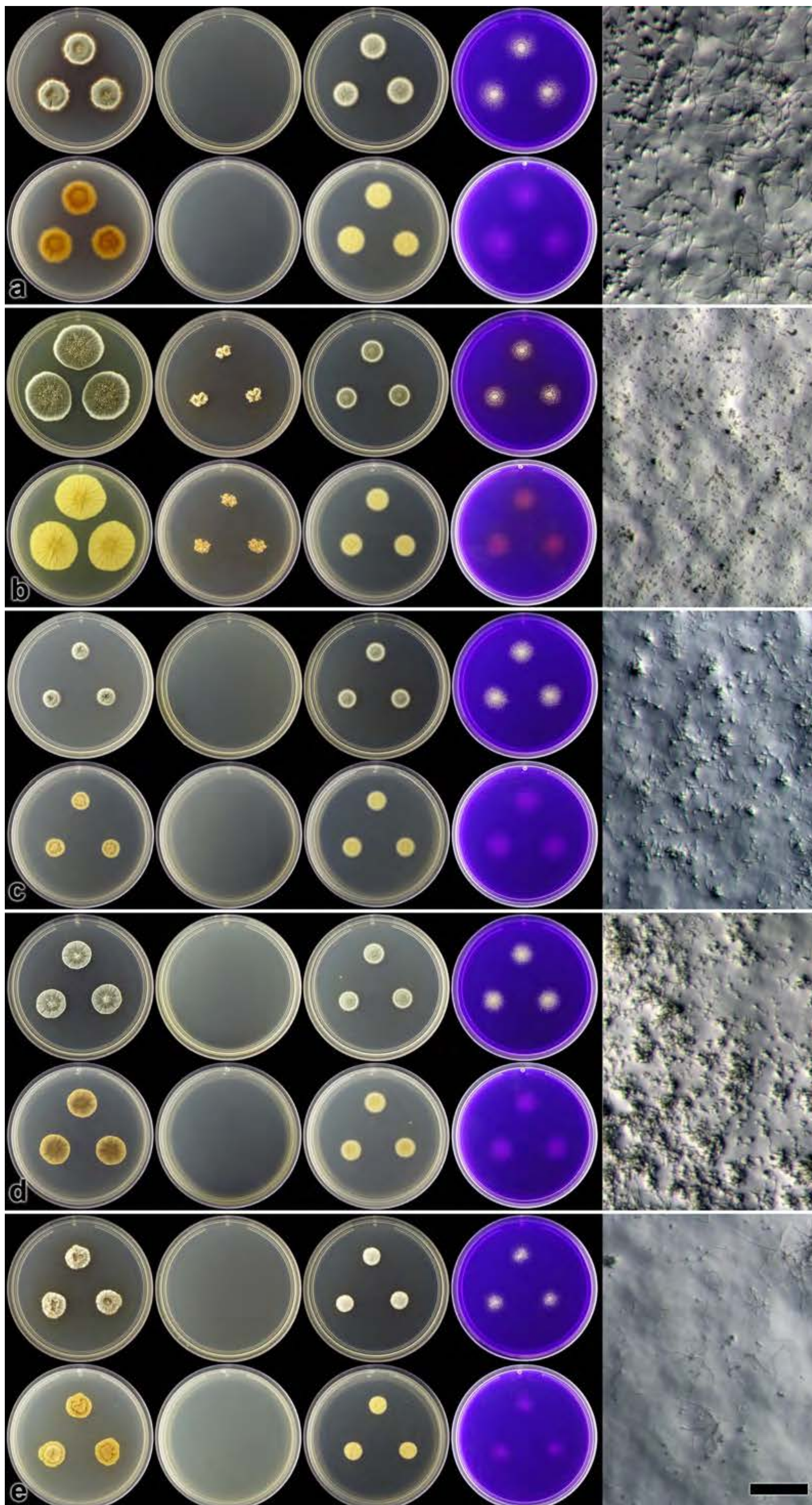

PLATE 105. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *P. cairnsense*. b. *P. citrinum*. c. *P. pancosmium*. d. *P. pasqualense*. e. *P. sanguifluum.*

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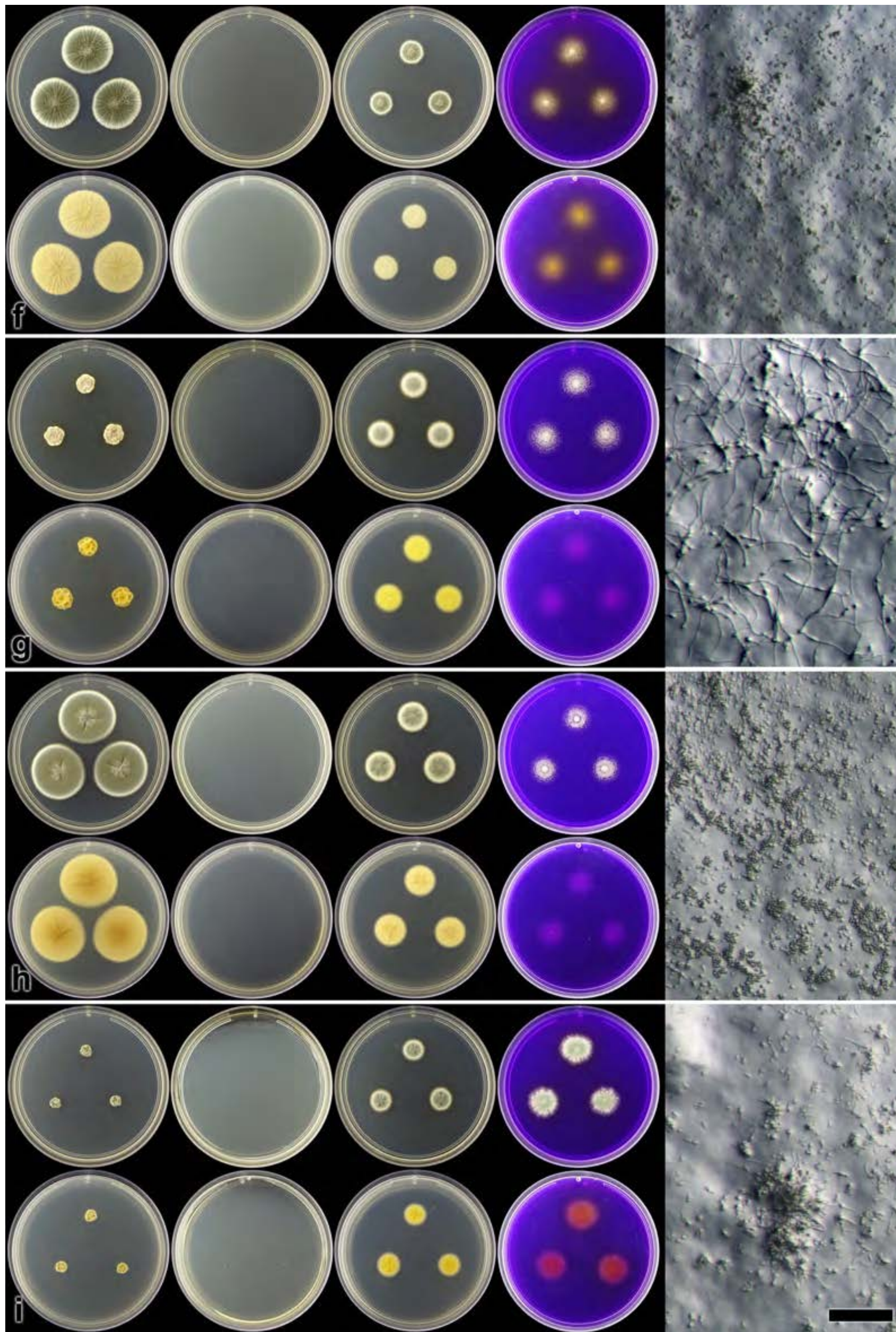

PLATE 105. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). f. *P. sizovae*. g. *P. sumatrense*. h. *P. sucrivorum*. i. *P. ubiquetum.*

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The section *Ramigena* Thom

The Penicillia 225. 1930.

SPECIES TREATED: *Penicillium cyaneum*

Thom (1930) introduced section *Ramigena* for species that was considered as monoverticillate, although subterminal branches are sometimes formed. Species in this section never produce an apical verticil of metulae and the conidiophores or branches have no specific organization or arrangement (Thom 1930, Houbraken & Samson 2011). The section also contained species that was described by Bainier & Sartory (1913) in the genus *Citromyces*. Pitt (1979) classified most of the species currently accommodated in section *Ramigena* (Houbraken & Samson 2011), in section *Exilicaulis*, series *Restricta* and *Citreonigra*. These groups were characterized by restricted growth, monoverticillate conidiophores with stipes that are non-vesiculate and that produce large (3–4 µm), smooth ellipsoidal (sometimes) pyriform conidia (Pitt 1979). However, phylogenetic data show that this group of species is distantly related to the section *Exilicaulis* (Houbraken & Samson, 2011) (FIGURE 1). Based on the Houbraken & Samson (2011) four-gene phylogeny, 7 species were included in the section.

The single species of section *Ramigena* isolated from Fynbos, was identified as *P. cyaneum*. Morphologically they were found to be similar to *P. dierckxii* and *P. sublateritium*. The strains resolved in a clade together with the type strains of *P. cyaneum* (NRRL775), *P. sublateritium* (CBS267.29) and *P. dierckxii* (NRRL755) (FIGURE 20). This was consistent for all phylogenies and the species are accepted here as synonyms, with *P. cyaneum* (= *C. cyaneus*) the older name. Thom (1930), Raper & Thom (1949) and Pitt (1979) accepted both *P. sublateritium* and *P. cyaneum* as distinct species. Pitt (1979) considered the blue conidia and floccose texture of *P. cyaneum* compared to velutinous and green conidia in *P. sublateritium*, although minor, enough to consider them distinct. *Penicillium ramusculum* was also considered as a synonym of *P. sublateritium*, but the phylogenies show that it is more similar to *P. brevissimum*. Pitt (1979) considered *P. dierckxii* as a synonym to *P. citreonigrum,* with Raper & Thom (1949) reducing it to synonymy with *P. fellutanum*. Although the strain we examined displayed poor sporulation, it clearly resembled colonies and conidiophores of *P. cyaneum*.

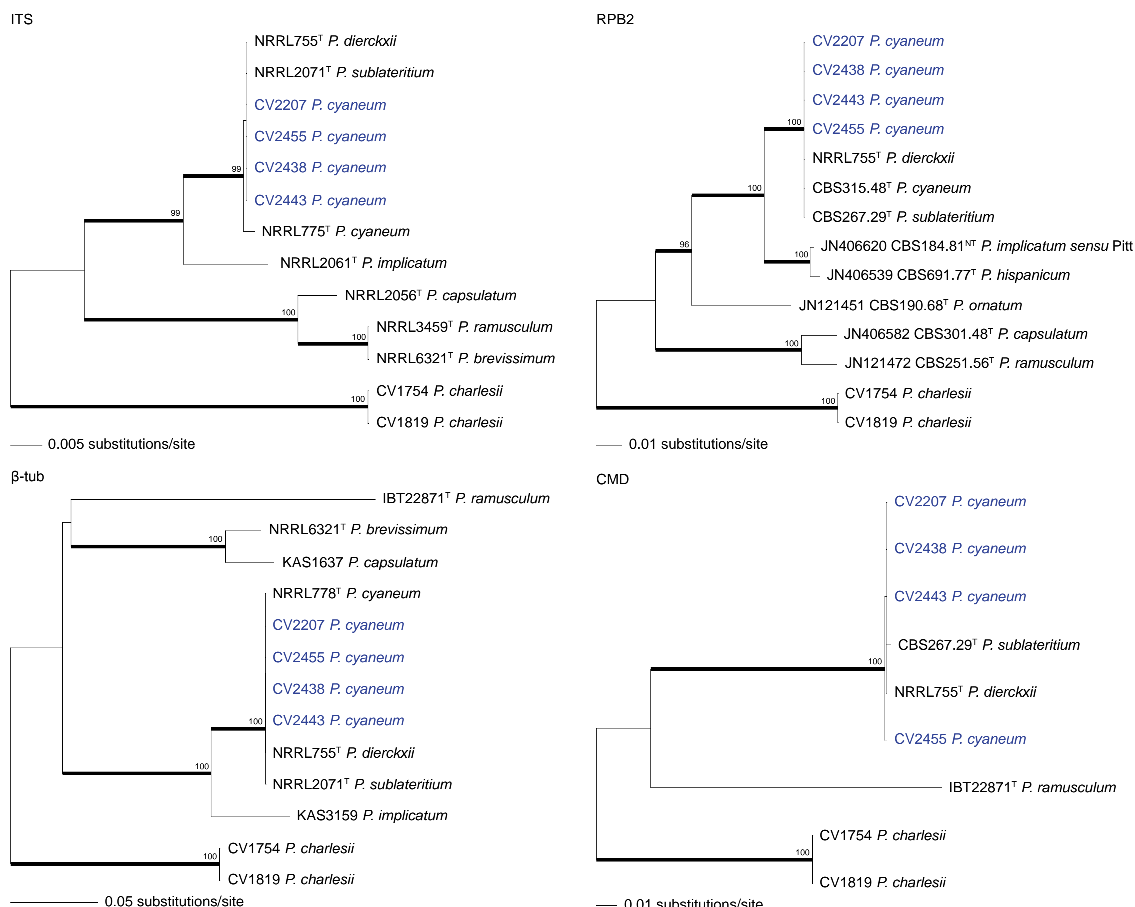

FIGURE 20: Phylogenetic trees based on ITS, RPB2, β-tubulin and Calmodulin, showing relationship of species in the section *Ramigena*. *Penicillium charlesii* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. ([T] = ex-type). Colored names indicate strains isolated from Fynbos.

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59. *Penicillium cyaneum* (Bainier & Sartory) Biourge PLATES 106, 107, 108
La Cellule 33: 102. 1923.

BASIONYM: *Citromyces cyaneus* Bainier & Sartory (Société Mycologique de France, Bulletin Trimestriel 29: 38. 1913)
SYNONYMS: = *P. dierckxii* Biourge (La Cellule 33: 313. 1923), = *P. sublateritium* Biourge (La Celulle 33: 315. 1923)
EX-TYPE: CBS315.48 = ATCC10432 = IMI039744 = NRRL775
TYPE ISOLATED FROM: Unrecorded source, France
SPECIMENS EXAMINED: NRRL775, NRRL2071, CV2207, CV2435, CV2438, CV2443
ISOLATED FROM: Mites and bracts from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 13–17 mm, moderately deep, radially and concentrically sulcate, raised at centre; margins low, very narrow, entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish to deep turquoise (24E6–24E8); exudate absent, soluble pigment absent, reverse pigmentation at centre brownish yellow (5C7–5C8), becoming orange (5B7–5B8) to greyish yellow (4C7) at margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 5–8 mm, moderately deep, irregularly shaped, olive yellow to greyish yellow (3C3–3C6); low, very narrow, entire; mycelia light yellow; texture velutinous; sporulation sparse, conidia *en masse* seems greyish green, but difficult to determine; exudate absent, soluble pigment absent, reverse pigmentation ring of light brown (5D7–5D8) amongst the normal yellowish grey to greyish yellow (4B3–4B4).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 14–20 mm, low, plane; margins low, narrow, entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish to deep turquoise (24E6–24E8); exudate absent, soluble pigment absent, reverse pigmentation orange yellow (4B7–4B8) at centre fading into olive brown (4D7–4D8).

YES, 25 °C, 7d: Colonies 18–22 mm, moderately deep, radially and concentrically sulcate, rising towards centre, but then sunken in at centre; margins low, narrow, entire; mycelia white; texture velutinous, conidia breaking of in crusts; sporulation dense, conidia *en masse* greyish turquoise to deep turquoise (24E6–24E8); exudate absent, soluble pigment absent, reverse pigmentation Golden Yellow (5B7) at centre becoming greyish yellow (4B6) to pale yellow (3A3) at margin.

G25N, 25 °C, 7d: Colonies 7–10 mm, low, plane, slightly raised at centre; margins low, very narrow entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* greyish turquoise to deep turquoise (24E6–24E8); exudate absent, soluble pigment absent, reverse pigmentation dark green (28F5–28F7) at centre, greyish green (28B3) elsewhere.

CREA, 25 °C, 7d: Colonies 6–8 mm, acid moderately produced.

Micromorphology — Conidiophores strictly monoverticillate; stipes smooth walled, 45–120 × 2–3 µm, vesicle 3–5 [3.97±0.45] µm, vesicle/stipe width 1.2–2 [1.5±0.18]; phialides acerose-ampulliform, sometimes very long and thin, mostly appressed, sometimes divergent, 5–8(–12) per stipe, 8.5–14.5 × 2.5–3.5 [10.65±1.12 × 2.8±0.23] µm; conidia smooth walled, connective scars visible with connectives seen when in chains, subsperoidal to broadly ellipsoidal, larger conidia pyriform, 2.5–4(–4.5) × 2–3(–3.5) [3.1±0.4 × 2.3±0.29] µm, average width/length ± stdev = 0.76±0.07, n = 93.

Notes — *Penicillium cyaneum* displays slow growth and compact colonies that have a typical turquoise conidial color. Conidiophores are strictly monoverticillate, non-vesiculate, with all features smooth walled. Pitt (1979) reported stipes of up to 300 µm long. However, the longest stipe measured for the Fynbos strains was 120 µm. *Penicillium cyaneum* is closely related to *P. implicatum, P. capsulatum* and *P. ramusculum* (FIGURE 20). *Penicillium implicatum* and *P. ramusculum* typically grew well at 37 °C, compared to *P. cyaneum* strains that did not germinate. *Penicilium cyaneum* also on average produced bigger conidia than *P. implicatum* strains. The *P. ramusculum* strain examined was badly deteriorated, but did show similar growth patterns to *P. cyaneum*. Therefore, morphologically this species could not be distinguished from *P. cyaneum* even though phylogenetically it is distinct.

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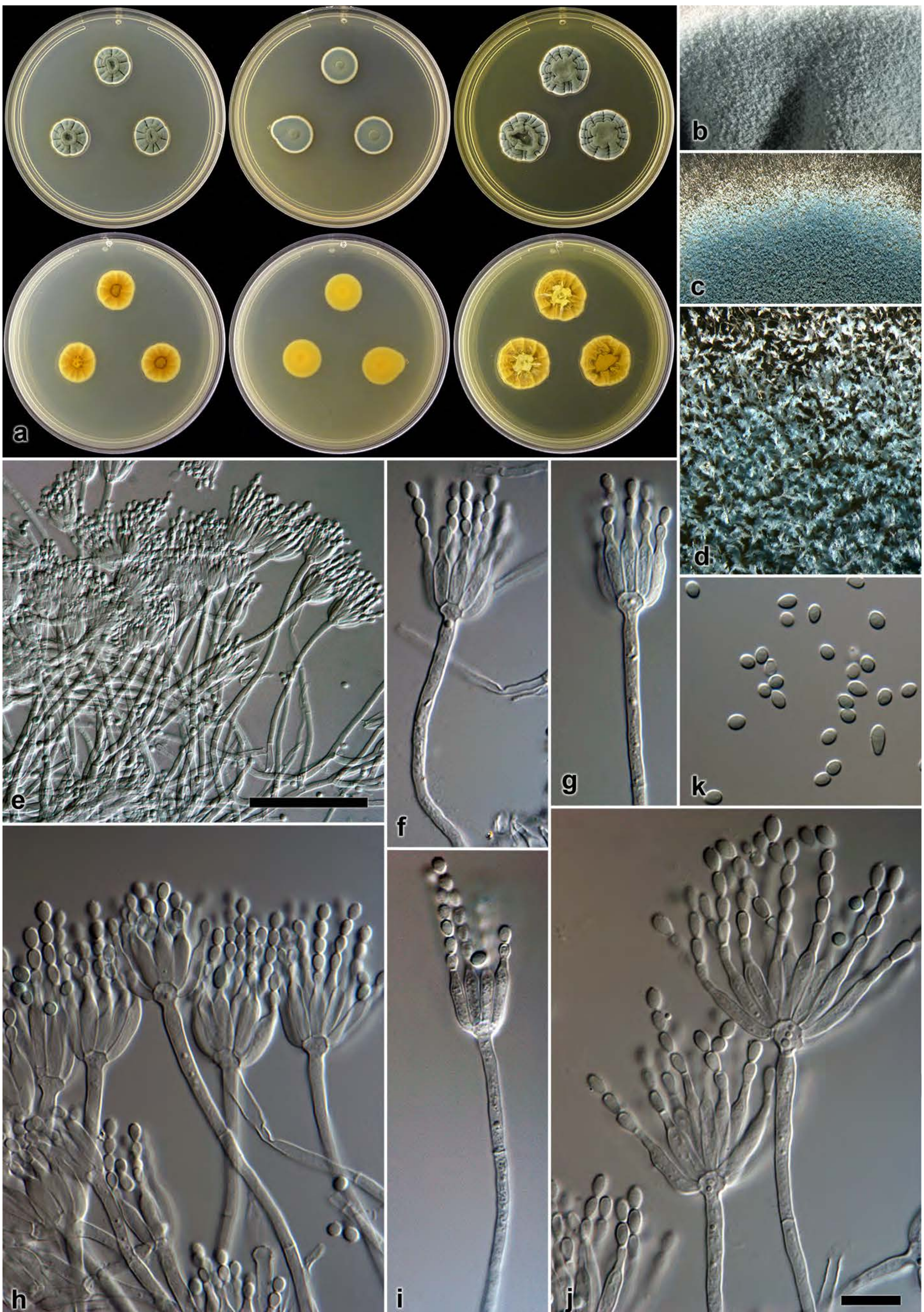

PLATE 106. *Penicillium cyaneum* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c, d. Texture on MEA. e–j. Conidiophores. k. Conidia (— Scale bar in e = 50 μm; — Scale bar in j = 10 μm, applies to f–k).

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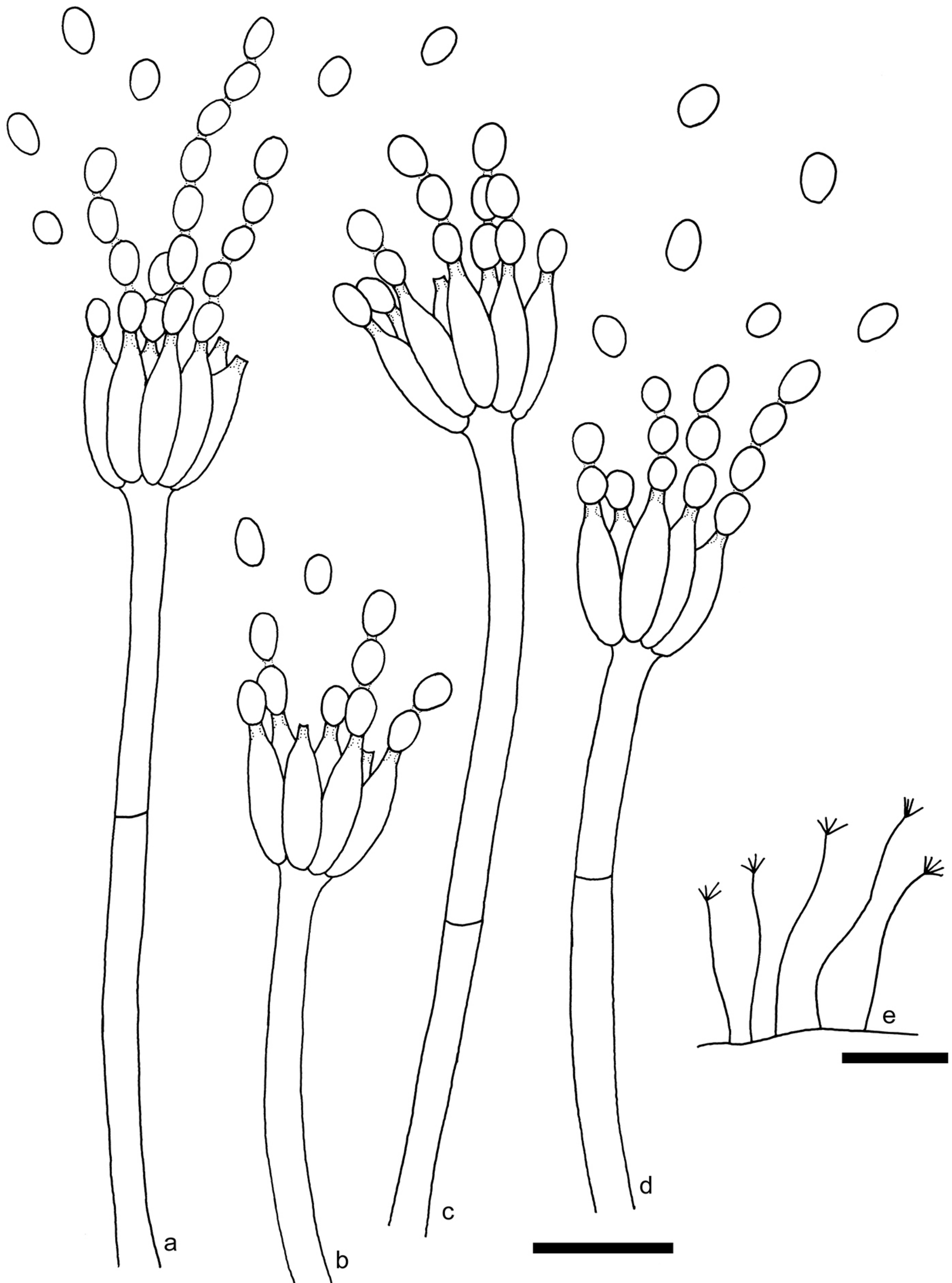

PLATE 107. Line drawing of *P. cyaneum*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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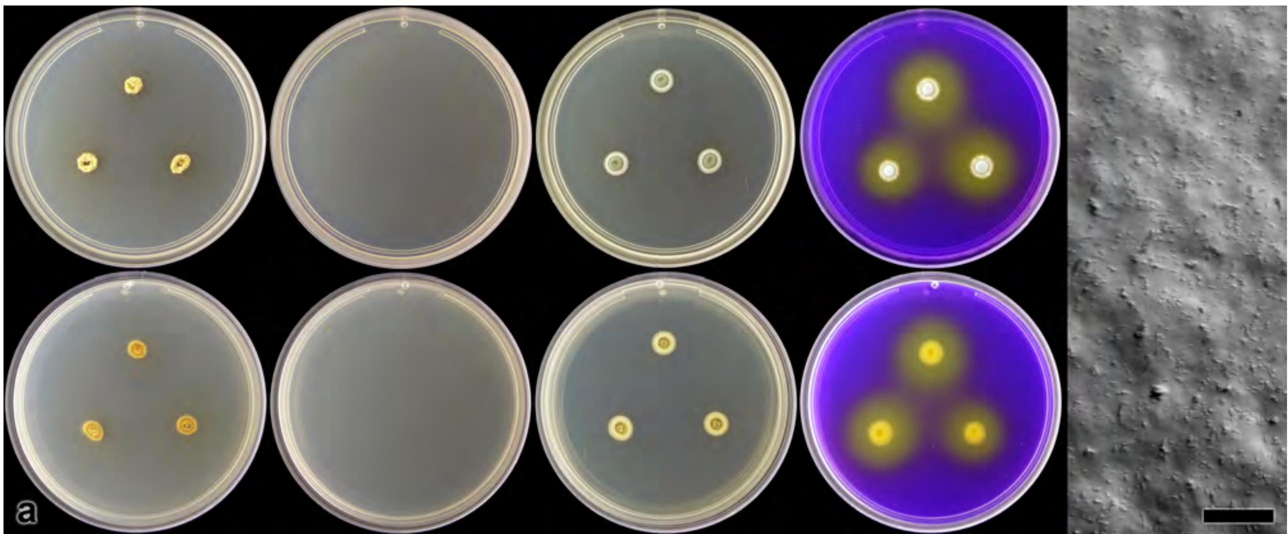

PLATE 108. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium cyaneum.*

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The section *Charlesia* Houbraken & Samson

Studies in Mycology 70: 33. 2011.

SPECIES TREATED: *Penicillium charlesii, P. fellutanum*

Houbraken & Samson (2011) introduced section *Charlesia* for species that typically produce monoverticillate conidiophores that have apical swellings. However, *Penicillium charlesii* and *P. fellutanum* do produce a minor proportion of irregular biverticillate conidiophores. Peterson *et al*. (2005) studied this group of species and their phylogenetic relationships and described a number of new species. Although Houbraken & Samson (2011) showed that section *Charlesia* is monophyletic, this was not observed for the ITS phylogeny present here. For instance, Section *Ramigena* splits section *Charlesia*, with *P. charlesii* and *P. fellutanum* that form one clade, distant from *P. chermesinum, P. indicum* and *P. coffeae* (FIGURE 1g). ITS sequence alignments across a diverse genus like *Penicillium* can often be problematic because of gaps in the datasets (Seifert *et al*. 2007, Houbraken & Samson 2011). In the *P. chermesinum* clade, it was observed that its position did shift in other ITS phylogenies, meaning that there is not much support for this section in ITS.

Fynbos strains from section *Charlesia* conformed to the characters of *P. charlesii* and *P. fellutanum*. Raper & Thom (1949) classified *P. charlesii* in section *Ramigena* but placed *P. fellutanum* in the *P. decumbens* series, which were characterized by a group of species that are strictly monoverticillate and have small conidiophores borne laterally from aerial hyphae (Raper & Thom 1949). Section *Ramigena* on the other hand incorporated species that typically have short side branches. Raper & Thom (1949) did no consider *P. fellutanum* and *P. charlesii* as close relatives. However, Pitt (1979) noticed the irregular branching patterns in the two species and considered the two as synonyms. He accepted *P. fellutanum* as the valid name and classified it in his subgenus *Furcatum* section *Divaricata*, series *Fellutana*. This group contained species that display typical slow growth on media and have divergent, irregular conidiophores, which now included the species *P. charlesii*.

Recent phylogenetic data suggest that these two species, although closely related, are distinct. However, morphologically these two are difficult to distinguish. The irregular biverticillate conidiophores were more often observed for *P. fellutanum* than *P. charlesii*. Also, *P. fellutanum* produce subterminal branches that is much longer than those observed in *P. charlesii.* Differences in growth rates on CYA at 30 °C (*P. fellutanum* 13–16 mm; *P. charlesii* 9–12 mm) and conidial color on MEA (*P. fellutanum* 26D4–26D6–26E6; *P. charlesii* 25E7–25F7) were also found to consistently vary for strains studied. However, in this case, concordance was observed for all the genes studied, which confirmed these two species as unique (FIGURE 21).

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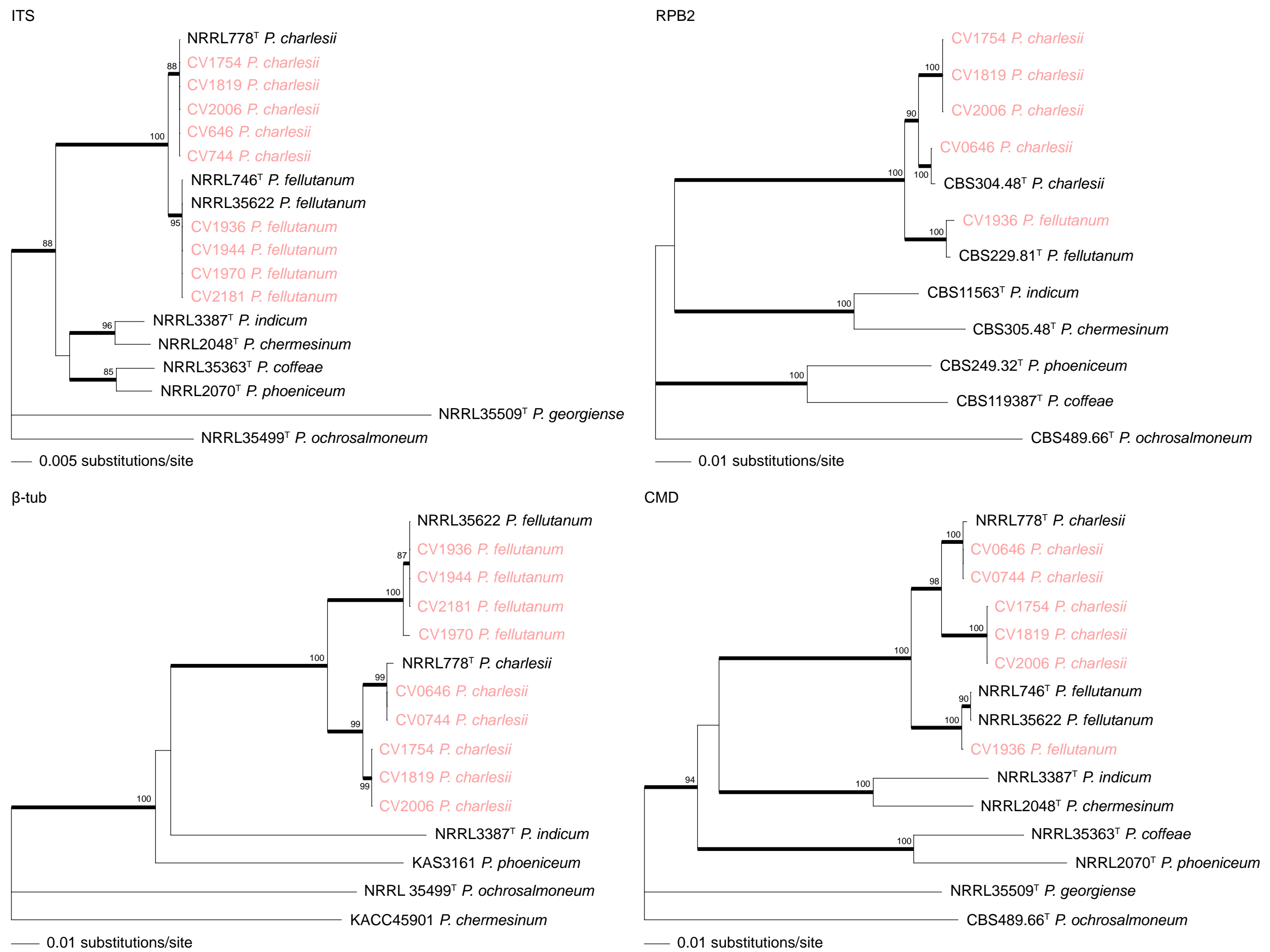

FIGURE 21: Phylogenetic trees based on ITS, RPB2, β-tubulin and Calmodulin, showing relationship of species in the section *Charlesia*. *Penicillium ochrosalmoneum* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type). Colored names indicate strains isolated from Fynbos.

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60. *Penicillium charlesii* Smith PLATES 109, 110, 113a

Transactions of the British Mycological Society 18: 90. 1933.

EX-TYPE: CBS304.48 = ATCC 8730 = CBS342.51 = IMI040232 = NRRL1887 = NRRL778
TYPE ISOLATED FROM: Unknown source, United Kingdom
SPECIMENS EXAMINED: NRRL778, CV1754, CV1819, CV2006, CV646, CV744.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 18–22 mm, moderately deep, sulcate; margins low, very narrow, entire; mycelia white; texture floccose with some velutinous regions; sporulation sparse to moderately dense in some regions, conidia *en masse* dull to greyish green (26E4–26E7); exudate clear, soluble pigment absent, reverse pigmentation dull to pale yellow (3B4–3A3).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 9–12 mm, moderately deep, randomly sulcate, sunken in at centre; margins low, very narrow, entire; mycelia white; sporulation absent; exudate absent, soluble pigment absent, reverse pigmentation pale to light yellow (4A3–4A5).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 14–16 mm, low to moderately deep near centre, plane; margins low, narrow, entire; mycelia white; texture velutinous near margin, floccose elsewhere; sporulation moderately dense, conidia *en masse* greyish green to Myrtle green (25E7–25F7); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at centre, fading into Absinthe Yellow (3C5) towards margin.

YES, 25 °C, 7d: Colonies 25–28 mm, moderately deep, sulcate, raised at centre; margins low, narrow, entire; mycelia white; texture floccose with some velutinous regions; sporulation mostly absent, but CV646 and CV2006 sometimes sporulating, conidia *en masse* dull to greyish green (26E4–26E7); exudate clear minute droplets sometimes produced, soluble pigment absent, dull to pale yellow (3B3–3A3).

G25N, 25 °C, 7d: Colonies 7–10 mm, moderately deep, plane; margins low, very narrow, entire; mycelia white; texture floccose; sporulation moderate, conidia *en masse* dull green to greyish green (25D4–25D6); exudate absent, soluble pigment absent, reverse pigmentation greyish green (30B3–30B4).

CREA, 25 °C, 7d: Colonies 9–12 mm, acid not produced.

Micromorphology — Conidiophores mostly biverticillate, with minor proportion monoverticillate, branching irregular; Stipes smooth walled, length very difficult to determine, when monoverticillate 30–280 × 2–3 µm, when biverticillate 50–350 × 2–3 µm, Branches 17–35 × 2–3 µm; Metulae mostly two per stipe, sometimes three, 10–30 × 2–3 [19.77±4.6 × 2.48±0.28] µm, metulae vesicle 3–5.5 [4.18±0.5] µm; Phialides ampulliform, some long and acerose-like, 8–12 per metula/stipe, 6.5–9.5 × 2–3.5 [7.85±0.66 × 2.76±0.24] µm, Conidia lightly rough walled, connectives and connective scars visible, spheroid to subspheroidal, 2–3 × 2–3 [2.43±0.22 × 2.36±0.25] µm, average width/length 0.85±0.06, n = 93.

Notes — *Penicillium charlesii* is similar to *P. fellutanum*, especially based on colony morphology. However, the latter species consistently produced colonies on MEA with lighter conidial colors. *Penicillium charlesii* also grows more restricted than *P. fellutanum* on CYA at 30 °C. *Penicillium charlesii*, typically produces conidiophores with metulae often borne subterminally. Conidiophore dimensions between the two species are very similar. However, *P. charlesii* conidiophores are much less irregular than *P. fellutanum*. This is seen in conidiophores of *P. charlesii* that has metulae borne on the same stage, whereas P. fellutanum conidiophores are often so irregularly branched that it is difficult to determine whether it is a metulae or very short stipes.

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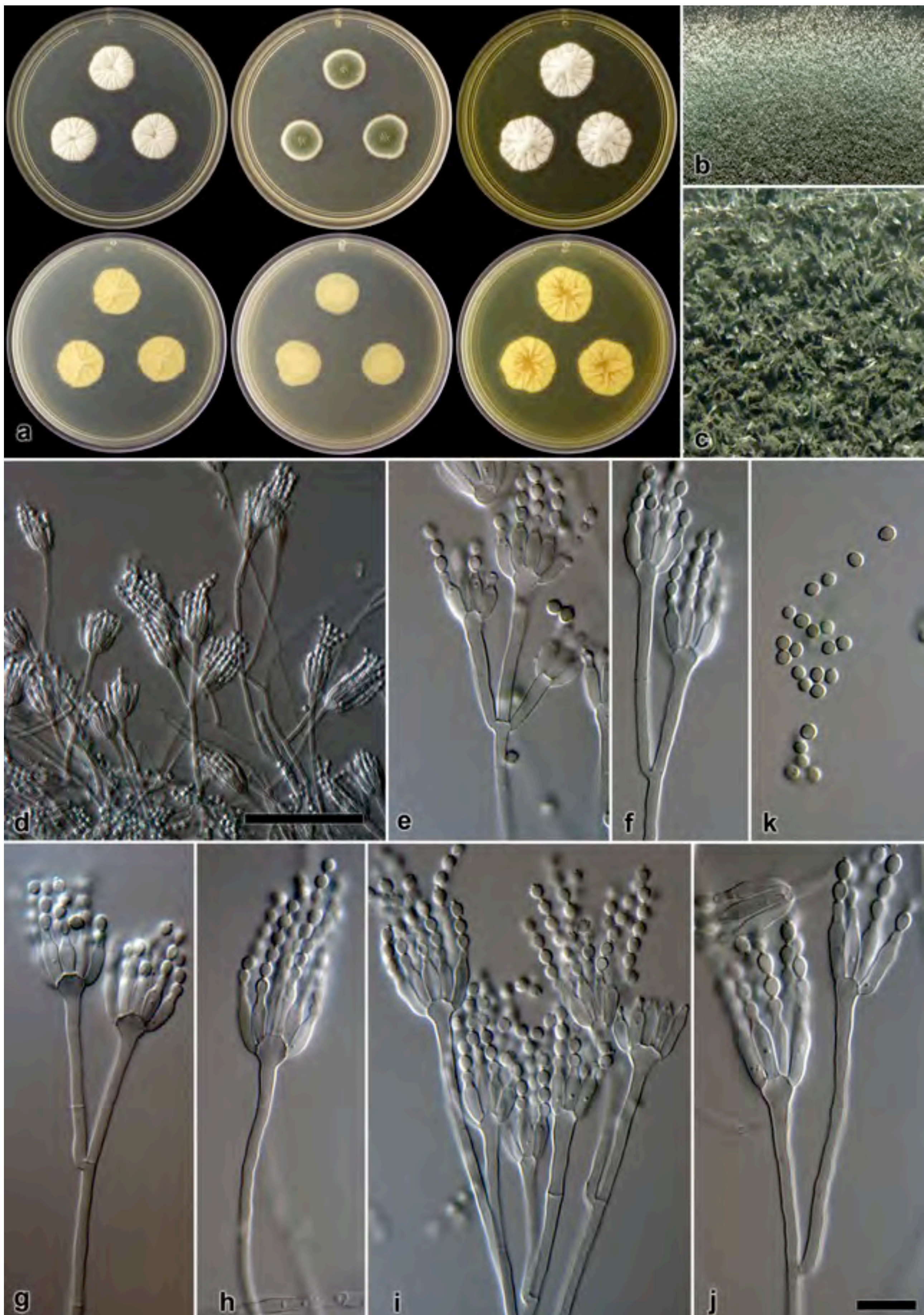

PLATE 109. *Penicillium charlesii* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–j. Conidiophores. k. Conidia (— Scale bar in d = 50 µm; — Scale bar in j = 10 µm, applies to e–k).

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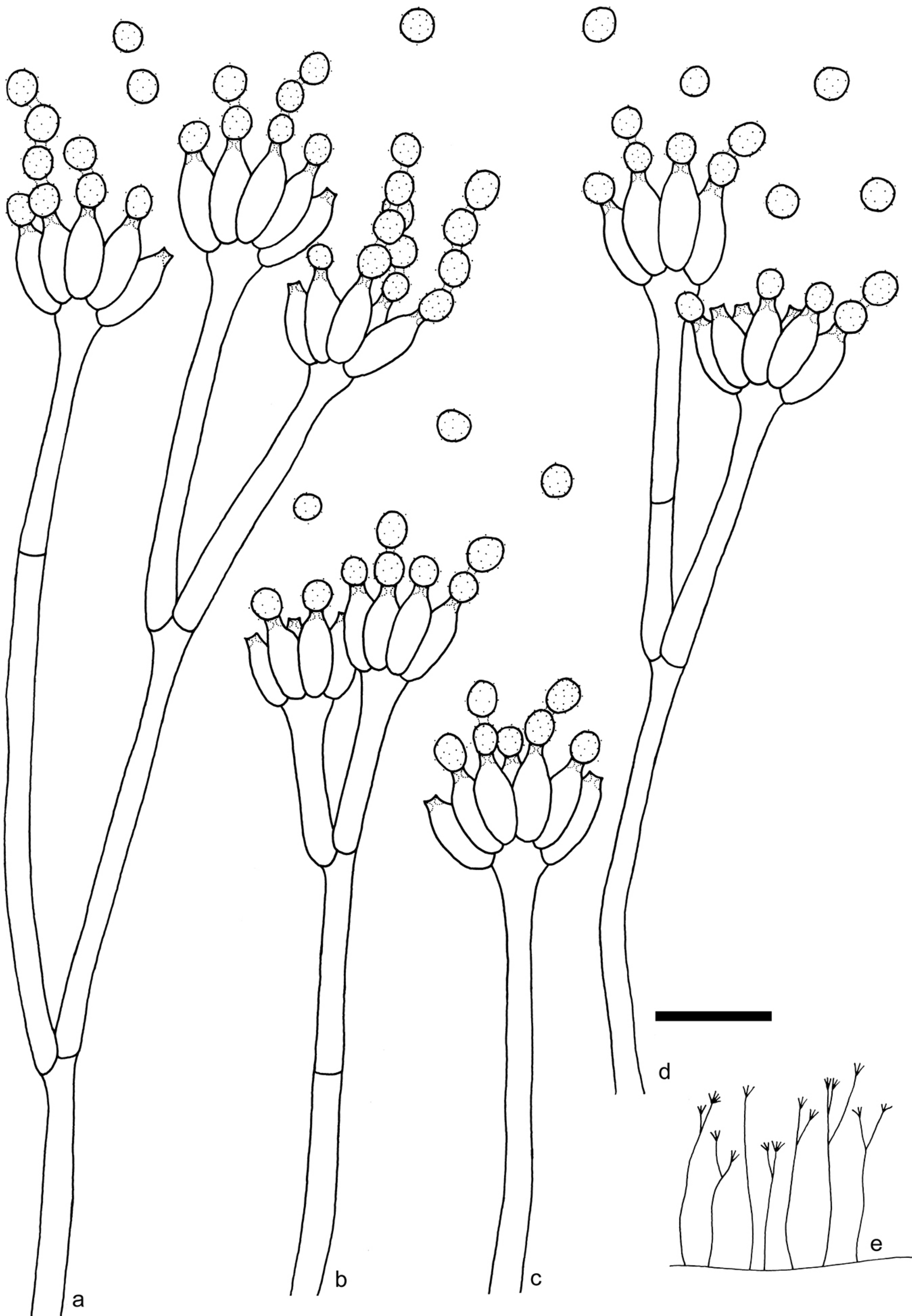

PLATE 110. Line drawing of *P. charlesii*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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61. *Penicillium fellutanum* Biourge — PLATES 111, 112, 113b

La Cellule 33: 262. 1923.

EX-TYPE: CBS326.48 = NRRL746 = ATCC10443 = FRR746 = IMI039734
TYPE ISOLATED FROM: Unknown source, United Kingdom
SPECIMENS EXAMINED: CV1936, CV1944, CV1970, CV2181.
ISOLATED FROM: Mites and bracts from *Protea repens* infructescence, Struisbaai

Macromorphology — CYA, 25 °C, 7d: Colonies 18–22 mm, moderately deep, radially and concentrically sulcate, raised at centre; margins low, very narrow, entire; mycelia white; texture floccose with some velutinous regions; sporulation sparse in CV1936, in KAS2285 moderate, conidia *en masse* light to greyish turquoise (24A4–24B4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A3), in KAS2285 greyish green (30C5) regions present.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 13–16 mm, moderately deep, radially and concentrically radiate; margins low, very narrow, entire; mycelia white; texture floccose; sporulation sparse to moderate, conidia *en masse* greyish green to dull green (25C4–25D4); exudate absent, soluble pigment absent, reverse pigmentation light yellow (3A5) at centre, olive grey (1D2–1F2) elsewhere.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 15–19 mm, moderately deep, plane; margins moderately deep, very narrow (1 mm), entire; mycelia white; texture floccose, velutinous near margin; sporulation moderately dense, conidia *en masse* dull to greyish green (26D4–26D6–26E6); exudate absent, soluble pigment absent, reverse pigmentation olive brown (4E7–4F7) at centre, greyish yellow (4B3–4B4) elsewhere.

YES, 25 °C, 7d: Colonies 25–27 mm, moderately deep, radially and concentrically sulcate; margins low, very narrow, entire; mycelia white; texture floccose with some velutinous regions; sporulation absent in CV1936, moderate in KAS2285, conidia *en masse* light to greyish turquoise (24A4–24B4); exudate absent, soluble pigment absent, reverse pigmentation pale yellow (3A3), in KAS2285 greyish green (30C5) regions present.

G25N, 25 °C, 7d: Colonies 7–9 mm, moderately deep, plane; margins low, very narrow, entire; mycelia white; texture floccose; sporulation moderate, conidia *en masse* greyish green (25B4–25B5); exudate absent, soluble pigment absent, reverse pigmentation (30B3–30B4), dull green (30D4) ring present.

CREA, 25 °C, 7d: Colonies 6–9 mm, acid not produced.

Micromorphology — Conidiophores monoverticillate and irregularly biverticillate, pattern however difficult to identify because of variance in length of metulae making it difficult to determine whether a side branch is a stipe from monoverticillate conidiophore or metulae from biverticillate conidiophore; Stipes smooth walled, almost impossible to determine for biverticillate conidiophores, when definitely monoverticillate stipes 30–220 × 1.5–2.5 µm; Branches 20–50 × 1.5–2.5 µm; Metulae when present only two, 8–37 × 1.5–2.5 [21.24±6.3 × 2.17±0.21] µm, metulae vesicle 2.5–5 [4.12 ± 0.65] µm; Phialides ampulliform, some long and acerose-like, 8–12 per metula/stipe, 6.5–10×2–3 [8.52±0.75 × 2.57±0.21] µm; Conidia lightly rough walled, connectives and connective scars visible, spheroidal to subspheroidal, 2–3 × 2–3 [2.54±0.14 × 2.19±0.17] µm, average width/length = 0.86±0.05, n = 81.

Notes — *Penicillium fellutanum* produces irregular conidiophores, where the metulae is often indistinguishable from the stipes. Interestingly, branches are often borne at the same stage with metulae that can become quite long and almost resemble that of a short conidiophore (PLATE 112e). This branching pattern, together with the lighter colored conidial colors on MEA, makes it distinct from its closest relative, *P. charlesii.*

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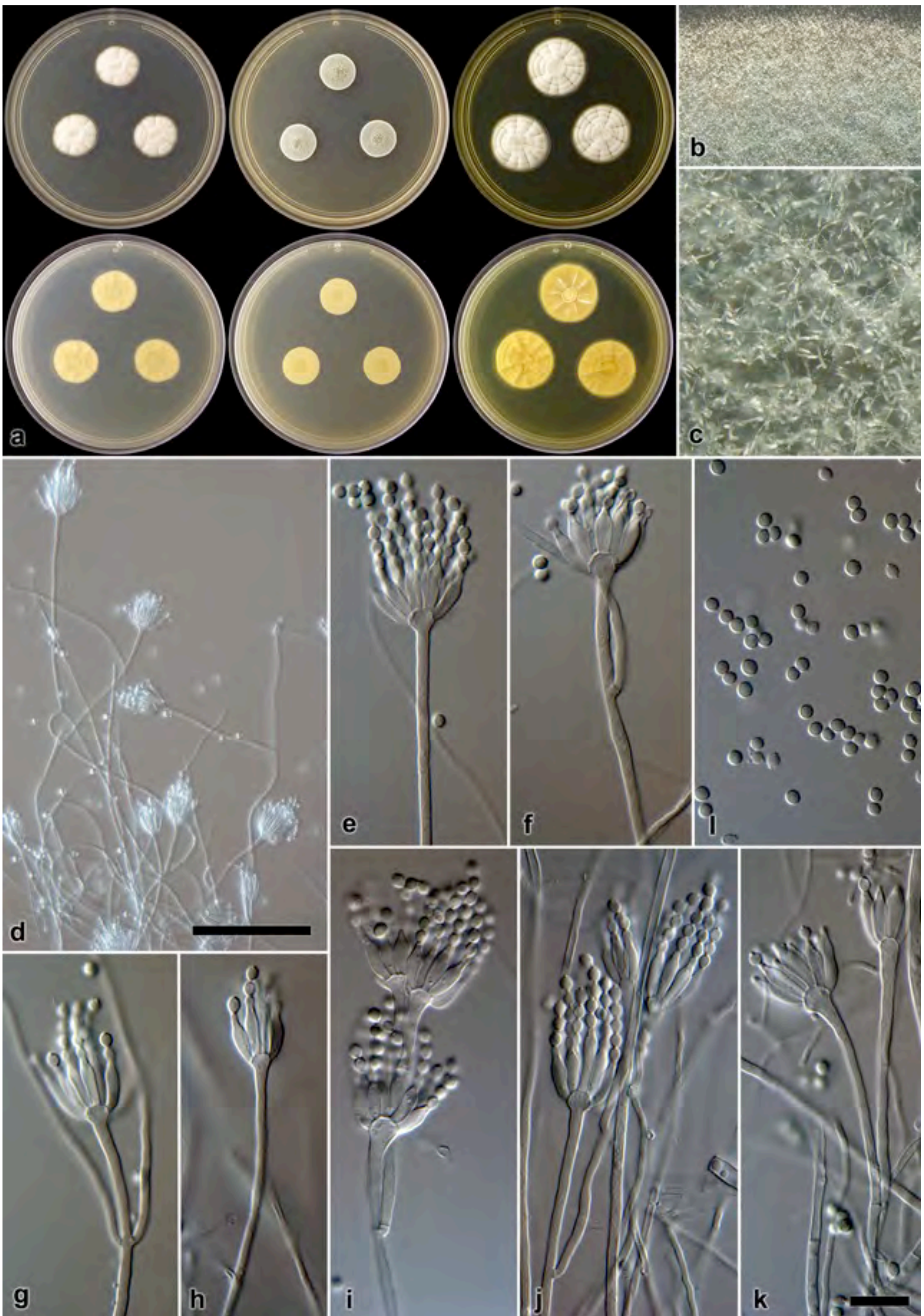

PLATE 111. *Penicillium fellutanum.* a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c. Texture on MEA. d–k. Conidiophores. l. Conidia (— Scale bar in d = 50 µm; — Scale bar in k = 10 µm, applies to e–l).

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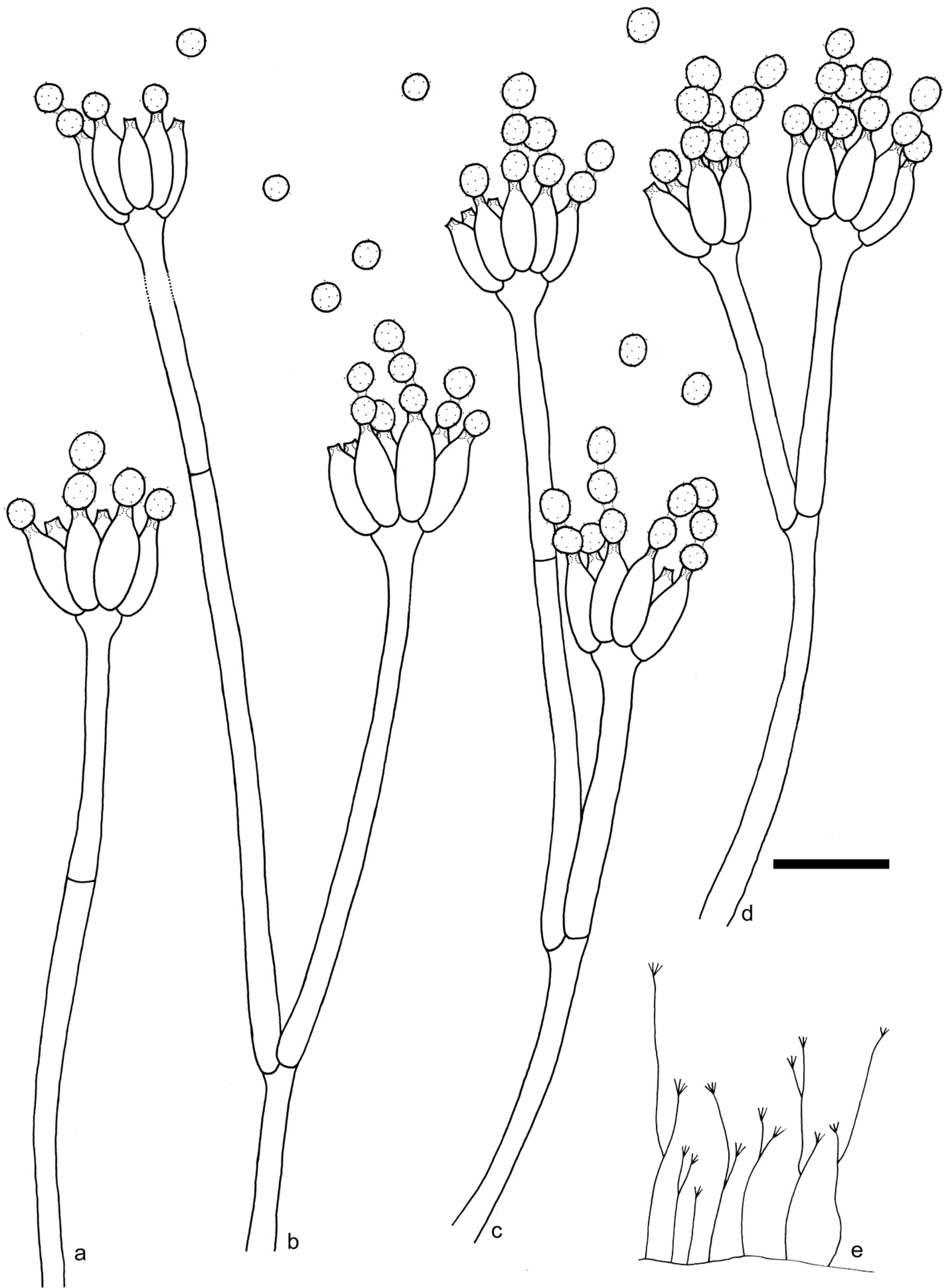

PLATE 112. Line drawing of *P. fellutanum*. a–d. Conidiophores (— Scale bar = 10 μm). e. Conidiophore branching (— Scale bar = 50 μm).

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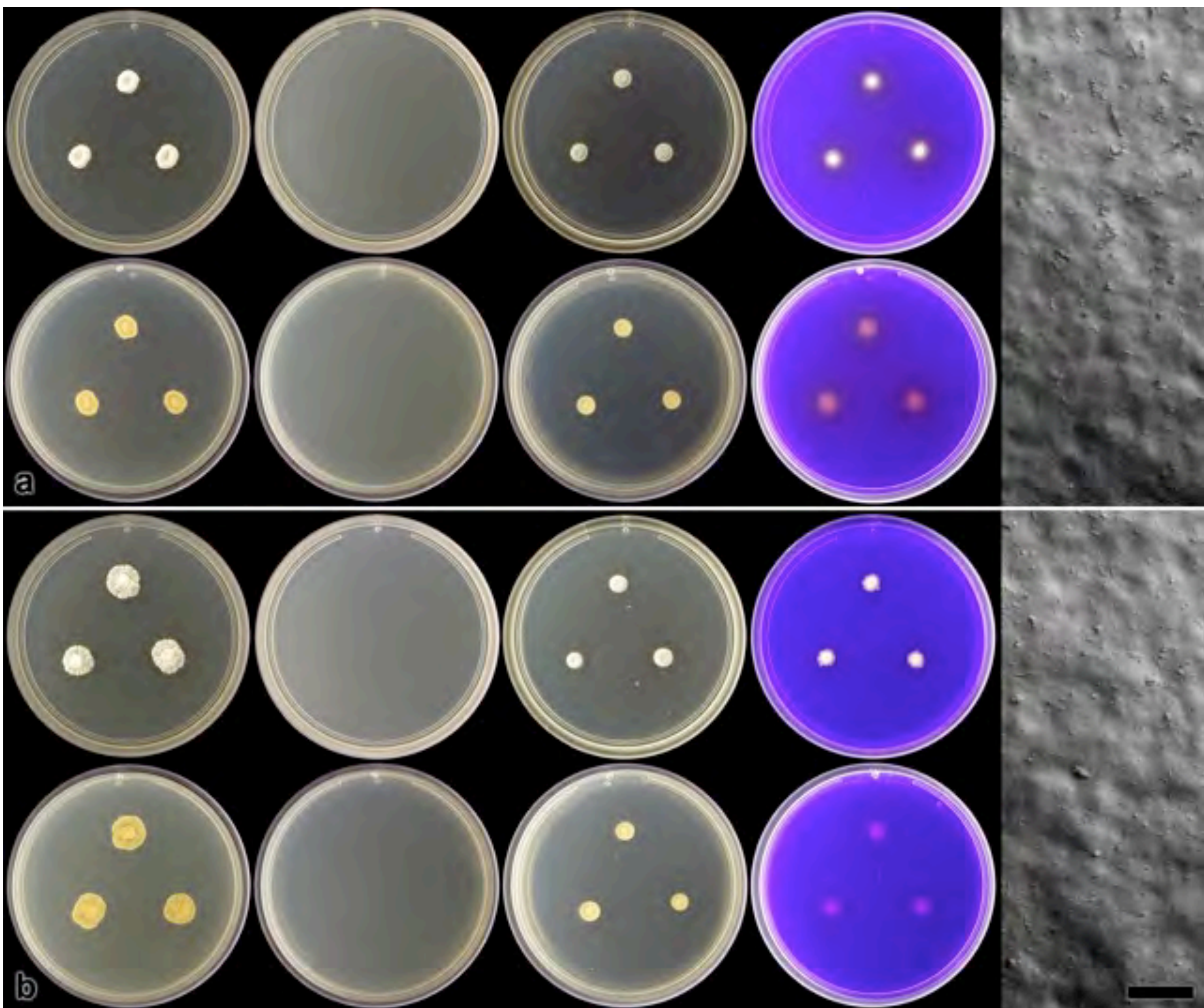

PLATE 113. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *Penicillium charlesii.* b. *Penicillium fellutanum.*

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Table 4: Strains used for phylogenetic analysis in this study

Strains		Species	GenBank accession number			
			ITS	Btub	CMD	RPB2
DAOM226268		*P. abeanum*				
CBS246.67	T	*P. abidjanum*	GU981582	GU981650		JN121469
CBS209.28 = NRRL737	T	*P. adametzii*	JN714929	JN625957		JN121455
AS34470		*P. adametzii*			AY678540	
AS35708		*P. adametzii*			AY678535	
KAS3463		*P. adametzii*	JN714930	JN625958		
CBS313.59 = NRRL3405	T	*P. adametzioides*	JN686433	JN799642	JN686387	JN406578
DAOM239916		*P. adametzioides*	JN686434	JN799643	JN686388	
CBS484.48	T	*P. aethiopicum*	AY371635	AY495983		
CBS254.69	T	*P. albidum*				
NRRL25744		*P. albocinerascens*	AF033459			
IBT10682	T	*P. albocoremium*	AJ004819			
NRRL35755	T	*P. alicantinum*	EU427299			
IBT3056		*P. allii*	AJ005484			
CBS317.67 = NRRL5812	T	*P. alutaceum*	AF033454			JN121489
CBS479.66 = NRRL5820	T	*P. anatolicum*	AF033425	JN606849	JN606571	
CBS478.66		*P. anatolicum*		JN606848	JN606570	
NRRL28157	T	*P. angulare*	AF125937			JN406554
NRRL35630		*P. angulare*	EF200087	EF198554	EF198582	
NRRL35633		*P. angulare*	EF200088	EF198555	EF198583	
CV0037 = DTO180G7 = KAS4119	T	*P. annulatum*	JX091426	JX091514	JX141545	
CV0187 = DTO181C3 = KAS4052		*P. annulatum*	JX091425	JX091515	JX141546	
CV0548 = DTO181I1 = KAS5143		*P. annulatum*	JX091423	JX091516	JX141547	
CV1707 = DTO183E9 = KAS4028		*P. annulatum*	JX091424	JX091517	JX141548	
KAS2148		*P. antarcticum*				
KAS2149		*P. antarcticum*				
KAS2151		*P. antarcticum*				
KAS2175		*P. antarcticum*				
CBS100492	T	*P. antarticum*				JN406653
CBS414.69	T	*P. arabicum*				
CBS113149	T	*P. araracuarense*	GU981597	GU981642		
CBS113148		*P. araracuarense*	GU981596	GU981641		
CBS497.73	T	*P. ardesiacum*				JN406547
KAS3057		*P. ardesiacum*				
CBS130371	T	*P. argentinense*		JN606815	JN606549	
CBS130381		*P. argentinense*		JN606806	JN606542	
NRRL3411	T	*P. asperosporum*	AF033412			
NRRL795	T	*P. atramentosum*	AF033483			
CBS109.66	T	*P. atrofulvum*	JN617663	JN606677	JN606387	
CBS261.64		*P. atrofulvum*		JN606676	JN606388	
CV55 = DTO180H4 = KAS4155 = DAOM241083	T	*P. atrolazulinum*	JX140913	JX141077	JX157416	
CV1063 = DTO182G8 = KAS3967		*P. atrolazulinum*	JX140901	JX141108	JX157481	
CV1091 = DTO182G9 = KAS3968		*P. atrolazulinum*	JX140902	JX141109	JX157482	
CV120 = DTO181A8 = KAS3982 = DAOM241124		*P. atrolazulinum*	JX140903	JX141078	JX157422	
CV125 = DTO184E8		*P. atrolazulinum*		JX141079	JX157423	
CV1414 = DTO183A5 = KAS4002 = DAOM241087		*P. atrolazulinum*	JX140904	JX141110	JX157484	
CV146 = DTO181B5 = KAS4010 = DAOM241086		*P. atrolazulinum*	JX140905	JX141111	JX157426	
CV1736 = DTO186D2		*P. atrolazulinum*		JX141112	JX157498	
CV1776 = DTO183D6 = KAS4034		*P. atrolazulinum*	JX140906	JX141113	JX157500	
CV1778 = DTO183D7 = KAS4035 = DAOM241085		*P. atrolazulinum*	JX140907	JX141114	JX157501	
CV1791 = DTO186D7		*P. atrolazulinum*		JX141115	JX157502	
CV1822 = DTO183E2 = KAS4042		*P. atrolazulinum*	JX140909	JX141116	JX157503	
CV1854 = DTO186E3		*P. atrolazulinum*		JX141117	JX157505	
CV2222 = DTO186H9		*P. atrolazulinum*		JX141119	JX157513	
CV244 = DTO181D3 = KAS4091 = DAOM241088		*P. atrolazulinum*	JX140911	JX141090	JX157436	
CV264 = DTO184G9		*P. atrolazulinum*		JX141092	JX157438	
CV336 = DTO181E5 = KAS4112 = DAOM241092		*P. atrolazulinum*	JX140912	JX141098	JX157445	
CV365 = DTO184H9		*P. atrolazulinum*		JX141099	JX157446	
CV438 = DTO184I2		*P. atrolazulinum*		JX141100	JX157448	
CV458 = DTO184I4		*P. atrolazulinum*		JX141101	JX157449	
CV602 = DTO181I6 = KAS4159 = DAOM241091		*P. atrolazulinum*	JX140914	JX141104	JX157455	
CV991 = DTO182G1 = KAS4230 = DAOM241089		*P. atrolazulinum*	JX140915	JX141107	JX157478	
CBS380.75	T	*P. atrosanguineum*	JN617706			JN406557
CBS241.56 = NRRL2571	T	*P. atrovenetum*	AF033492	JX140944	JX157410	JN121467
CBS243.56		*P. atrovenetum*		JX140945	JX157411	
CBS126228	T	*P. aurantiacobrunneum*	JN617670	JN606702	JN606522	
CBS126230		*P. aurantiacobrunneum*		JN606703	JN606519	
CBS324.89 = NRRL971	T	*P. aurantiogriseum*	AF033476	AY674296		JN406573
CBS642.95		*P. aurantiogriseum*		AY674297		
CBS792.95		*P. aurantiogriseum*		AY674298		
CV48 = DTO180H2 = KAS4135		*P. aurantiogriseum*	JX091395	JX091501	JX141571	
CV72 = DTO180H8 = KAS4168		*P. aurantiogriseum*	JX091396	JX091531	JX141572	
CV1842 = DTO183E6 = KAS4047 = DAOM241066	T	*P. austricola*	JX091466	JX091579	JX141600	
CV1808 = DTO183D8 = KAS4038 = DAOM241065		*P. austricola*	JX091465	JX091584	JX141598	
CV1811 = DTO186D8		*P. austricola*		JX091578	JX141599	
CV1902 = DTO183F5 = KAS4056		*P. austricola*	JX091467	JX091586	JX141602	
CV1943 = DTO183F8 = KAS4059 = DAOM241067		*P. austricola*	JX091468	JX091587	JX141603	
CV1954 = DTO183F9 = KAS4060		*P. austricola*	JX091469	JX091588	JX141604	
CV1968 = DTO186G3		*P. austricola*		JX091589	JX141605	
NRRL2086	T	*P. baarnense*	AF033481			

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Table 4: Continued

Strains		Species	GenBank accession number			
			ITS	Btub	CMD	RPB2
CBS227.28 = NRRL865	T	*P. bialowiezense*	EU587315	AY674439		
CBS110104		*P. bialowiezense*		AY674440		
CBS112882		*P. bialowiezense*		AY674441		
CBS221.66 = NRRL3391	T	*P. bilaiae*	JN714937	JN625966	JN626009	JN406610
ATCC20851		*P. bilaiae*	JN714934	JN625964	JN626005	
ATCC22348		*P. bilaiae*	JN714935	JN625962	JN626007	
CV0255 = DTO181D8 = KAS4103		*P. bilaiae*	JX091437	JX091565	JX141560	
CV0291 = DTO181E2 = KAS4107		*P. bilaiae*	JX091438			
CV0335 = DTO181E4 = KAS4111 = DAOM241114		*P. bilaiae*	JX091439	JX091566	JX141561	
CV1213 = DTO182H8 = KAS3983		*P. bilaiae*	JX091446	JX091569	JX141564	
CV1273 = DTO182I4 = KAS3990		*P. bilaiae*	JX091447	JX091570	JX141565	
CV1429 = DTO183A8 = KAS4005		*P. bilaiae*	JX091448	JX091571	JX141566	
CV231 = DTO181D1 = KAS4081		*P. bilaiae*	JX091449	JX091564	JX141559	
CV387 = DTO181F4 = KAS4122		*P. bilaiae*	JX091450	JX091567	JX141562	
CV988 = DTO182F8 = KAS4228		*P. bilaiae*	JX091451	JX091568	JX141563	
DAOM197974		*P. bilaiae*	JN714936	JN625965	JN626008	
NRRL2013	T	*P. biourgeianum*	AY484897			
NRRL28088		*P. biourgeianum*	AY484898			
NRRL28103		*P. biourgeianum*	AY484899			
NRRL31002	T	*P. boreae*	AF481122			
RMF9598		*P. bovifimosum*	AF263347			
CV2842 = DTO180D3 = KAS3937 = DAOM241159	T	*P. brachycaulon*	FJ231021	JX091526	JX141536	
CV2857 = DTO184D1 = KAS3951		*P. brachycaulon*	FJ231022	JX091523	JX141537	
CV2858 = DTO180F2 = KAS3952		*P. brachycaulon*	FJ231023	JX091522	JX141538	
CBS253.55	T	*P. brasilianum*	GU981577	GU981629		
CBS235.81 = IMI216895 = NRRL710	T	*P. brefeldianum*	GU981580	GU981623	FJ530980	
CBS257.29 = NRRL2011	T	*P. brevicompactum*	AY484912	AY674437	AY484817	JN406594
CBS110067		*P. brevicompactum*		AY674438		
CBS110068		*P. brevicompactum*		AY674436		
CBS110069		*P. brevicompactum*		AY674435		
CBS480.84		*P. brevicompactum*		AY674434		
CV144 = DTO181B4 = KAS4006		*P. brevicompactum*	JX091397	JX091532	JX141573	
CV1492 = DTO183B6 = KAS4014		*P. brevicompactum*	JX091398	JX091533	JX141574	
CV1821 = DTO183E1 = KAS4041		*P. brevicompactum*	JX091399	JX091534	JX141575	
CV2475 = DTO183I8 = KAS4099		*P. brevicompactum*	JX091400	JX091535		
NRRL2012		*P. brevicompactum*	AY484913			
NRRL5276		*P. brevicompactum*	AY484921			
NRRL867		*P. brevicompactum*	AY484926			
CBS763.68 = NRRL6321	T	*P. brevissimum*	EF433764	EU427270		JN406534
AS36887		*P. brevistipitatum*	DQ221696			
NRRL31472	T	*P. brocae*	AF484396		AY741737	JN406639
NRRL31462		*P. brocae*	AF484391		AY741732	
CV949 = DTO182E4 = KAS4214 = DAOM241359	T	*P. brunneoconidia*	JX140768	JX271529	JX157309	
CV1702 = DTO186H9		*P. brunneoconidia*		JX271524	JX157339	
CV875 = DTO182B7 = KAS4187		*P. brunneoconidia*	JX140771	JX271517	JX157294	
CV901 = DTO182C6 = KAS4196		*P. brunneoconidia*	JX140772	JX271519	JX157296	
CV902 = DTO185E8		*P. brunneoconidia*		JX271527	JX157297	
CV907 = DTO185F1		*P. brunneoconidia*		JX271526	JX157299	
CV914 = DTO182D1 = KAS4200 = DAOM241358		*P. brunneoconidia*	JX140766	JX271520	JX157300	
CV915 = DTO185F4		*P. brunneoconidia*		JX271522	JX157301	
CV921 = DTO185F6		*P. brunneoconidia*		JX271518	JX157302	
CV935 = DTO182D8 = KAS4209		*P. brunneoconidia*	JX140767	JX271516	JX157305	
CV946 = DTO182E2 = KAS4212		*P. brunneoconidia*	JX140773	JX271521	JX157307	
CV947 = DTO185G4		*P. brunneoconidia*		JX271528	JX157308	
CV950 = DTO185G5		*P. brunneoconidia*		JX271530	JX157310	
CV954 = DTO185G7		*P. brunneoconidia*		JX271523	JX157312	
CV966 = DTO185H2		*P. brunneoconidia*		JX271525	JX157317	
CV970 = DTO182F2 = KAS4222		*P. brunneoconidia*	JX140769	JX271515	JX157318	
CBS325.89	T	*P. burgense*				JN406572
DAOM239914	T	*P. cainii*	JN686435	JN686366	JN686389	
DAOM239915		*P. cainii*	JN686436	JN686367	JN686390	
CBS124325	T	*P. cairnsense*	JN617669	JN606693	JN606512	
CBS118028		*P. cairnsense*		JN606696	JN606516	
CV2340 = DTO187A1		*P. cairnsense*	JX140855			
CV2344 = DTO183H6 = KAS4083 = DAOM241041		*P. cairnsense*	JX140856	JX141009	JX141501	
CBS123.80 = NRRL874	T	*P. camemberti*	AF034453			
NRRL31003	T	*P. canariense*	AF481121			
CBS300.48 = NRRL910	T	*P. canescens*	AF033493	JX140946		JN121485
NRRL910	T	*P. canescens*	AF033493			JN121485
IMI149218		*P. canescens*				
NRRL35656		*P. canescens*	DQ658168			
CBS233.81	T	*P. caperatum*	GU981615	GU981659		JN121465
CBS301.48 = ATCC10420 = IMI040576 = NRRL2056	T	*P. capsulatum*	AF033429			JN406582
KAS1637		*P. capsulatum*				
NRRL25170	T	*P. carneum*	DQ339566			
CV110 = DTO181A3 = KAS3970 = DAOM241130		*P. caseidecus*	JX140741	JX271494	JX157273	
CV111 = DTO181A4 = KAS3971 = DAOM241131		*P. caseidecus*	JX140742	JX271495	JX157274	
CBS352.67	T	*P. catenatum*				JN121504
CBS255.87	T	*P. chalybeum*				JN406596
CBS304.48 = ATCC 8730 = CBS342.51 = NRRL1887 = NRRL778	T	*P. charlesii*	AF033400	JX091508	AY741754	JN121486

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Table 4: Continued

Strains	Species	GenBank accession number			
		ITS	Btub	CMD	RPB2
CV1754 = DTO183D4 = KAS4032	*P. charlesii*	JX091505	JX091505	JX141532	
CV1754 = KAS4032 = DTO183D4	*P. charlesii*	JX091414	JX091505	JX141532	
CV1819 = DTO183D9 = KAS4040	*P. charlesii*	JX091506	JX091506	JX141533	
CV1819 = KAS4040 = DTO183D9	*P. charlesii*	JX091415	JX091506	JX141533	
CV2006 = KAS4063 = DTO183G1	*P. charlesii*	JX091416	JX091507	JX141534	
CV646 = KAS4161 = DTO181I8	*P. charlesii*	JX091417	JX091503	JX141530	
CV744 = KAS4171 = DTO182A4	*P. charlesii*	JX091418	JX091504	JX141531	
CBS305.48 = NRRL2048	[T] *P. chermesinum*	AY742693		AY741728	JN406581
NRRL735	*P. chermesinum*	AF033413			
KACC45901	*P. chermisinum*		JF521507		
CBS126236	[T] *P. christenseniae*	JN617674	JN606680	JN606373	
CBS126237	*P. christenseniae*		JN606679	JN606374	
CBS306.48 = NRRL807	[T] *P. chrysogenum*	AF033465	AY495981	JF909973	JN121487
ATCC10108	*P. chrysogenum*	AY371634			
CBS122151	*P. chrysogenum*	DQ674380			
CBS478.84	*P. chrysogenum*		AY495988		
CBS776.95	*P. chrysogenum*		AY495986		
CBS217.28	[T] *P. chrzaszczii*	GU944603	JN606758	JN606423	
CBS176.81	*P. chrzaszczii*		JN606759	JN606420	
CBS275.83	[T] *P. ciegleri*	GU981601	GU981671		
IMI092234 = NRRL748	[T] *P. cinerascens*	AF033455	JX141041		
CBS222.66	[T] *P. cinereoatrum*				JN406608
NRRL162	[T] *P. cinnamopurpureum*	EF626950			
CBS258.29 = NRRL761	[T] *P. citreonigrum*	AF033456			JN121474
NRRL1187	*P. citreonigrum*	EF198646	EF198622	F198626	
NRRL2046	*P. citreonigrum*	EF198647	EF198623		
CBS320.59	[T] *P. citreovirens*				
CBS321.59	[T] *P. citreoviride var. aeneum*				
CBS139.45 = NRRL1841	[T] *P. citrinum*	AF033422	GU944545	GU944638	
CV0184 = DTO181C2 = KAS4045	*P. citrinum*	JX140859	JX141011	JX141502	
CV0480 = DTO181G4 = KAS4136	*P. citrinum*	JX140860	JX141012	JX141503	
CV0506 = DTO181H1 = KAS4142	*P. citrinum*	JX140861	JX141013	JX141504	
CV1276 = DTO182I5 = KAS3991	*P. citrinum*		JX141014	JX141505	
NRRL1003	[T] *P. clavigerum*	DQ339555			
CV550 = DTO181I3 = KAS4156 = DAOM241129	*P. clavistipa*	JX140770	JX271513	JX157288	
CV552 = DTO185A9	*P. clavistipa*		JX271512	JX157289	
CV83 = DTO184E2	*P. clavistipa*		JX271511	JX157270	
CV957 = DTO182E6 = KAS4217 = DAOM241127	*P. clavistipa*	JX140774	JX271514	JX157314	
CBS141.45	[T] *P. coeruleum*	GU981606	GU981655		
CBS119387 = IBT27866 = NRRL35363	[T] *P. coffeae*	AY742702		AY741747	JN121436
CBS311.48 = ATCC10428	[T] *P. commune*	AY373905			
CV1875 = DTO183F3 = KAS4053 = DAOM241034	[T] *P. compactum*	JX091443	JX091563	JX141557	
CV112 = DTO181A5 = KAS3972 = DAOM241032	*P. compactum*	JX091441	JX091559	JX141553	
CV1722 = DTO183D1 = KAS4029	*P. compactum*	JX091442	JX091562	JX141567	
CV204 = DTO181C6 = KAS4066	*P. compactum*	JX091444	JX091560	JX141554	
CV227 = DTO181C9 = KAS4078 = DAOM241033	*P. compactum*	JX091445	JX091561	JX141555	
CV401 = DTO181F7 = KAS4126 = DAOM241031	*P. compactum*	JX091440	JX091558	JX141556	
NRRL2034	*P. concentricum*	DQ339561			
P11.1	*P. concentricum*	EU833217			
CBS171.87	[T] *P. confertum*		AY674373		
CV547 = DTO181H9 = KAS4152 = DAOM241072	[T] *P. consobrinum*	JX140888	JX141135	JX157453	
CV1095 = DTO185I4	*P. consobrinum*		JX141145	JX157483	
CV1457 = DTO186A6	*P. consobrinum*		JX141146	JX157486	
CV436 = DTO181F8 = KAS4130 = DAOM241071	*P. consobrinum*	JX140887	JX141134	JX157447	
CV865 = DTO182B5 = KAS4185 = DAOM241073	*P. consobrinum*	JX140889	JX141138	JX157463	
CV888 = DTO185E2	*P. consobrinum*		JX141139	JX157465	
CV911 = DTO182C9 = KAS4199 = DAOM241074	*P. consobrinum*	JX140890	JX141141	JX157469	
CV977 = DTO182F5 = KAS4225 = DAOM241075	*P. consobrinum*	JX140891	JX141144	JX157477	
NRRL13626	[T] *P. coprobium*	DQ339559			
NRRL13627	[T] *P. coprophilum*	AF033469			
CBS127355	[T] *P. copticola*	JN617685	JN606817	JN606553	
CBS127356	*P. copticola*		JN606805	JN606532	
CBS123.65	[T] *P. coralligerum*	JN617667			JN406632
CBS114.69	*P. coralligerum*	AJ010484			
CBS162.81	[T] *P. cordubense*	AF527055			
CBS312.48 = NRRL802	[T] *P. corylophilum*	AF033450	JX141042		
CBS231.38	*P. corylophilum*	JN617696			
CBS259.67	*P. corylophilum*				
CBS330.79	*P. corylophilum*	GU944557			JN406569
CBS330.79	*P. corylophilum*	GU944557	GU944519	GU944607	
CV2852 = DTO184C9	*P. corylophilum*	FJ230998	JX141043	JX157521	
CV2853 = DTO180E4	*P. corylophilum*	FJ230997	JX141044	JX157523	
CV2854 = DTO180E5	*P. corylophilum*	FJ230996	JX141045	JX157522	
DAOM221130	*P. corylophilum*	JN942912			
CBS256.87	[T] *P. corynephorum*				
CBS126995	[T] *P. cosmopolitanum*		JN606733	JN606472	
CBS124316	*P. cosmopolitanum*		JN606734	JN606487	
CBS127038	*P. cosmopolitanum*	JN617682			
CBS200.86	*P. cosmopolitanum*	JN617691			
CV92 = DTO180I5 = KAS4202 = DAOM241082	*P. cravenianum*	JX140900	JX141076	JX157418	

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Table 4: Continued

Strains		Species	GenBank accession number			
			ITS	Btub	CMD	RPB2
CBS223.66	T	*P. cremeogriseum*	GU981586	GU981624	JX141539	
CV0071 = DTO180H7 = KAS4167		*P. cremeogriseum*	JX091429	JX091518	JX141549	
CV0095 = DTO180I7 = KAS4215		*P. cremeogriseum*	JX091430	JX091519	JX141550	
CV0102 = DTO180I9 = KAS3962		*P. cremeogriseum*	JX091427	JX091520	JX141551	
CV0391 = DTO181F5 = KAS4123		*P. cremeogriseum*	JX091428	JX091521	JX141552	
NRRL6175		*P. crocicola*	EU427290			
NRRL3332	T	*P. crustaceum*	AF033466			
NRRL3332	T	*P. crustaceum*	AF033466			
CBS115503 = NRRL968 = IMI91917	T	*P. crustosum*	AF033472	AY674353	DQ911132	
CBS101025		*P. crustosum*		AY674351		
CBS471.84		*P. crustosum*		AY674352		
CV1267 = DTO182I3 = KAS3989		*P. crustosum*	JX091401	JX091537	JX141578	
CV1529 = DTO183C4 = KAS4023		*P. crustosum*	JX091402	JX091538	JX141579	
CV241 = DTO181D2 = KAS4088		*P. crustosum*	JX091403	JX091536	JX141576	
CV251 = DTO181D6 = KAS4101		*P. crustosum*	JX091404	JX091530	JX141577	
CBS271.89 = ATCC60138 = IMI296794 = NRRL13460	T	*P. cryptum*				JN121478
CV1036 = DTO182G6 = KAS3964 = DAOM241148		*P. cumulacinatum*	JX140778	JX271557	JX157321	
CV122 = DTO181A9 = KAS3984 = DAOM241026		*P. cumulacinatum*	JX140760	JX271548	JX157275	
CV1293 = DTO182I7 = KAS3993 = DAOM241121		*P. cumulacinatum*	JX140761	JX271550	JX157330	
CV1299 = DTO182I8 = KAS3994		*P. cumulacinatum*	JX140762	JX271551	JX157331	
CV1300 = DTO182I9 = KAS3995 = DAOM241123		*P. cumulacinatum*	JX140763	JX271552	JX157332	
CV1301 = DTO183A1 = KAS3996		*P. cumulacinatum*	JX140764	JX271553	JX157333	
CV1335 = DTO183A3 = KAS3999 = DAOM241147		*P. cumulacinatum*	JX140780	JX271559	JX157334	
CV1585 = DTO183C6 = KAS4025		*P. cumulacinatum*	JX140781	JX271556	JX157338	
CV209 = DTO181C7 = KAS4069 = DAOM241122		*P. cumulacinatum*	JX140782	JX271554	JX157277	
CV214 = DTO181C8 = KAS4070		*P. cumulacinatum*	JX140783	JX271555	JX157278	
CV2816 = DTO180C4 = KAS3933		*P. cumulacinatum*		JX271560	JX157343	
CV2829 = DTO180C8 = KAS3957 = DAOM241120		*P. cumulacinatum*		JX271561	JX157344	
CV2840 = DTO180D2 = KAS3935 = DAOM241118		*P. cumulacinatum*	FJ231029	JX271562	JX157345	
CV2843 = DTO180D6 = KAS3940		*P. cumulacinatum*	FJ231028	JX271563	JX157346	
CV2847 = DTO180D9 = KAS3943 = DAOM241119		*P. cumulacinatum*	FJ231027	JX271564	JX157347	
CV2859 = DTO180F5 = KAS3955		*P. cumulacinatum*		JX271565	JX157348	
CV885 = DTO182B8 = KAS4188 = DAOM241149		*P. cumulacinatum*	JX140784	JX271558	JX157295	
CV997 = DTO182G2 = KAS4231		*P. cumulacinatum*	JX140765	JX271549	JX157320	
CBS315.48 = ATCC10432 = IMI034910 = MUCL29234 = NRRL775	T	*P. cyaneum*	AF033427	JX091552		JN406575
CV2207 = DTO183H1 = KAS4075		*P. cyaneum*	JX091433	JX091553	JX141497	
CV2438 = DTO183I3 = KAS4090		*P. cyaneum*	JX091435	JX091555	JX141499	
CV2443 = DTO183I4 = KAS4092		*P. cyaneum*	JX091436	JX091556	JX141500	
CV2455 = DTO183I5 = KAS4094		*P. cyaneum*	JX091434	JX091554	JX141498	
CBS144.45	T	*P. cyclopium*		AY674310		
ATCC46511		*P. cyclopium*	GU319999			
CBS101136		*P. cyclopium*		AY674308		
CBS211.28	T	*P. daleae*	GU981583	GU981649		
CBS117509 = nRRL28160	T	*P. decaturense*	GU944604	JN606685	JN606413	
CBS117504		*P. decaturense*		JN606688	JN606414	
CBS230.81 = NRRL741	T	*P. decumbens*	AY157490			JN406601
DTO23D8 = CBS185.81 = IMI092216 = MUCL28665 = NRRL755	T	*P. dierckxii*	EF634444	EF634442	EF634443	JN406619
NRRL786	T	*P. digitatum*	AF033471			
CBS456.70 = NRRL5207	T	*P. dimorphosporum*	AF081804			JN121517
CBS173.87 = NRRL13487	T	*P. dipodomyicola*	AY371616	AY674409		
CBS110421		*P. dipodomyicola*		AY674411		
CBS110413 = NRRL13485	T	*P. dipodomyis*	AY371615	AY495990		
CBS474.84 = IBT3087	T	*P. discolor*	AJ004816	AY674348		
CBS278.97		*P. discolor*		AY674349		
NRRL5562	T	*P. donkii*	AF033445			
F01V25	T	*P. dravuni*	AY494856			
NRRL917	T	*P. echinatum*		JX140952	JX157407	
CBS317.48 = NRRL1151	T	*P. echinulatum*	AF033473	AY674341		
CBS328.59	T	*P. echinulonalgiovense*	GU981587	GU981631		
NRRL2090	T	*P. egyptiacum*	AF033467			
CBS324.48 = NRRL708	T	*P. ehrlichii*	GU981578	GU981652		
CBS118135	T	*P. elleniae*	GU981612	GU981661		
CBS118134		*P. elleniae*	GU981610	GU981662		
CBS318.67 = NRRL6223	T	*P. erubescens*	AF033464	HQ646566		JN121490
CBS323.71	T	*P. euglaucum*	JN617699	JN606856	JN606564	
CBS130372		*P. euglaucum*		JN606808	JN606550	
CBS325.48 = ATCC7861 = NRRL976	T	*P. expansum*	AY373912	AY674400	DQ911134	JF417427
CV2860 = DTO180F6		*P. expansum*	FJ230989	JX091539	CV2860	
CV2861 = DTO180F7		*P. expansum*	FJ230990	JX091540	CV2861	
CBS689.77 = NRRL28201	T	*P. fagi*	AF481124			JN406540
CBS449.73	T	*P. farinosum*	AF527057			
CBS229.81 = NRRL746 = ATCC10443 = CBS326.48 = IMI039734	T	*P. fellutanum*	AF033399		AY741753	JN121460
CV1936 = KAS4058 = DTO183F7		*P. fellutanum*	JX091419	JX091509	JX141535	
CV1944		*P. fellutanum*	JX091420	JX091510		
CV1970		*P. fellutanum*	JX091421	JX091511		
CV2181		*P. fellutanum*	JX091422	JX091512		
NRRL35619		*P. fellutanum*	EF200079			
NRRL35622		*P. fellutanum*	EF200082	EF198548	EF198576	
NRRL35622		*P. fellutanum*	EF200082			
CBS260.29		*P. flavidorsum*				

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Table 4: Continued

Strains	Species	GenBank accession number ITS	Btub	CMD	RPB2
CBS202.87	[T] *P. flavidostipitatum*				
CBS419.89	[T] *P. flavigenum*		AY495993		
CBS110406	*P. flavigenum*		AY495994		
CBS110407	*P. flavigenum*		AY495995		
CV100 = DTO180I8 = KAS3958 = DAOM241157	*P. flavosclerotia*		JX271508	JX157272	
CV537 = DTO181H7 = KAS4149 = DAOM241156	*P. flavosclerotia*	JX140756	JX271496	JX157285	
CV545 = DTO185A5	*P. flavosclerotia*		JX271498	JX157286	
CV553 = DTO185B1	*P. flavosclerotia*		JX271507	JX157290	
CV65 = DTO184D8	*P. flavosclerotia*		JX271510	JX157266	
CV76 = DTO184D9	*P. flavosclerotia*		JX271506	JX157267	
CV77 = DTO180I1 = KAS4173 = DAOM241158	*P. flavosclerotia*	JX140757	JX271497	JX157268	
CV80 = DTO184E1	*P. flavosclerotia*		JX271509	JX157269	
CV839 = DTO182B2 = KAS4181 = DAOM241155	*P. flavosclerotia*	JX140758	JX271499	JX157292	
CV924 = DTO182D3 = KAS4203 = DAOM241154	*P. flavosclerotia*	JX140775	JX271500	JX157303	
CV925 = DTO182D4 = KAS4204 = DAOM241153	*P. flavosclerotia*	JX140776	JX271501	JX157304	
CV938 = DTO182D9 = KAS4210 = DAOM241152	*P. flavosclerotia*	JX140777	JX271502	JX157306	
CV955 = DTO185G8	*P. flavosclerotia*		JX271504	JX157313	
CV971 = DTO182F3 = KAS4223 = DAOM241151	*P. flavosclerotia*	JX140759	JX271503	JX157319	
CV99 = DTO184E5	*P. flavosclerotia*		JX271505	JX157271	
CBS112292	*P. freii*		AY674292		
CBS794.95	*P. freii*		AY674290		
IBT3464	*P. freii*	AJ005479			
CBS105.11	[T] *P. frequentans*				
CV531 = DTO181H5 = KAS4147 = DAOM241356	*P. fuscum*	JX140750	JX271493	JX157284	
DAOM229848	*P. fuscum*				
NRRL721	*P. fuscum*	AF033443			
CBS295.62 = NRRL3008	[T] *P. fuscum (ex-type of P. pinetorum)*	AF033411			JN121483
CV931 = DTO182D5 = KAS4026 = DAOM241348	[T] *P. fynbosense*	JX140830	JX140992	JX157384	
CV1163 = DTO182H4 = KAS3976 = DAOM241353	*P. fynbosense*	JX140819	JX141001	JX157394	
CV1502 = DTO183B9 = KAS4018 = DAOM241162	*P. fynbosense*	JX140820	JX141002	JX157396	
CV1512 = DTO186B4	*P. fynbosense*	JX140821	JX141003	JX157397	
CV1704 = DTO183C8 = KAS4027 = DAOM241349	*P. fynbosense*	JX140822	JX141004	JX157399	
CV30 = DTO180G5 = KAS4109 = DAOM241192	*P. fynbosense*	JX140823	JX140977	JX157351	
CV475 = DTO181G3 = KAS4134 = DAOM241352	*P. fynbosense*	JX140824	JX140979	JX157365	
CV487 = DTO181G5 = KAS4137 = DAOM241350	*P. fynbosense*	JX140825	JX140980	JX157366	
CV75 = DTO180H9 = KAS4172	*P. fynbosense*	JX140826	JX140978	JX157355	
CV864 = DTO182B4 = KAS4184 = DAOM241163	*P. fynbosense*	JX140827	JX140981	JX157371	
CV874 = DTO185D8	*P. fynbosense*	JX140807	JX140982	JX157372	
CV881 = DTO185E1	*P. fynbosense*		JX140983	JX157373	
CV890 = DTO185E3	*P. fynbosense*	JX140808	JX140984	JX157374	
CV892 = DTO185E2	*P. fynbosense*	JX140809	JX140985	JX157375	
CV893 = DTO182C2 = KAS4191 = DAOM241164	*P. fynbosense*	JX140828	JX140986	JX157376	
CV897 = DTO185E6	*P. fynbosense*	JX140810	JX140987	JX157377	
CV898 = DTO185E7	*P. fynbosense*		JX140988	JX157378	
CV899 = DTO182C4 = KAS4193 = DAOM241351	*P. fynbosense*	JX140829	JX140989	JX157379	
CV929 = DTO185F7	*P. fynbosense*	JX140811	JX140990	JX157382	
CV930 = DTO185F8	*P. fynbosense*	JX140812	JX140991	JX157383	
CV937 = DTO185G1	*P. fynbosense*	JX140813	JX140993	JX157385	
CV939 = DTO185G2	*P. fynbosense*	JX140814	JX140994	JX157386	
CV953 = DTO185G6	*P. fynbosense*		JX140995	JX157387	
CV958 = DTO185H1	*P. fynbosense*	JX140815	JX140996	JX157388	
CV967 = DTO185H3	*P. fynbosense*	JX140816	JX140997	JX157389	
CV969 = DTO185H4	*P. fynbosense*		JX140998	JX157390	
CV975 = DTO185H7	*P. fynbosense*	JX140817	JX140999	JX157391	
CV978 = DTO185H8	*P. fynbosense*	JX140818	JX141000	JX157392	
CBS167.81	[T] *P. gallaicum*	JN617690	JN606837	JN606548	
CBS164.81	*P. gallaicum*		JN606836	JN606547	
NRRL35509	[T] *P. georgiense*	EF422851		EF506239	
CBS125543	[T] *P. glabrum*	GU981567	GU981619		
CBS125543 = IMI91944	[T] *P. glabrum*	GU981567	GU981619		JF417447
CV1038 = DTO185I2	*P. glabrum*	JX140789	JX271540	JX157322	
CV1181 = DTO182H6 = KAS3980 = DAOM241365	*P. glabrum*	JX140796	JX271546	JX157326	
CV1239 = DTO182I1 = KAS3987 = DAOM241360	*P. glabrum*	JX140797	JX271545	JX157329	
CV1494 = DTO183B7 = KAS4015 = DAOM241364	*P. glabrum*	JX140798	JX271542	JX157336	
CV15 = DTO180G2 = KAS4017 = DAOM241135	*P. glabrum*	JX140799	JX271534	JX157261	
CV188 = DTO181C4 = KAS4054 = DAOM241132	*P. glabrum*	JX140790	JX271541	JX157276	
CV2082 = DTO183G5 = KAS4068 = DAOM241355	*P. glabrum*	JX140747	JX271543	JX157341	
CV341 = DTO184H8	*P. glabrum*	JX140792	JX271539	JX157280	
CV36 = DTO180G6 = KAS4116 = DAOM241366	*P. glabrum*	JX140801	JX271544	JX157264	
CV4 = DTO180F8 = KAS4125 = DAOM241361	*P. glabrum*	JX140802	JX271536	JX157258	
CV43 = DTO180H1 = KAS4129 = DAOM241363	*P. glabrum*	JX140793	JX271537	JX157265	
CV504 = DTO181G9 = KAS4141 = DAOM241362	*P. glabrum*	JX140794	JX271538	JX157283	
CV6 = DTO184D3	*P. glabrum*		JX271532	JX157259	
CV7 = DTO180F9 = KAS4166 = DAOM241133	*P. glabrum*	JX140803	JX271533	JX157260	
CV728 = DTO182A2 = KAS4169 = DAOM241134	*P. glabrum*	JX140795	JX271535	JX157291	
DAOM215352	*P. glabrum*				
DAOM215354	*P. glabrum*				
DAOM230098	*P. glabrum*				
DAOM230099	*P. glabrum*				
DAOM230100	*P. glabrum*				

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Table 4: Continued

Strains	Species	GenBank accession number			
		ITS	Btub	CMD	RPB2
DAOM231134	*P. glabrum*				
KAS1730	*P. glabrum*				
KAS2345	*P. glabrum*				
KAS2346	*P. glabrum*				
KAS3065	*P. glabrum*				
KAS3456	*P. glabrum*				
NRRL.35621	*P. glabrum*		EF198547		
NRRL.35626	*P. glabrum*		EF198552		
NRRL.35684	*P. glabrum*		EF198564		
CBS324.83	[T] *P. glabrum (ex-type of P. asperosporum)*	AF033412			JN406574
CBS260.29	*P. glabrum (ex-type of P. flavidorsum)*		GQ367515	GQ367541	
CBS105.11	*P. glabrum (ex-type of P. frequentas)*		GQ367501	GQ367525	JN406647
CBS213.28	*P. glabrum (ex-type of P. oledzskii)*		GQ367516	GQ367542	
CBS229.28	*P. glabrum (ex-type of P. paczowskii)*		GQ367506	GQ367531	JN406602
CBS344.59	*P. glabrum (ex-type of P. spinuloramigenum)*		GQ367502	GQ367526	
CBS228.28	*P. glabrum (ex-type of P. terlikowskii)*		GQ367519	GQ367532	
CBS328.48	*P. glabrum (ex-type of P. trzebinskii)*				
NRRL939	[T] *P. gladioli*	AF033480			
NRRL985	*P. glandicola*	DQ339573			
CBS215.28	[T] *P. godlewskii*	JN617692	JN606768	JN606443	
CBS218.28	*P. godlewskii*		JN606769	JN606437	
CBS408.69	[T] *P. gorlenkoanum*	GU944581	GU944520	GU944608	
CBS411.69	*P. gorlenkoanum*		GU944521	GU944609	
CBS166.81	[T] *P. granatense*				
CBS185.27 = NRRL2300	[T] *P. griseofulvum*	AF033468	AY674432	JF909960	JN121449
CBS110420	*P. griseofulvum*		AY674431		
CBS485.84	*P. griseofulvum*		AY674430		
CV148 = DTO181B6	*P. griseofulvum*	JX091406	JX091544	JX141586	
CV41 = DTO180G8 = KAS4127	*P. griseofulvum*	JX091407	JX091541	JX141582	
CV51 = DTO180H3 = KAS4144	*P. griseofulvum*	JX091408	JX091542	JX141583	
CV69	*P. griseofulvum*	JX091405	JX091543	JX141584	
CV78 = DTO180I2 = KAS4174	*P. griseofulvum*	JX091409	JX091502	JX141585	
NRRL2671	[T] *P. griseolum*	EF422848			
CCT6241	*P. griseoroseum*	AY425983			
DAOM239912	[T] *P. guanacastense*	JN626098	JN625967	JN626010	
DAOM239913	*P. guanacastense*	JN626099	JN625968	JN626011	
NRRL907	[T] *P. guttulosum*	HQ646592	HQ646576	HQ646587	
CBS412.69	[T] *P. harmonense*		AY495996		
CV2845 = DTO180D8 = KAS3942 = DAOM241098	[T] *P. hemitrachum*	FJ231003	JX141048	JX157526	
CV2844 = DTO180D7 = KAS3941 = DAOM241097	*P. hemitrachum*	FJ231002	JX141047	JX157525	
CV964 = DTO182F1 = KAS4221 = DAOM241099	*P. hemitrachum*	JX140916	JX141046	JX157475	
CBS336.48 = NRRL1040	[T] *P. herquei*	JN626101	JN625970	JN626013	JN121494
CBS136.22	*P. herquei*	JN626100	JN625969	JN626012	
CBS226.89	[T] *P. heteromorphum*				JN406605
CBS122392	[T] *P. hetheringtonii*	GU944558	GU944538	GU944642	
CBS124287 = DTO32E3	*P. hetheringtonii*		GU944540	GU944644	
CBS229.60 = NRRL143	[T] *P. hirayamae*	JN626095	JN625955	JN626003	JN121459
CBS238.65	*P. hirayamae*	JN626096	JN625956	JN626004	
CV877 =	*P. hirayamae*	JX091452			
CV887 = DTO182B9 = KAS4189 = DAOM241115	*P. hirayamae*	JX091453	JX091572	JX141568	
CV916 = DTO182D2 = KAS4201 = DAOM241116	*P. hirayamae*	JX091454	JX091573	JX141569	
IBT10628	[T] *P. hirsutum*	AJ004818			
CBS691.77 = ATCC = 38667 = IMI253785	[T] *P. hispanicum*				JN406539
CBS701.68	*P. hordei*		AY674347		
CBS788.70	*P. hordei*		AY674345		
IBT3083	*P. hordei*	AJ004817			
NRRL5274	*P. idahoense*	EF626955			
KAS3159	*P. implicatum*				
CBS184.81 = FRR2061 = IMI190235 = NRRL2061	[T] *P. implicatum sensu Pitt*	AF033428			JN406620
CBS115.63 = ATCC18324 = IMI166620 = NRRL3387	[T] *P. indicum*	AY742699	EU427263	AY741744	JN406640
CV1518 = DTO183C3 = KAS4022 = DAOM241145	*P. infra-aurantiacum*	JX140746	JX271568	JX157337	
CV362 = DTO181F1 = KAS4118 = DAOM241146	*P. infra-aurantiacum*	JX140749	JX271569	JX157281	
ATCC48114	*P. italicum*	AY373920			
DAOM239937	[T] *P. jacksonii*	JN686437	JN686368	JN686391	
DAOM239938	*P. jacksonii*	JN686438	JN686369	JN686392	
IBT24411	[T] *P. jamesonlandense*	DQ267912			
CBS221.28	[T] *P. janczewskii*	AY157487			JN406612
CBS279.47	*P. janczewskii*				
CBS413.68	*P. janczewskii*				
CBS414.68	*P. janczewskii*				
CBS458.69	*P. janczewskii*				
CBS340.48 = NRRL2016	[T] *P. janthinellum*	GU981585	GU981625		
DTO92D2	*P. janthinellum*				
CBS341.48	[T] *P. javanicum*	GU981613	GU981657		JN121498
AS35706	*P. javanicum*			AY678588	
AS36549	*P. javanicum*			AY678589	
DTO10C3	*P. javanicum*				
DTO111A8 = IBT29369	*P. javanicum*				
CBS216.28	[T] *P. jensenii*				JN406614
CBS342.48	[T] *P. jensenii*	JN617693			

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Table 4: Continued

			GenBank accession number			
Strains		**Species**	**ITS**	**Btub**	**CMD**	**RPB2**
NRRL909	T	*P. jensenii*	AY443470		AY443490	JN406614
NRRL909		*P. jensenii*	AY443470	JX140954		
DAOM239943	T	*P. johnkrugii*	JN686447	JN686378	JN686401	
DAOM239944		*P. johnkrugii*	JN686448	JN686379	JN686402	
CBS192.87	T	*P. jugoslavicum*				JN406618
DAOM216105		*P. kananaskensis*				
CBS218.28	T	*P. kapuscinskii*		JX140955	JX157404	
CBS247.67 = NRRL5182	T	*P. katangense*	AF033458			JN121471
NRRL3442	T	*P. kojigenum*	AF033489			
CBS625.67	T	*P. kurssanovi*				
CBS185.65 = MUCL8221	T	*P. lagena*				JN121450
CBS110532		*P. lagena*				
CBS120415		*P. lagena*				
CBS337.97		*P. lagena*				
DTO92D1		*P. lagena*				
NRRL2009	T	*P. lanosum*	DQ304540			
CBS343.48 = NRRL718	T	*P. lapidosum*	AF033409		EU644070	JN121500
CBS277.70 = ATCC22054 = IMI148395 = NRRL5272	T	*P. lassenii*	AF033430		EU644071	JN121481
CBS345.48	T	*P. levitum*	GU981607	GU981654		
NRRL705	T	*P. levitum*	JN626097	JN714938	JN714939	
CBS339.97	T	*P. limosum*	GU981568	GU981621		
CBS188.77	T	*P. lineolatum*	GU981579	GU981620		
CBS347.48	T	*P. lividum*	AF033406			JN406563
DAOM195063		*P. lividum*				
DAOM212615		*P. lividum*				
KAS1583		*P. lividum*				
CBS347.51	T	*P. luteocoeruleum*				JN406562
NRRL5824	T	*P. luzoniacum*	AF033446			
CBS196.81	T	*P. maclennaniae*				
NRRL3452	T	*P. madriti*	AF033482			
NRRL35754	T	*P. malacaense*	EU427300			
CBS647.95	T	*P. malachiteum*				JN121543
CV2855 = DTO180E6 = KAS3947 = DAOM241161	T	*P. malacosphaerula*	FJ231026	JX091524	JX141542	
CV2836 = DTO180D1 = KAS3931		*P. malacosphaerula*	FJ231024	JX091525	JX141540	
CV2848 = DTO180E1 = KAS3944		*P. malacosphaerula*	FJ231025	JX091527	JX141541	
CBS500.73	T	*P. mali*	AF527056			
CV1180 = DTO182H5 = KAS3979 = DAOM241144		*P. malmesburiensis*	JX140743	JX271566	JX157325	
CV1422 = DTO183A6 = KAS4003 = DAOM241143		*P. malmesburiensis*	JX140745	JX271567	JX157335	
DAOM239917	T	*P. malochii*	JN626104	JN625973	JN626016	
DAOM239925		*P. malochii*	JN626112	JN625980	JN626023	
CBS253.31 = NRRL2134	T	*P. manginii*	GU944599	JN606651	JN606381	
CBS407.65		*P. manginii*		JN606649	JN606382	
CBS271.83	T	*P. mariaecrucis*	GU981593	GU981630		
CBS641.95	T	*P. melanoconidium*				JN406542
CBS115506		*P. melanoconidium*		AY674304		
CBS218.90		*P. melanoconidium*		AY674302		
CBS640.95		*P. melanoconidium*		AY674303		
CV1331 = DTO183A2 = KAS3998		*P. melanoconidium*	JX091410	JX091545	JX141587	
IBT3442		*P. melanoconidium*	AJ005483			
CBS218.30 = NRRL2041	T	*P. melinii*	AF033449			JN406613
CBS340.61		*P. melinii*				
CV2393 = DTO183H8 = KAS4085 = DAOM241102		*P. melinii*	JX140917	JX141052	JX157527	
CV2404 = DTO183I1 = KAS4087 = DAOM241103		*P. melinii*	JX140918	JX141053	JX157528	
CV535 = DTO181H6 = KAS4148 = DAOM241100		*P. melinii*	JX140919	JX141050	JX157451	
CV542 = DTO181H8 = KAS4150 = DAOM241101		*P. melinii*	JX140920	JX141051	JX157452	
CBS544.88		*P. melinii-like*				
CBS546.88		*P. melinii-like*				
CBS445.74 = NHL6468	T	*P. meloforme*	GU981605	GU981656	EU644066	
NRRL50410	T	*P. menonorum*	HQ646591	HQ646573	HQ646584	
CBS314.67 = NRRL5814	T	*P. meridianum*	AF033451			JN406576
CBS220.28 = NRRL1077	T	*P. miczynskii*	GU944600	JN606706	JN606526	
CBS126223		*P. miczynskii*		JN606707	JN606528	
FSU6293		*P. molle*	GQ221148			
CBS172.87	T	*P. mononematosum*		AY495997		
CBS112104		*P. mononematosum*		AY495998		
CBS310.63 = NRRL3407	T	*P. montanense*	AF527058			JN406579
KAS1583		*P. montanense*				
KAS1584		*P. montanense*				
KAS905		*P. montanense*				
CBS501.73 = NRRL2060	T	*P. multicolor*	EU427298	JN799645	JN799646	EU427262
CBS352.48 = NRRL911	T	*P. nalgiovense*	AY371617	AY495999		
CBS353.48 = NRRL1070	T	*P. namyslowskii*	AF033463	JX141067		JF417430
CBS169.87 = IBT3439	T	*P. neoechinulatum*	AJ005481	AY674301		
CBS126231	T	*P. neomiczynskii*	JN617671	JN606705	JN606523	
CBS203.84	T	*P. nepalense*				JN121453
CBS354.48	T	*P. nigricans*				
CBS744.70	T	*P. nigricans var. sulphuratum*				
CBS130383	T	*P. nothofagi*	JN617712	JN606732	JN606507	
CBS127004		*P. nothofagi*		JN606731	JN606508	
CBS137.41	T	*P. novaezeelandiae*	JN617688			JN406628

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Table 4: Continued

Strains	Species	GenBank accession number			
		ITS	Btub	CMD	RPB2
CBS546.77	*P. novaezeelandiae*				
CV117 = DTO181A6 = KAS3977 = DAOM241113	*P. novaezeelandiae*	JX140846	JX140958	JX157356	
CV129 = DTO184F1	*P. novaezeelandiae*	JX140836	JX140959	JX157357	
CV1290 = DTO186A3	*P. novaezeelandiae*	JX140847	JX140972	JX157395	
CV147 = DTO184F5	*P. novaezeelandiae*	JX140837	JX140960	JX157358	
CV1560 = DTO186B7	*P. novaezeelandiae*	JX140848	JX140973	JX157398	
CV1812 = DTO186D9	*P. novaezeelandiae*	JX140849	JX140974	JX157400	
CV200 = DTO184G5	*P. novaezeelandiae*	JX140838	JX140961	JX157360	
CV2029 = DTO186H1	*P. novaezeelandiae*	JX140850	JX140975	JX157401	
CV2051 = DTO186H2	*P. novaezeelandiae*	JX140851	JX140976	JX157402	
CV337 = DTO184H7	*P. novaezeelandiae*	JX140839	JX140962	JX157361	
CV355 = DTO181E8	*P. novaezeelandiae*	JX140852	JX140963	JX157362	
CV406 = DTO184I1	*P. novaezeelandiae*	JX140840	JX140964	JX157363	
CV42 = DTO180G9 = KAS4128 = DAOM241112	*P. novaezeelandiae*	JX140853	JX140956	JX157352	
CV452 = DTO184I3	*P. novaezeelandiae*		JX140965	JX157364	
CV47 = DTO184D5	*P. novaezeelandiae*	JX140835	JX140957	JX157353	
CV587 = DTO185B4	*P. novaezeelandiae*	JX140841	JX140966	JX157367	
CV616 = DTO185B7	*P. novaezeelandiae*	JX140842	JX140967	JX157368	
CV818 = DTO185D6	*P. novaezeelandiae*	JX140843	JX140968	JX157370	
CV909 = DTO182C8 = KAS4198 = DAOM241354	*P. novaezeelandiae*	JX140854	JX140969	JX157380	
CV910 = DTO185F3	*P. novaezeelandiae*	JX140844	JX140970	JX157381	
CV992 = DTO185H9	*P. novaezeelandiae*	JX140845	JX140971	JX157393	
NRRL35618	*P. novaezeelandiae*	EF200078			
NRRL793	[T] *P. obscurum*		JX141068		
CBS357.48 = NRRL926	[T] *P. ochrochloron*	GU981604	GU981672		
CBS489.66 = ATCC18338 = IMI116248II = NRRL35499	[T] *P. ochrosalmoneum*	EF626961	EF506212	EU644067	JN121524
NRRL35497	*P. ochrosalmoneum*	EF626959			
NRRL35498	*P. ochrosalmoneum*	EF626960			
CBS294.62 = KAS808	[T] *P. odoratum*				JN406583
CBS349.51	[T] *P. oligosporum*	GU981614	GU981658		
CBS232.60	[T] *P. olsonii*	EU587341	AY674445		
ATCC64639	*P. olsonii*	AY373925			
CBS349.61	*P. olsonii*		AY674443		
CBS381.75	*P. olsonii*		AY674444		
CBS174.81	[T] *P. onobense*	GU981575	GU981627		
CBS190.68 = ATCC18608 = IMI137977 = NRRL3471	[T] *P. ornatum*				JN121758
NRRL5922	[T] *P. osmophilum*	EU427295			
CBS219.30 = NRRL787	[T] *P. oxalicum*	AF033438			JN121456
AS35705	*P. oxalicum*			AY678546	
CV822 = DTO182B1 = KAS4180	*P. oxalicum*	JX091431	JX091528	JX141543	
DAOM178627	*P. oxalicum*				
CV2224 = DTO183H2 = KAS4076 = DAOM241069	[T] *P. pagulum*	JX140898	JX141070	JX157519	
CV2236 = DTO183H3 = KAS4077 = DAOM241070	*P. pagulum*	JX140899	JX141071	JX157520	
CBS336.79	[T] *P. palmense*			GQ367534	JN406566
CBS687.77	*P. palmense (ex-type of P. grancanariae)*			GQ367533	
CBS276.75	[T] *P. pancosmium*	JN617660	JN606790	JN606446	
CBS126434	*P. pancosmium*		JN606787	JN606452	
CV0134 = DTO181B3 = KAS4000 = DAOM241039	*P. pancosmium*	JX140862	JX141016	JX141507	
CV0693 = DTO182A1 = KAS4165 = DAOM241040	*P. pancosmium*	JX140863	JX141017	JX141508	
IBT12392	[T] *P. paneum*	X82360			
CBS570.73	[T] *P. papuaneum*				JN406546
CBS430.65 = ATCC22354	[T] *P. paraherquei*	AF178511	GU981628		
CV1360 = DTO183A4 = KAS4001 = DAOM241061	*P. parviverruscosum-like*	JX091458	JX091576	JX141594	
CV1448 = DTO183A9 = KAS4007 = DAOM241063	*P. parviverruscosum-like*	JX091459	JX091582	JX141595	
CV1450 = DTO183B1 = KAS4008 = DAOM241064	*P. parviverruscosum-like*	JX091460	JX091583	JX141596	
CV1583 = DTO186B8	*P. parviverruscosum-like*		JX091577	JX141597	
CV1851 = DTO183E8	*P. parviverruscosum-like*	JX091461	JX091585	JX141601	
CV2815 = DTO184A1	*P. parviverruscosum-like*		JX091590	JX141606	
CV2819 = DTO184A4	*P. parviverruscosum-like*	JX091462	JX091591	JX141607	
CV2822 = DTO184A6	*P. parviverruscosum-like*	JX091463	JX091592	JX141608	
CV2831 = DTO184B4	*P. parviverruscosum-like*		JX091593	JX141612	
CV2833 = DTO184B6	*P. parviverruscosum-like*	JX091464	JX091594	JX141613	
CV2839 = DTO184C2	*P. parviverruscosum-like*		JX091595	JX141615	
CV2849 = DTO184C6	*P. parviverruscosum-like*		JX091596	JX141616	
CV933 = DTO185F9	*P. parviverruscosum-like*		JX091575	JX141591	
CV959 = DTO182E7 = KAS4218 = DAOM241059	*P. parviverruscosum-like*	JX091456	JX091581	JX141592	
CV979 = DTO182F6 = KAS4226 = DAOM241060	*P. parviverruscosum-like*	JX091457	JX091580	JX141593	
NRRL35504	[T] *P. parvulum*	EF422845			
CBS359.48 = NRRL2095	[T] *P. parvum*	AF033460	HQ646568		JN406559
CBS126330	[T] *P. pasqualense*	JN617676	JN606673	JN606394	
CBS122402	*P. pasqualense*		JN606674	JN606393	
CV2387 = DTO183H7 = KAS4084 = DAOM241036	*P. pasqualense*	JX140864	JX141018	JX141509	
CBS260.87	*P. patens*				JN406593
CBS360.48 = NRRL2008	[T] *P. paxilli*	GU944577	JN606844	JN606566	
CBS162.96	*P. paxilli*		JN606804	JN606545	
CBS113178	[T] *P. penarojense*	GU981570	GU981646		
CBS113133	*P. penarojense*	GU981569	GU981645		
CBS623.72	[T] *P. philippinense*				JN406543
CBS249.32 = ATCC10481 = IMI040585 = NRRL2070	[T] *P. phoeniceum*	AY742694		AY741729	JN406597
KAS3161	*P. phoeniceum*				

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Table 4: Continued

Strains		Species	GenBank accession number			
			ITS	Btub	CMD	RPB2
CBS102479 = NRRL25542	T	*P. pimiteouiense*	AF037431	HQ646569		JN406650
CBS362.48 = NRRL1075	T	*P. piscarium*	GU981600	GU981668		
CBS222.28 = NRRL995	T	*P. polonicum*	AF033475	AY674305		
CBS690.77		*P. polonicum*		AY674307		
IBT11388		*P. polonicum*	AJ005492			
CBS382.64	T	*P. porphyreum*				
CV2189 = DTO183G7 = KAS4072 = DAOM241107	T	*P. pseudoantarcticum*	JX140804	JX141006	JX157413	
CV2191 = DTO183G8 = KAS4073 = DAOM241108		*P. pseudoantarcticum*	JX140805	JX141007	JX157414	
CV1758 = DTO183D5 = KAS4033 = DAOM241106	T	*P. pseudoatrovenetum*	JX140831	JX141005	JX157412	
CV816 = DTO182A9 = KAS4179 = DAOM241111	T	*P. pseudocanescens*	JX140834	JX140949	JX157369	
CBS280.39 = NRRL2026	T	*P. pulvillorum*	GU981602	GU981670		
CBS366.48	T	*P. purpurascens*	AF033408		GQ367538	
CBS126.64		*P. purpurascens*			GQ367537	
NRRL720	T	*P. purpurescens*	AF033408			
CV26 = DTO180G4 = KAS4104 = DAOM241136		*P. purpuroides*	JX140791	JX271547	JX157263	
NRRL2498	T	*P. pusillum*	EF626951			
CBS101623	T	*P. quebecense*	JN617661	JN606700	JN606509	
CBS417.69 = NRRL3758	T	*P. quercetorum*	AY443471			JN406552
CBS224.28 = NRRL2150	T	*P. raciborskii*	AF033447	JX141069		JN406607
CBS340.79	T	*P. radiatolobatum*				
CV198 = DTO184G3 = KAS4062 = DAOM241110		*P. radiatolobatum*	JX140832	JX140948	JX157359	
CV68 = DTO180H5 = KAS4162 = DAOM241109		*P. radiatolobatum*	JX140833	JX140947	JX157354	
CBS280.58	T	*P. radulatum*				JN406586
CBS285.65		*P. radulatum*				
NRRL2039	T	*P. raistrickii*	AF033491			
DTO193H2 = CBS251.56 = ATCC12292 = NRRL3459 = IBT22871	T	*P. ramusculum*	EF433765		JX141496	JN121472
CBS281.58 = NRRL2674	T	*P. raperi*	AF033433	GU981622		
CBS126234	T	*P. raphiae*	JN617673	JN606657	JN606409	
CBS367.48 = NRRL1748	T	*P. restrictum*	AF033457			JN121506
CV101 = DTO184E6		*P. restrictum*		JX141057	JX157421	
CV1695 = DTO186C6		*P. restrictum*		JX141066	JX157496	
CV799 = DTO185D3		*P. restrictum*		JX141058	JX157460	
CV872 = DTO182B6 = KAS4186 = DAOM241057		*P. restrictum*	JX140925	JX141059	JX157464	
CV896 = DTO182C3 = KAS4192 = DAOM241052		*P. restrictum*	JX140926	JX141060	JX157467	
CV90 = DTO180I4 = KAS4194 = DAOM241050		*P. restrictum*	JX140928	JX141054	JX157417	
CV900 = DTO182C5 = KAS4195 = DAOM241055		*P. restrictum*	JX140927	JX141061	JX157468	
CV93 = DTO180I6 = KAS4205 = DAOM241054		*P. restrictum*	JX140930	JX141055	JX157419	
CV932 = DTO182D6 = KAS4207 = DAOM241056		*P. restrictum*	JX140929	JX141062	JX157471	
CV943 = DTO182E1 = KAS4211 = DAOM241058		*P. restrictum*	JX140931	JX141063	JX157473	
CV948 = DTO182E3 = KAS4213 = DAOM241051		*P. restrictum*	JX140932	JX141064	JX157474	
CV96 = DTO184E4		*P. restrictum*		JX141056	JX157420	
CV972 = DTO182F4 = KAS4224 = DAOM241053		*P. restrictum*	JX140933	JX141065	JX157476	
CBS121.68 = NRRL3447	T	*P. reticulisporum*	GU981617	GU981665		
CBS513.74		*P. reticulisporum*		GU981666		
IBT16537	T	*P. ribeum*	DQ267916			
NRRL906	T	*P. rivolii*	AF033419			
CBS368.48 = NRRL1078	T	*P. rolfsii*	JN617705	GU981667		
NRRL849	T	*P. roqueforti*	EU427296			
CBS266.29 = NRRL2064	T	*P. roseopurpureum*	GU944605	JN606838	JN606556	
CBS281.39		*P. roseopurpureum*		JN606839	JN606557	
CBS145.83	T	*P. rubefaciens*				JN406627
CV1015 = DTO182G4 = KAS3960 = DAOM241077		*P. rubefaciens*	JX140895	JX141073	JX157479	
CV1479 = DTO186A8		*P. rubefaciens*		JX141147	JX157487	
CV1486 = DTO183B5 = KAS4013 = DAOM241079		*P. rubefaciens*	JX140892	JX141149	JX157489	
CV1495 = DTO183B8 = KAS4016 = DAOM241080		*P. rubefaciens*	JX140893	JX141150	JX157490	
CV1514 = DTO183C2 = KAS4020 = DAOM241081		*P. rubefaciens*	JX140894	JX141151	JX157491	
CV1546 = DTO186B5		*P. rubefaciens*		JX141152	JX157493	
CV1558 = DTO186B6		*P. rubefaciens*		JX141133	JX157494	
CV2817 = DTO180C5 = KAS3934 = DAOM241093		*P. rubefaciens*	FJ231004	JX141158	JX157533	
CV2820 = DTO180C6 = KAS3936 = DAOM241094		*P. rubefaciens*	FJ231005	JX141159	JX157534	
CV2826 = DTO180C7 = KAS3956 = DAOM241095		*P. rubefaciens*	FJ231007	JX141160	JX157535	
CV2835 = DTO180C9 = KAS3930 = DAOM241096		*P. rubefaciens*	FJ231006	JX141044	JX157532	
CV597 = DTO181I5 = KAS4158 = DAOM241076		*P. rubefaciens*	JX140896	JX141072	JX157454	
CV795 = DTO182A7 = KAS4177 = DAOM241078		*P. rubefaciens*	JX140934	JX141157	JX157459	
NRRL792	T	*P. rubens*		JF909949	JF909967	
CBS197.46 = NRRL832		*P. rubens*		JF909945	JF909963	
CBS205.57 = NRRL824		*P. rubens*	AY371609	JF909954	JF909972	JN406616
CBS307.48 = NRRL1951		*P. rubens*		JF909944	JF909962	JF909926
CV349 = DTO181E7 = KAS4114		*P. rubens*	JX091411	JX091546	JX141588	
CV361 = DTO181E9 = KAS4117		*P. rubens*	JX091412	JX091547	JX141589	
CV378 = DTO181F2 = KAS4120		*P. rubens*	JX091413	JX091548	JX141590	
NRRL824		*P. rubens*	AY371609			
CBS609.73 = NRRL6033	T	*P. rubidurum*	AF033462	HQ646574		JN406545
CBS261.87	T	*P. sabulosum*				
CBS127032	T	*P. sanguifluum*	JN617681	JN606819	JN606555	
CBS110.64		*P. sanguifluum*		JN606829	JN606533	
CV0530 = DTO181H4 = KAS4146		*P. sanguifluum*	JX140867	JX141019	JX141510	
CV1856 = DTO183E9 = KAS4049		*P. sanguifluum*	JX140865	JX141020	JX141511	
CV1865 = DTO183F2 = KAS4051		*P. sanguifluum*	JX140866	JX141021		
NRRL783	T	*P. sartoryi*	AF033421			

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Table 4: Continued

Strains	Species	GenBank accession number			
		ITS	Btub	CMD	RPB2
IBT30335	*P. saturniforme*				
DAOM214786 [T]	*P. scabrosum*	DQ267906			
NRRL3461 [T]	*P. sclerotigenum*	AF033470			
CBS287.36 = NRRL2074 [T]	*P. sclerotiorum*	JN626132	JN626001	JN626044	JN406585
CBS118889	*P. sclerotiorum*	JN686454	JN686385	JN686408	
CBS128.65	*P. sclerotiorum*	JN686452	JN686383	JN686406	
CBS258.55	*P. sclerotiorum*	JN686453	JN686384	JN686407	
CV934 = DTO182D7 = KAS4208 = DAOM241117	*P. sclerotiorum*	JX091455	JX091574	JX141570	
CBS290.48 = NRRL715 [T]	*P. shearii*	GU944606	JN606840	JN606560	
CBS578.70	*P. shearii*		JN606852	JN606575	
CBS235.60 [T]	*P. silvaticum*				
CBS372.48 = NRRL1075 [T]	*P. simplicissimum*	GU981588	GU981632		JN121507
AS35751	*P. simplicissimum*			EU644073	
AS36550	*P. simplicissimum*			AY678551	
ATCC48681	*P. simplicissimum*			DQ911128	
IBT15303	*P. simplicissimum*		DQ834935		
CBS413.69 [T]	*P. sizovae*	GU944588	GU944535	GU944618	
CV0987 = DTO182F7 = KAS4227	*P. sizovae*	JX140870	JX141023	JX141513	
CV0989 = DTO182F9 = KAS4229	*P. sizovae*	JX140871	JX141024	JX141514	
CV1285 = DTO182I6 = KAS3992	*P. sizovae*	JX140869	JX141025	JX141515	
CBS439.75 = NRRL13055 [T]	*P. skrjabinii*	GU981576	GU981626		
CV0085 = DTO180I3 = KAS4182	*P. skrjabinii*	JX091432	JX091529	JX141544	
KAS2160	*P. skrjabinii*				
KAS2161	*P. skrjabinii*				
KAS2179	*P. skrjabinii*				
KAS2190	*P. skrjabinii*				
KAS2200	*P. skrjabinii*			EU427280	
CBS276.83 [T]	*P. smithii*				JN406589
CBS424.89 = FRR937 [T]	*P. solitum*	AY373932	AY674354		
NRRL2023 [T]	*P. soppii*	AF033488			
KAS1721	*P. sp.*				
KAS3349	*P. sp.*				
KAS3349	*P. sp.*				
CBS374.48 = NRRL1750 [T]	*P. spinulosum*	AF033410			JN406558
CBS223.28	*P. spinulosum*			GQ367536	
CBS268.35	*P. spinulosum (ex-type of P. mediocre)*				
CBS269.35	*P. spinulosum (ex-type of P. mucosum)*				
CBS289.36	*P. spinulosum (ex-type of P. tannophagum)*			GQ367528	
CBS271.35	*P. spinulosum (ex-type of P. tannophilum)*			GQ367530	
CBS260.55 [T]	*P. steckii*	GU944597	GU944522	GU944611	
CBS325.59	*P. steckii*		GU944527	GU944617	
NRRL35367	*P. steckii*	DQ123665			
NRRL35463	*P. steckii*	EF634431			
NRRL5816 [T]	*P. stolkiae*	AF033444			
CBS705.68 = NRRL26877 [T]	*P. striatisporum*	AF038938	JX141156		
NRRL31108 [T]	*P. subarcticum*	AF481120			JN406538
CBS125096 [T]	*P. subericola*				
IBT23009	*P. subericola*			GQ367546	JN406621
IBT30068	*P. subericola*			GQ369760	
IBT30068	*P. subericola*				
KAS1289	*P. subericola*			GU991609	
KAS1724	*P. subericola*				
KAS1731	*P. subericola*				
CBS267.29 = ATCC10502 = IMI040594 = MUCL28655 = NRRL2071 [T]	*P. sublateritium*	EY437288	*JX091557*	AB566102	
CV1840 = DTO183E5 = KAS4046 = DAOM241042	*P. sucrivorum*	JX140872	JX141015	JX141506	
CBS281.36 = NRRL779 [T]	*P. sumatrense*	GU944578	JN606639	JN606368	JN406590
CBS335.59	*P. sumatrense*		JN606640	JN606369	
CV0179 = DTO181B9 = KAS4037 = DAOM 241048	*P. sumatrense*	JX140873	JX141026	JX141516	
CV0460 = DTO181G1 = KAS4132	*P. sumatrense*	JX140882	JX141027	JX141517	
CV0503 = DTO181G8 = KAS4140	*P. sumatrense*	JX140883	JX141028	JX141518	
CV0684 = DTO181I9 = KAS4163 = DAOM241049	*P. sumatrense*	JX140884	JX141029	JX141519	
CV1828 = DTO183E4 = KAS4044	*P. sumatrense*	JX140874	JX141030	JX141520	
CV1882 = DTO183F4 = KAS4055 = DAOM241046	*P. sumatrense*	JX140875	JX141031	JX141521	
CV2184 = DTO183G6 = KAS4071 = DAOM241044	*P. sumatrense*	JX140876	JX141032	JX141522	
CV2201 = DTO183G9 = KAS4074 = DAOM241043	*P. sumatrense*	JX140877	JX141033	JX141523	
CV2285 = DTO183H4 = KAS4080	*P. sumatrense*	JX140878	JX141034	JX141524	
CV2328 = DTO183H5 = KAS4082 = DAOM241047	*P. sumatrense*	JX140879	JX141035	JX141525	
CV2403 = DTO183H9 = KAS4086 = DAOM241045	*P. sumatrense*	JX140880	JX141036	JX141526	
CBS122416 [T]	*P. svalbardense*	GU981603	GU981669		
NRRL918 [T]	*P. swiecickii*	AF033490			
NRRL918 [T]	*P. swiecickii*		JX141008	JX157409	
NRRL3759 [T]	*P. syriacum*	EF634448			
CBS2282.8	*P. terlikowski*				
CBS313.67 [T]	*P. terrenum*	AM992111			
DAOM178626	*P. terricola*				JN406577
CBS127354 [T]	*P. terrigenum*	JN617684	JN606810	JN606583	
CBS347.59 = NRRL2077 [T]	*P. thomii*	AF034448		GQ367535	
CV1189 = DTO182H7 = KAS3981 = DAOM241142	*P. thomii*	JX140744	JX271570	JX157327	
CV461 = DTO181G2 = KAS4133 = DAOM241137	*P. thomii*	JX140786	JX271571	JX157282	
KAS1388	*P. thomii*				JN121501

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Table 4: Continued

Strains	Species	GenBank accession number ITS	Btub	CMD	RPB2
KAS2344	*P. thomii*				
KAS2379	*P. thomii*				
KAS2793	*P. thomii*				
CBS745.70 = NRRL6175	*P. thomii (ex-type of P. crocicola)*	EU427290			
CBS350.59	*P. thomii (ex-type of P. yezoense)*			GQ367548	JN406535
CV1000 = DTO182G3 = KAS3959	*P. toxicarium*	JX140935	JX141164	JX157536	
CV11 = DTO180G1 = KAS3969	*P. toxicarium*	JX140937	JX141162	JX157415	
CV11 = DTO180G1 = KAS3969	*P. toxicarium*	JX140937	JX141162	JX157415	
CV1226 = DTO182H9 = KAS3985	*P. toxicarium*	JX140938	JX141166	JX157328	
CV1454 = DTO183B2 = KAS4009	*P. toxicarium*	JX140939	JX141167	JX157485	
CV1532 = DTO183C5 = KAS4024	*P. toxicarium*	JX140940	JX141168	JX157492	
CV2015 = DTO183G2 = KAS4064	*P. toxicarium*	JX140942	JX141169	JX157537	
CV283 = DTO181E1 = KAS4106	*P. toxicarium*	JX140943	JX141163	JX157441	
CV283 = DTO181E1 = KAS4106	*P. toxicarium*	JX140943	JX141163	JX157441	
NRRL2047	*P. toxicarium*		EF198605		
NRRL2579	*P. toxicarium*		EF198606		
NRRL31271	*P. toxicarium*	EF198660		F198641	
NRRL6172	*P. toxicarium*	EF198650		F198631	EF198486
CBS635.95	[T] *P. tricolor*	JN942704	AY674313		EF198499
ATCC10413	*P. tricolor*	AY373935			
CBS636.95	*P. tricolor*		AY674311		
IBT12471	*P. tricolor*	AJ005489			
CBS122410	[T] *P. tropicoides*	GU944584	GU944531	GU944624	
CBS122436	*P. tropicoides*		GU944530	GU944623	
CBS112584	[T] *P. tropicum*	GU944582			
CBS431.65	*P. trzebinskianum*				
CBS432.65	*P. trzebinskianum*				
NRRL5273	[T] *P. tularense*	AF033487			
NRRL759	[T] *P. turbatum*	AF033452			
CBS126437	[T] *P. ubiquetum*	JN617680	JN606800	JN606460	
CBS126439	*P. ubiquetum*		JN606797	JN606457	
CV0451 = DTO181F9 = KAS4131 = DAOM241038	*P. ubiquetum*	JX140885	JX141038	JX141528	
CV0588 = DTO181I4 = KAS4157 = DAOM241037	*P. ubiquetum*	JX140886	JX141039	JX141529	
NRRL2159	*P. urticae*	AF514301			
CV25 = DTO180G3 = KAS4100 = DAOM241357	*P. vagum*	JX140748	JX271531	JX157262	
CBS126323	[T] *P. vancouverense*	JN617675	JN606663	JN606399	
CBS126322	*P. vancouverense*		JN606661	JN606398	
CBS126216	[T] *P. vanderhammenii*		GU981647		
CBS116296	*P. vanderhammenii*	GU981573	GU981648		
CBS339.79	[T] *P. vasconiae*	GU981599	GU981653		
CBS250.32 = NRRL2069	[T] *P. velutinum*	AF033448	JX141170		
IBT5464	*P. venetum*	AJ005485			
FRR965	[T] *P. verrucosum*	AY373938			
CBS389.48 = NRRL739	[T] *P. vinaceum*	AF033461	HQ646575	HQ646586	
BBA65745	[T] *P. virgatum*	AJ748692			JN406555
CBS390.48 = FRR963	[T] *P. viridicatum*	AY373939	AY674295		
JCM17636	[T] *P. viticola*	AB606414	AB540174	AB540173	
DAOM239933	*P. viticola*	JN686439	JN686370	JN686393	
CV1145 = DTO182H2 = KAS3974 = DAOM241140	*P. vulgaris*	JX140779	JX271574	JX157323	
CV1148 = DTO182H3 = KAS3975 = DAOM241139	*P. vulgaris*	JX140785	JX271573	JX157324	
CV2850 = DTO180E2 = KAS3945	*P. vulgaris*	FJ231032	JX271575	JX157349	
CV2851 = DTO180E3 = KAS3946	*P. vulgaris*	FJ231033	JX271576	JX157350	
CV851 = DTO182B3 = KAS4183 = DAOM241138	*P. vulgaris*	JX140787	JX271572	JX157293	
CV905 = DTO182C7 = KAS4197 = DAOM241141	*P. vulgaris*	JX140788	JX271577	JX157298	
NRRL1001	[T] *P. vulpinum*	EU427294			
NRRL2031	*P. vulpinum*	AF506012			
CBS230.28 = NRRL777	[T] *P. waksmanii*	GU944602	JN606779	JN606431	
CBS126428	*P. waksmanii*		JN606778	JN606424	
CBS130375	*P. wellingtonense*		JN606670	JN606395	
CBS231.28 = NRRL800	[T] *P. westlingii*	GU944601	JN606718	JN606500	
CBS688.77	*P. westlingii*		JN606729	JN606503	
CBS118171	[T] *P. wotroi*	GU981591	GU981637		
CBS118138	*P. wotroi*	GU981590	GU981636		
CV1677 = DTO183C7 = KAS4026 = DAOM241104	[T] *P. xanthomelinii*	JX140921	JX141120	JX157495	
CV1745 = DTO183D3	*P. xanthomelinii*	JX140922	JX141121	JX157499	
CV1844 = DTO183E7	*P. xanthomelinii*	JX140923	JX141122	JX157504	
CV1871 = DTO186E5	*P. xanthomelinii*		JX141123	JX157506	
CV1886 = DTO186E8	*P. xanthomelinii*		JX141124	JX157507	
CV1905 = DTO183F6 = KAS4057 = DAOM241105	*P. xanthomelinii*	JX140924	JX141125	JX157508	
CV1923 = DTO186F4	*P. xanthomelinii*		JX141126	JX157509	
CV1942 = DTO186F9	*P. xanthomelinii*		JX141128	JX157510	
CV1969 = DTO186G4	*P. xanthomelinii*		JX141129	JX157511	
CV2329 = DTO186I9	*P. xanthomelinii*		JX141131	JX157515	
CBS410.69	[T] *P. yarmokense*				
CBS992.72	[T] *P. zonatum*	GU981581	GU981651		JN406553

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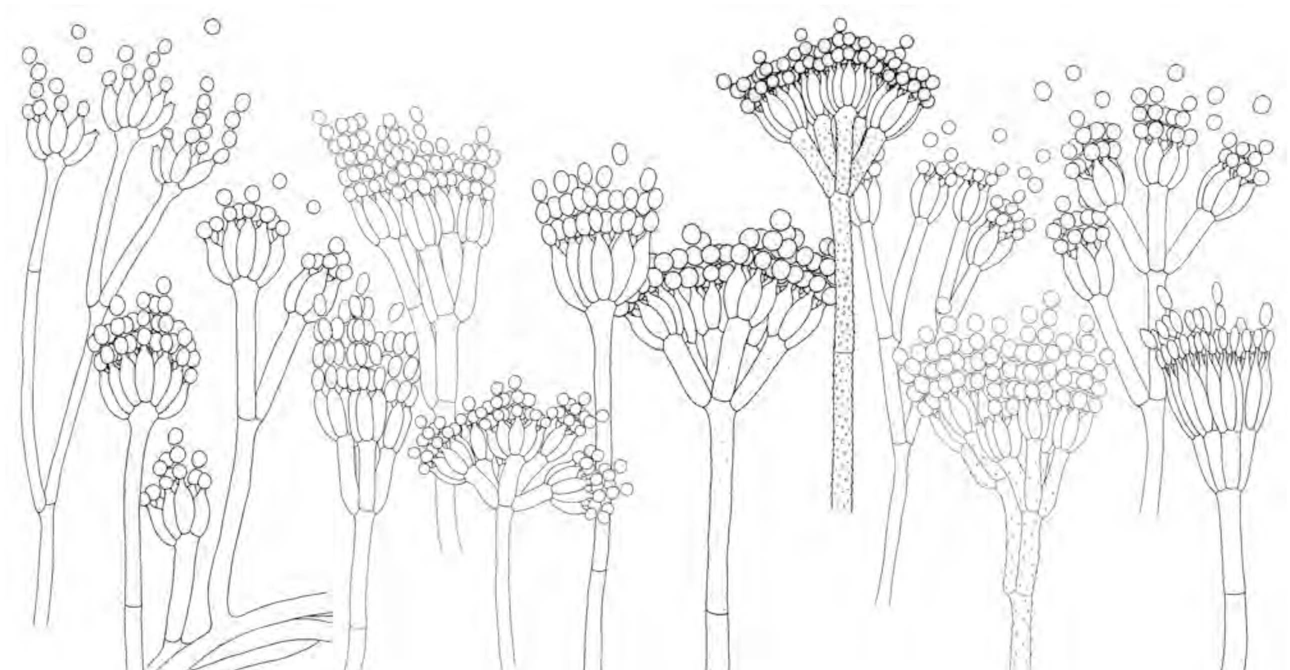
CHAPTER 3
Talaromyces spp. from the Fynbos in the Western Cape, South Africa, including five new species



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Talaromyces spp. from the Fynbos in the Western Cape, South Africa, including five new species

ABSTRACT — A microfungal survey in the diverse Fynbos biome situated in the Western Cape, South Africa, resulted in a large number of isolates that belong to the genus *Talaromyces*. Based on morphological and phylogenetic characterization, fifteen species were found, five of which are considered novel. The aims of this paper were to described these new species and compare them to close relatives using a polyphasic taxonomic approach. *Talaromyces stellenbossiensis* prov. nom. is characterized by ampulliform phialides that taper into fine apical pores and produce rough walled conidia. *Talaromyces crassa* prov. nom. produces deep floccose yellow colonies on CYA and MEA, making it distinct from its close relatives. *Talaromyces parvaurantica* prov. nom. produces restricted growth on CYA, as well as orange mycelia, a character not observed for other species in this particular clade. Two of the new Fynbos species are closely related to *T. rugulosus.* However, they are easily distinguished from *T. rugulosus* based on conidia *en masse* color and floccose colony texture. *Talaromyces vermiculicola* prov. nom. is distinguished from *T. infrolivacea* prov. nom. based on restricted growth rate, funiculose colonies and lack of an olive reverse pigmentation in colonies of the latter species'. Morphological identifications were confirmed with the multigene phylogenies.

KEYWORDS — Biodiversity hotspot, Multigene phylogeny, Internal transcribed spacer region, β-tubulin, Calmodulin.

Introduction

Benjamin (1955) introduced *Talaromyces* for teleomorphic *Penicillium* species that produces soft ascocarps with a cleistothecial wall of interwoven hyphae and typically yellow ascocarps. Species produces mostly spiny ascospores in ovate to globose asci. Benjamin (1955) typified the genus with *T. vermiculatus* (Dangeard) (NRRL 2098, SUI 1725) as type species, but the material was subsequently lost. In response, Samson *et al*. (2011) designated the Dangeard (1907) Plate XVIII of this species as lectotype. *Talaromyces* used to be historically associated with the anamorph genera *Paecilomyces*, *Penicillium* subgenus *Biverticillium* and *Geosmithia* (Pitt *et al*. 2000). Until very recently, it was well known that *Penicillium* sensu lato was polyphyletic and that subgenus *Biverticillium* was distinct from the other three of Pitt's (1979) subgenera *Aspergilloides*, *Furcatum* and *Penicillium*. This was based on morphology, physiology, secondary metabolites and phylogenetic data (Frisvad *et al*. 1990a, Frisvad *et al*. 1990b, LuBuglio *et al*. 1993, Berbee *et al*. 1995, Ogawa *et al*. 1997, Ogawa & Sugiyama 2000, Peterson 2000, Heredia *et al*. 2001, Seifert *et al*. 2004, Wang & Zhuang 2007). With changes made to the ICBN, which provided for single-name nomenclature in fungi (Hawksworth *et al*. 2011, Norvell 2011), Houbraken *et al*. (2011a) evaluated genera in the family *Trichocomaceae* using a four-gene phylogeny and redefined many genera within the family. They also split the family into three, na[1]mely the *Trichocomaceae* (i.e. *Talaromyces* and *Trichocoma*), *Aspergillaceae* (i.e. *Aspergillus* and *Penicillium*) and *Thermoascaceae* (i.e. *Byssochlamys*, *Paecilomyces* and *Thermoascus*). Subsequently, *Penicillium* subgenus *Biverticillium* was transferred into *Talaromyces* sensu stricto (Samson *et al*. 2011).

Thermophillic *Talaromyces* species were shown to be distinct from *Talaromyces* s.str. (Houbraken *et al*. 2011a) and were incorporated into the new genus *Rasamsonia* (Houbraken *et al*. 2011b). In addition, previous *Talaromyces* s.l. associated anamorphs, such as *Paecilomyces,* was shown to belong to a *Paecilomyces*/*Byssochlamys* clade in the family *Thermoascaceae*. *Geosmithia* was shown to be polyphyletic, with a number of species that belong to the *Hypocreales* and the remaining species dispersed between *Penicillium* s.str., *Talaromyces* s.str. and *Rasamsonia* (Houbraken *et al*. 2011a). *Talaromyces* s.str. thus now accommodate species that produces typical *Talaromyces* cleistothecia, as well as the symmetrical, biverticillate and typically acerose phialides of subgenus *Biverticillium*.

The Fynbos biome is situated at the tip of southern Africa and has been identified as one of the world's 25 biodiversity hotspots (Myers *et al*. 2000). Although the area covers only 0.5% of Africa, it contains close to 20% of the continent's plant species, boasting ±9 030 species of which approximately 70% are endemic (Goldblatt & Manning 2002, Midgeley *et al*. 2002, Crous *et al*. 2006, Mucina & Rutherford 2006). Estimates predict 171 500 fungal species to occur in South Africa and approximately 63 210 species in the Fynbos (Crous *et al*. 2006). Unfortunately, only a proportion of this estimate has been isolated and described to date. From previous surveys in the area, it is known that *Penicillium* and *Talaromyces* spp. are commonly isolated from soil and plants (Allsopp *et al*. 1987, Visagie *et al*. 2009, Roets personal communication, Visagie & Jacobs 2012). In the current project fifteen *Talaromyces* species were isolated from soil, air and *Protea repens* infructescence samples. Five of these species were found to represent novel species. The aim of this paper is thus to describe these species and compare them to their closest relatives, using morphology

[1] Repetition presented here are due to the preparation of individual manuscripts for publication

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and multigene phylogenies. An identification key to *Talaromyces* spp. from Fynbos is also provided.

Materials and Methods

Sampling and isolations

Soil, air and *Protea repens* infructescence samples were collected at three sites. These include Stellenbosch Mountain (S33°56'47; E18°52'49: 1 March 2009), Riverlands Nature Reserve near Malmesbury (S33°49'46; E18°35: 1 July 2009) and Struisbaai (S34°45'06; E19°58'59: 1 August 2009).

Air samples were collected with a MAS-100 Eco® air sampler. A volume of 50 L air was sampled in triplicate. For isolations from soil, 5 g soil were added to 100 mL dH_2O and diluted to 10^{-1} and 10^{-2}. Dilutions were plated out in triplicate. *Protea repens* infructescences were collected from three plants per site. At each *P. repens* plant, 1–, 2– and 3 year old infructescences were collected in triplicate. Strains were isolated from *Protea repens* as well as the mite populations inside the infructescences. Infructescences were cut open and mites shake out over 1% agar plates. Mites were then sedated by placing a tiny piece of chloroform soaked cotton wool (*ca*. 1 × 1 cm) inside petri dish. Mites were transferred onto isolation media using a fine steel needle. Plates were incubated on water traps. This prevented cross contamination of plates by mites. Interior parts of infructescences were suspended in 250 mL dH_2O. A dilution series was prepared up to 10^{-5} and plated out in the same manner as the soil dilutions. Direct isolations from infructescence bracts were done with the aid of a Nikon SMZ800 stereomicroscope.

All isolations were done from potato dextrose agar (PDA), supplemented with Chloramphenicol (100 ppm), Streptomycin (50 ppm) and Dichloran (2 ppm) as medium. Plates were incubated at 25 °C for 5–7 d. Colonies resembling *Talaromyces* were transferred onto malt extract agar (MEA) and incubated for a further 7 d. Single spore cultures were stored as water plugs at 4 °C.

Morphology

Strains were characterized with a strict standardized protocol. Inoculations were done from spore suspensions in semi-solid agar (0.1% agar; 0.05% Tween 80), using a pipet with inoculum size of ±1 µL. Strains were incubated on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), yeast sucrose agar (YES), 25% glycerol nitrate agar (G25N) and creatine sucrose agar (CREA). Media were supplemented with $CuSO_4 \cdot 5H_2O$ (0.0005 %) and $ZnSO_4 \cdot 7H_2O$ (0.001 %) (Frisvad & Samson 2004). Plates (90 mm) were incubated at 25 °C, for 7 d, in the dark, left unwrapped and not placed in boxes (Pitt 1979, Samson & Pitt 1985, Okada *et al*. 2000, Frisvad & Samson 2004). Additionally, strains were also incubated on CYA at 5, 30 and 37 °C. Colors in colonies were documented using color codes and names following Kornerup and Wanscher (1967). Cultures grown on MEA were used for microscopic examination. Microscopic characters were documented with an Olympus BX50 light microscope and Olympus SZX12 stereomicroscope, equipped with an Evolution MP digital microscope camera and ImagePro 6.0 software. Camera lucida drawings were prepared using a Nikon Eclipse E800 light microscope. For morphological comparisons, reference strains were obtained from the Centraal Bureau voor Schimmelcultures (CBS), the Netherlands.

Phylogeny

DNA extractions were prepared from strains grown on MEA for 8 d with the ZR Fungal/Bacterial DNA Kit (Zymo Research, California). This study used three genes for phylogenetic comparisons. The ITS gene region was amplified using primers ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White *et al*. 1990). In general, primers Bt2a (GGT AAC CAA ATC GGT GCT GCT TTC) and Bt2b (ACC CTC AGT GTA GTG ACC CTT GGC) were used for β-tubulin amplification (Glass & Donaldson 1995). However, in the *T. rugulosus* clade primers T10 (ACG ATA GGT TCA CCT CCA GAC) and T2 (AAT TGG TGC TGC TTT CTG GCA) were used with an annealing temperature of 52 °C. For Calmodulin, primers CMD5 (CCG AGT ACA AGG ARG CCT TC) and CMD6 (CCG ATR GAG GTC ATR ACG TGG) (Hong *et al*. 2006) were used. A standard PCR protocol was used, which had an initial denature step (94 °C – 3 min), followed by 35 cycles of (94 °C – 45 sec; 56 °C – 45 sec; 72 °C – 60 sec) and ending with a final elongation at 72 °C for 10 min. Sequence reactions were set up using the same primer pairs as the PCR with a Big Dye Terminator Cycle Premix Kit and products run on an ABI PRISM 310 automated sequencer (Applied Biosystems). Sequence contigs were assembled using CodonCode Aligner v4.0.1.

The fynbos strains was first compared to other *Talaromyces* spp. in an ITS phylogeny. The database contained mostly ex-type sequences (Samson *et al*. 2011, Visagie *et al*. 2009, & Visagie & Jacobs 2012). Multiple β-tubulin and Calmodulin databases were constructed relating to the different clades of the ITS phylogeny. Strains used for phylogenetic comparisons and GenBank accession numbers to sequences are summarized in TABLE 1. Datasets were aligned in MAFFT v6.850b (Katoh *et al*. 2009), with the L-INS-i option selected for ITS and G-INS-i for β-tubulin and Calmodulin. Sequence analysis were done in PAUP* v. 4.0b10 (Swofford 2000). Neighbor-joining analysis, with the BioNJ option selected (Gascuel 1997), was used for all the analyses. Confidence in nodes was determined using a bootstrap analysis with a 1000 replicates.

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Results

Isolations made from soil, air, mites and *Protea repens* infructescences resulted in the isolation of 199 *Talaromyces* strains. Based on colony characters on CYA and MEA, these were placed into tentative taxa. Taxa displayed the typical symmetric biverticillate conidiophores with acerose phialides with more or less the same length as its metulae. Representative strains from each taxon were further characterized by colony characters grown on a wide range of media at different conditions. Based on the results, 15 *Talaromyces* spp. were identified. Species included *T. amestolkiae* prov. nom. (Yilmaz *et al*. 2012 unpubl.), *T. chloroloma*, *T. dendriticus*, *T. minioluteus*, *T. pinophilus*, *T. ptychoconidium*, *T. radicus*, *T. ramulosus*, *T. solicola* and *T. variabilis*. However, five of the species displayed unique morphological features and are considered here as novel.

PCR resulted in amplicons of *ca*. 600bp, 500bp and 600bp lengths for ITS, β-tubulin and Calmodulin genes, respectively. ITS was used for determining species placement in the genus (FIGURE 1). β-tubulin and Calmodulin is more difficult to align across the entire genus. As such, these gene sequences were used in clade specific phylogenies (FIGURES 2–4). The aligned ITS dataset was 472bp long. *Trichocoma paradoxa* was chosen as suitable outgroup. Based on this phylogeny, 5 major clades were identified for *Talaromyces*. Three of these were resolved using β-tubulin and Calmodulin. *Talaromyces minioluteus* and *T. solicola* was used as outgroup of CLADE 1, *T. ptychoconidium* was used as outgroup for CLADE 2 and *T. piceus* as outgroup for CLADE 5 (FIGURE 4). The respective phylogenies confirmed all of the identifications made based on morphological characters. In addition, the five novel species resolved in well-defined clades separate from previously described species. The ITS dataset did not show much variation in CLADE 1. However, for CLADES 2–5 it was able to distinguish between most species. For the β-tubulin and Calmodulin phylogenies, species resolution are much higher than observed for ITS. As such, it aided in the identification of many species, as well as confirming the newly described species as coherent groups. The five new species are described below. Descriptions for the other species are also provided here in order to provide morphological data that is based on near wild-type cultures.

Table 1:Strains used for phylogenetic analyis of section *Talaromyces*

Strains examined		Name	GenBank nr. ITS	Btub	CMD
CBS252.87	T	*Geosmithia viridis*	JN899314		
CBS253.87	T	*Paecilomyces pascuus*	JN899321		
CBS184.27	T	*Penicillium crateriforme*	JN899373		
CBS762.68	T	*Penicillium korosum*	JN899347		
CBS624.72	T	*Penicillium mirabile*	JN899322		
CBS258.87	T	*Penicillium oblatum*	JN899364		
CBS270.35	T	*Penicillium purpurogenum var. rubrisclerotium*	JN899381		
DTO93C9 = CBS255.31	T	*Penicillium rugulosum var. atricolum*			JX140715
CBS137.84	T	*Penicillium samsonii*	JN899369		
CBS122434	T	*Penicillium sanguineum*			
CBS289.48	T	*Talaromyces aculeatus*	JN899378		JX140684
CBS100105		*Talaromyces aculeatus*	JN899389		
CBS563.92		*Talaromyces aculeatus*			
DTO166E5	T	*Talaromyces albobiverticillius*			
CBS453.93	T	*Talaromyces allahabadensis*	JN899345		
CBS132696 = KAS3883	T	*Talaromyces amestolkiae*			
DTO179E4 = KAS3874		*Talaromyces amestolkiae*			JX140685
DTO179F1 = KAS3880		*Talaromyces amestolkiae*			JX140686
DTO179F6 = KAS3884		*Talaromyces amestolkiae*			JX140687
CBS263.93		*Talaromyces amestolkiae*	JN899315		
CBS353.93		*Talaromyces amestolkiae*			
CBS312.59	T	*Talaromyces apiculatus*	JN899375	JX091378	
CBS227.60	T	*Talaromyces brunneus*	JN899365		
CBS112002	T	*Talaromyces calidicanius*	JN899319		JX140688
KAS504 = DAOM233329	T	*Talaromyces cecidicola*	AY787844	FJ753295	
CV2802 = KAS3953 = DAOM241016	T	*Talaromyces chloroloma*	FJ160273	GU385736	JX140690
CV2803 = KAS3954		*Talaromyces chloroloma*	FJ160273	GU385737	JX140691
CV785 = DTO182A5 = KAS 4175		*Talaromyces chloroloma*	JX091485	JX091597	JX140689
CBS103.83	T	*Talaromyces coalescens*	JN899366	JX091390	
CV131 = DTO181B2 = KAS 3997 = DAOM 241027	T	*Talaromyces crassa*	JX091474	JX091607	JX140725
CV123 = DTO181B1 = KAS 3986		*Talaromyces crassa*	JX091473	JX091606	JX140726
CV196 = DTO181C5 = KAS 4061		*Talaromyces crassa*	JX091472	JX091608	JX140727
CV2026 = DTO183G3 = KAS 4065		*Talaromyces dendriticus*	JX091486	JX091619	JX140692
CBS660.80	T	*Talaromyces dendriticus*	JN899339	JX091391	
CBS320.48	T	*Talaromyces diversus*	JN899341		
CBS644.80	T	*Talaromyces erythromellis*	JN899383	HQ156945	JX140681
CBMFA0942	T	*Talaromyces euchlorocarpius*	AB176617		
CBS102801	T	*Talaromyces flavovirens*	JN899392		
CBS310.38	T	*Talaromyces flavus*	JN899360		
CBS272.86	T	*Talaromyces funiculosus*	JN899377		
CBS751.74	T	*Talaromyces galapagensis*	JN899358		
CBS408.93	T	*Talaromyces homine*			
CBS169.91		*Talaromyces homine*			
CBS265.93		*Talaromyces homine*			
CBS624.93		*Talaromyces homine*			

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Table 1: Continued

Strains examined		Name	GenBank nr. ITS	Btub	CMD
CV1251 = DTO182I2 = KAS 3988 = DAOM 241024		*Talaromyces infrolivacea*	JX091481	JX091615	JX140734
CV1742 = DTO183D2 = KAS 4030 = DAOM 241023		*Talaromyces infrolivacea*	JX091482	JX091616	JX140735
CV1861 = DTO183F1 = KAS 4050 = DAOM 241030		*Talaromyces infrolivacea*	JX091483	JX091617	JX140736
CV2070 = DTO183G4 = KAS 4067		*Talaromyces infrolivacea*	JX091484	JX091618	JX140737
CBS338.48	T	*Talaromyces islandicus*	JN899318		
CBS189.68		*Talaromyces islandicus*			
CBS643.80	T	*Talaromyces loliensis*	JN899379		
CV383 = DTO181F3 = KAS 4121		*Talaromyces minioluteus*	JX091487	JX091620	JX140693
CBS642.68	T	*Talaromyces minioluteus*	JN899346		
DTO179C5 = KAS3859		*Talaromyces minioluteus*			JX140694
CBS128.89	T	*Talaromyces panamensis*	JN899362		JX140695
CV549 = DTO181I2 = KAS 4154 = DAOM 241020		*Talaromyces parvaurantica*	JX091475	JX091609	JX140728
CBS233.60	T	*Talaromyces phialosporus*	JN899340		
CBS361.48	T	*Talaromyces piceus*	JN899370		
CBS631.66	T	*Talaromyces pinophilus*	JN899382	JX091381	
CV2460 = DTO183I6 = KAS 4095		*Talaromyces pinophilus*	JX091488	JX091621	JX140697
CBS139.84	T	*Talaromyces pittii*	JN899325		
CBS321.48	T	*Talaromyces primulinus*	JN899317		
CBS470.70	T	*Talaromyces pseudostromaticus*	JN899371	HQ156950	JX140698
CV2808 = DTO180E7 = KAS3948 = DAOM241017	T	*Talaromyces ptychoconidium*	FJ160266	GU385733	JX140701
CV2806 = DTO180E9 = KAS3947		*Talaromyces ptychoconidium*	FJ160267	GU385734	JX140700
CV2807 = DTO180F1 = KAS3950		*Talaromyces ptychoconidium*	GQ414762	GU385735	JX140699
CBS475.71	T	*Talaromyces purpureus*	JN899328		
CBS286.36	T	*Talaromyces purpurogenus*	JN899372		
DTO189A1		*Talaromyces purpurogenus*			
CV245 = DTO181D4 = KAS 4093		*Talaromyces radicus*	JX091489	JX091622	JX140702
CV247 = DTO181D5 = KAS 4098		*Talaromyces radicus*	JX091490	JX091623	JX140703
CV253 = DTO181D7 = KAS 4102		*Talaromyces radicus*	JX091491	JX091624	JX140704
CV279 = DTO181D9 = KAS 4105		*Talaromyces radicus*	JX091492	JX091625	JX140705
CBS100489	T	*Talaromyces radicus*	JN899324		
CV1426 = DTO183A7 = KAS 4004		*Talaromyces ramulosus*	JX091493	JX091632	JX140710
CV314 = DTO181E3 = KAS 4110		*Talaromyces ramulosus*	JX091494	JX091626	JX140706
CV394 = DTO181F6 = KAS 4124		*Talaromyces ramulosus*	JX091495	JX091629	JX140707
CV735 = DTO182A3 = KAS 4170		*Talaromyces ramulosus*	JX091496	JX091630	JX140708
CV787 = DTO182A6 = KAS 4176		*Talaromyces ramulosus*	JX091497	JX091631	JX140709
CV2837 = DTO184B8 = DAOM241660	T	*Talaromyces ramulosus*	EU795706	FJ753290	JX140711
CBS369.48	T	*Talaromyces rotundus*	JN899353		
CBS370.48	T	*Talaromyces rubrus*			
CBS132704		*Talaromyces rubrus*			
CBS196.88		*Talaromyces rubrus*	JN899312		
CBS237.93		*Talaromyces rubrus*			
CBS371.48	T	*Talaromyces rugulosus*	JN899374		
DTO11B6		*Talaromyces rugulosus*			
DTO14A2		*Talaromyces rugulosus*			JX140718
DTO179H4 = KAS3895 = Dust204		*Talaromyces rugulosus*			
DTO179I3 = KAS3903 = Dust219		*Talaromyces rugulosus*			
DTO180A4 = KAS3913		*Talaromyces rugulosus*			
DTO180A4 = KAS3913 = Dust229		*Talaromyces rugulosus*			
DTO180B9 = KAS3926 = Dust237		*Talaromyces rugulosus*			
DTO39F8 = CBS116761		*Talaromyces rugulosus*			JX140714
DTO55E5		*Talaromyces rugulosus*			JX140719
DTO61E8		*Talaromyces rugulosus*			JX140720
DTO63C7		*Talaromyces rugulosus*			JX140721
DTO66G6		*Talaromyces rugulosus*			JX140722
DTO93B8 = CBS111.65		*Talaromyces rugulosus*			JX140713
CV2800 = DTO180D4 = KAS3938 = DAOM241015		*Talaromyces solicola*	FJ160264	GU385731	
CV2801 = DTO180D5 = KAS3939		*Talaromyces solicola*	FJ160265	GU385732	
CV104 = DTO181A2 = KAS 3966 = DAOM 241021	T	*Talaromyces stellenbossiensis*	JX091471	JX091605	JX140683
CV103 = DTO181A1 = KAS 3963 = DAOM 241028		*Talaromyces stellenbossiensis*	JX091470	JX091604	JX140682
CBMFA0939T	T	*Talaromyces sublevisporus*	AB176638		
CBS250.94	T	*Talaromyces tardifaciens*	JN899361		
CBS579.72	T	*Talaromyces udagawae*	JN899350		
CV175 = DTO181B7 = KAS 4031 = DAOM 241018		*Talaromyces variabilis*	JX091498	JX091636	JX140738
CV507 = DTO181H2 = KAS 4143 = DAOM 241019		*Talaromyces variabilis*	JX091499	JX091637	JX140739
CV603 = DTO181I7 = KAS 4160		*Talaromyces variabilis*	JX091500	JX091638	JX140740
FRR1055		*Talaromyces variabilis*	L14507		
FRR1290		*Talaromyces variabilis*	AY373936		
CBS385.48	T	*Talaromyces variabilis*	JN899343		
NRRL6419		*Talaromyces variabilis*	HQ288049		
CV1467 = DTO183B3 = KAS 4011 = DAOM 241025		*Talaromyces vermiculicola*	JX091476	JX091610	JX140729
CV1469 = DTO183B4 = KAS 4012		*Talaromyces vermiculicola*	JX091477	JX091611	JX140730
CV1503 = DTO183C1 = KAS 4019 = DAOM 241029		*Talaromyces vermiculicola*	JX091477	JX091612	JX140731
CV1825 = DTO183E3 = KAS 4043 = DAOM 241022		*Talaromyces vermiculicola*	JX091479	JX091613	JX140732
CV2466		*Talaromyces vermiculicola*	JX091480	JX091614	JX140733
CBS388.48	T	*Talaromyces verruculosus*	JN899367		
CBS391.48	T	*Talaromyces wortmannii*	JN899352		
CBS788.83		*Trichocoma paradoxa*	JN899398		

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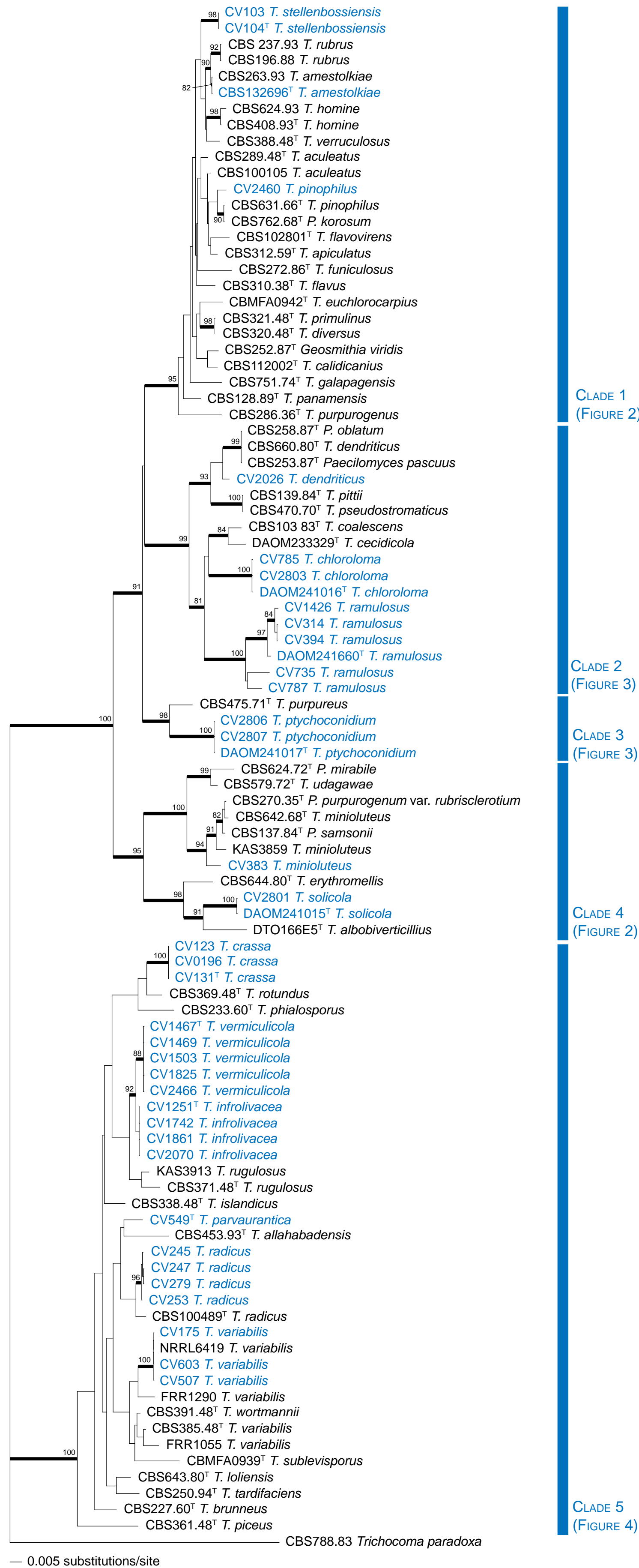

FIGURE 1. Phylogenetic tree, based on the ITS barcodes, showing relationship of the Fynbos *Talaromyces* spp. with the rest of the genus. The genus is divided into 5 clades. *Trichocoma paradoxa* was chosen as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type strain; species in color isolated from Fynbos). The aligned dataset was 476 bp long.

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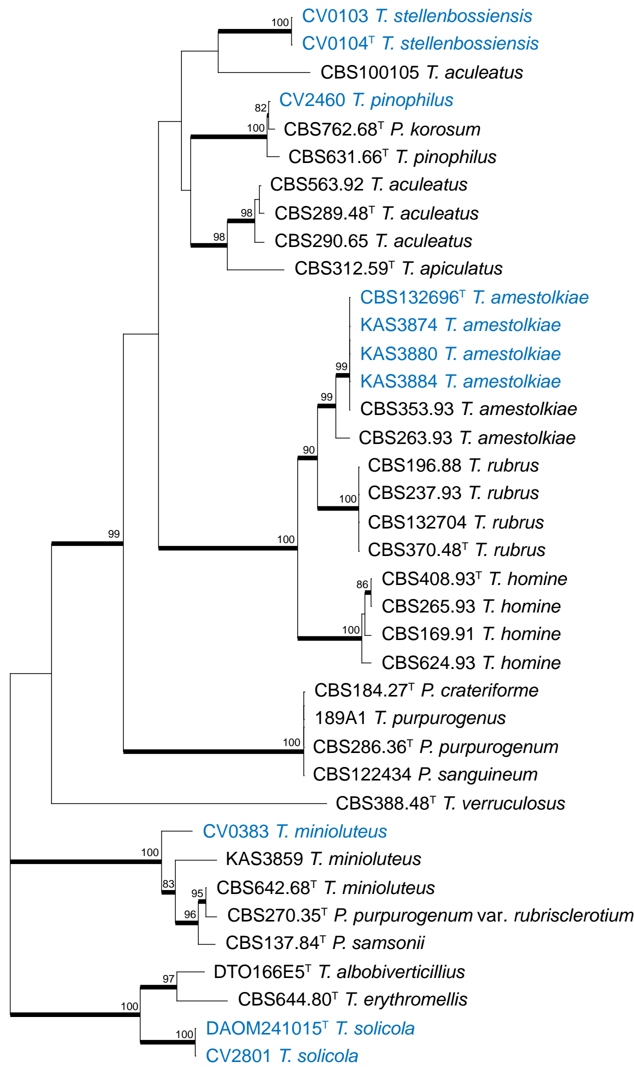

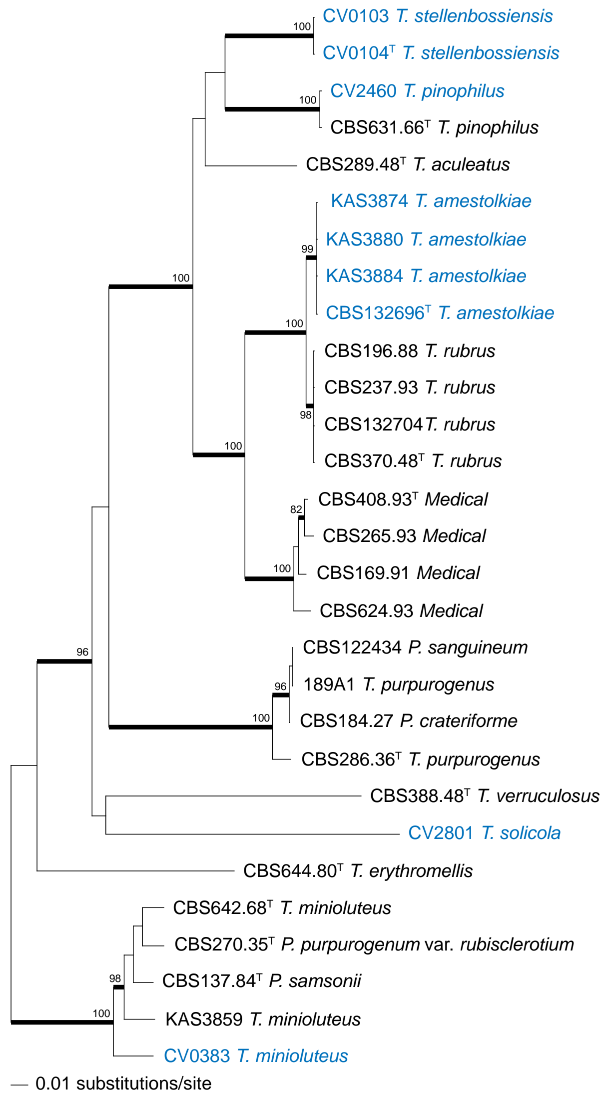

FIGURE 2. Phylogenetic trees, based on the β-tubulin (left) and Calmodulin (right) gene, showing relationship of the Fynbos *Talaromyces* spp. from FIGURE 1 CLADE 1 and close relatives. In both phylogenies, the *T. minioluteus* clade (FIGURE 1 CLADE 4) was used as outgroup. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type strain; species in color isolated from Fynbos). The aligned datasets for β-tubulin and Calmodulin were 346 and 505 bp long, respectively.

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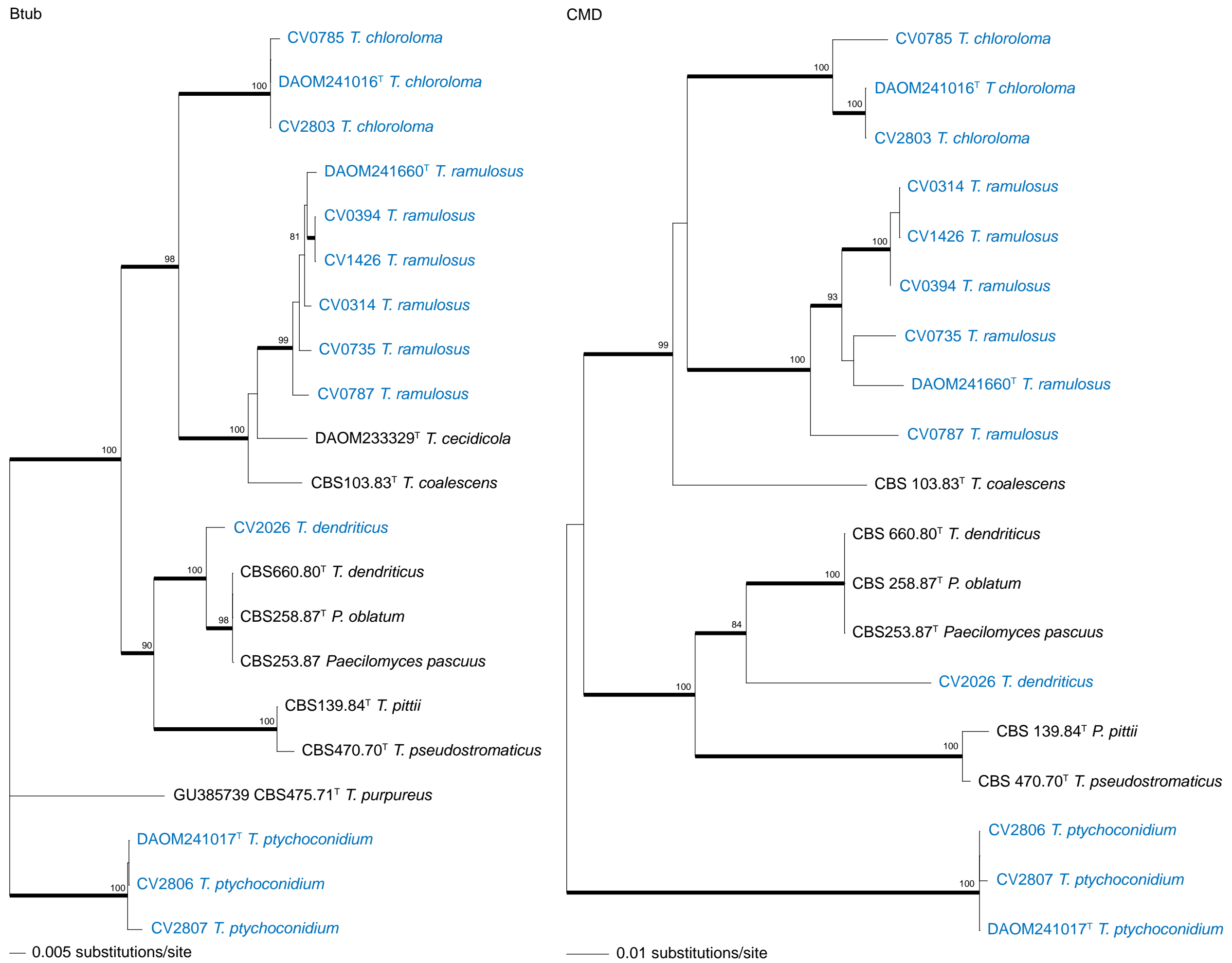

FIGURE 3. Phylogenetic trees, based on the β-tubulin (left) and Calmodulin (right) gene, showing relationship of the Fynbos *Talaromyces* spp. FIGURE 1 CLADE 2 and close relatives. In both phylogenies, the *T. ptychoconidium* clade (FIGURE 1 CLADE 3), was used as outgroup. Bootstrap values above 80% are indicated above thick branches. (T = ex-type strain; species in color isolated from Fynbos). The aligned datasets for β-tubulin and Calmodulin were 384 and 504 bp long, respectively.

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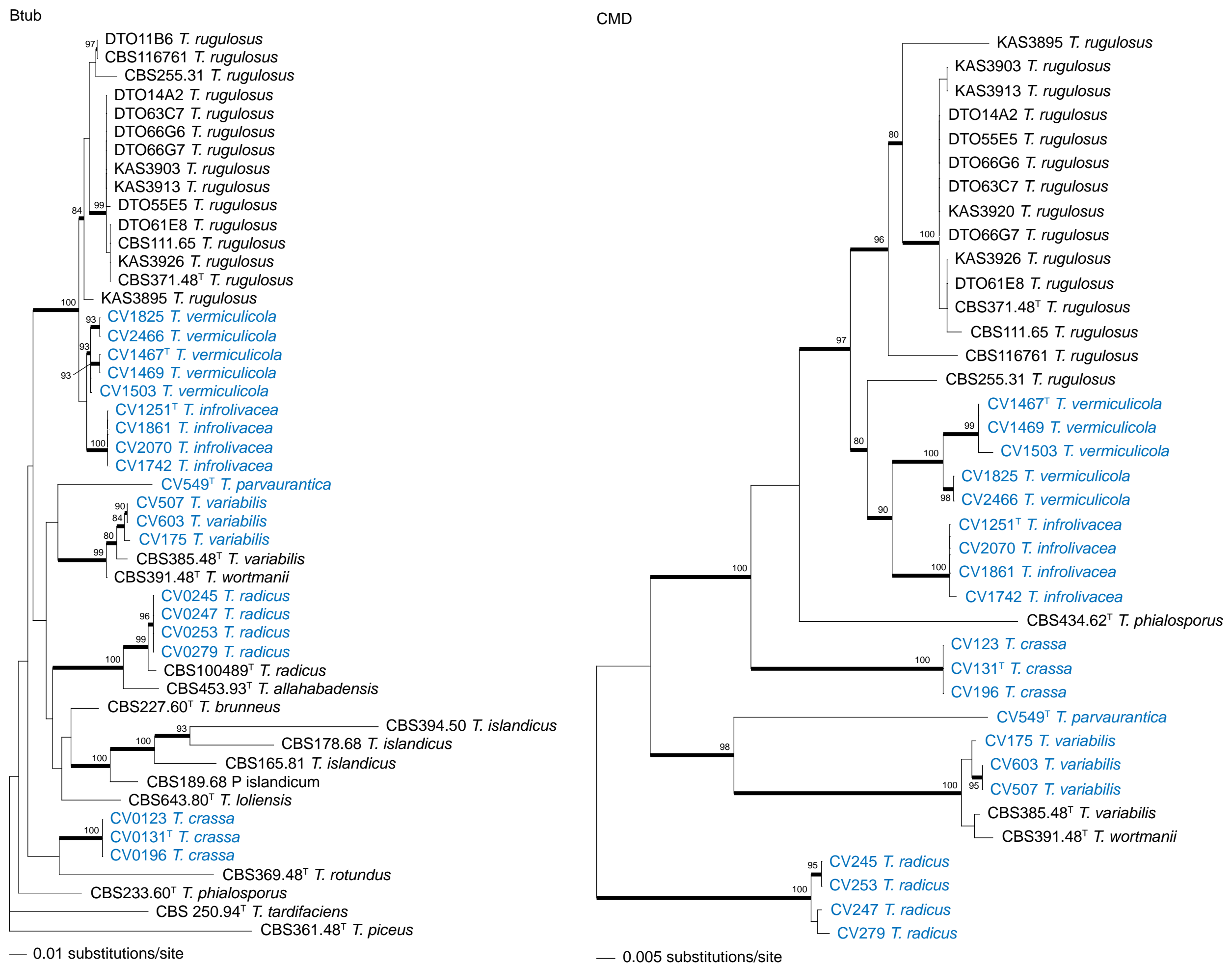

FIGURE 4. Phylogenetic tree, based on the β-tubulin (left) and Calmodulin (right) gene, showing relationship of the Fynbos *Talaromyces* spp. FIGURE 1 CLADE 5 and close relatives. *Talaromyces piceus* was used as outgroup in the β-tubulin phylogeny and *T. radicus* for the Calmodulin phylogeny. Bootstrap values above 80% are indicated above thick branches. (^{T} = ex-type strain; species in color isolated from Fynbos). The aligned datasets for β-tubulin and Calmodulin were 472 and 543 bp long, respectively.

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Taxonomy

Talaromyces amestolkiae Yilmaz **prov. nom.** PLATES 1, 2, 31a

EX-TYPE: CBS132696 = DTO179F5
ISOLATED FROM: House dust, Cape Town, South Africa
SPECIMENS EXAMINED: CV2811, CV2812, CV2813, DTO179E4, DTO179E5, DTO179E7, DTO179F1, DTO179F2, DTO179F5, DTO179F6, DTO179F7, DTO179F9, DTO179G1, DTO179G2, DTO179G3, DTO179G4, DTO179G5

Macromorphology — CYA, 25 °C, 7d: Colonies 25–29 mm, low, raised at centre, plane; margins low, wide (2–3 mm), entire; mycelia white and yellow; texture floccose, with overlaying funicles; sporulation moderately dense, conidia en masse greyish green (26E6–26E7); exudate absent, soluble pigment absent, reverse pigmentation dark brown to dark ruby (8F8–12F8) at centre, fading into pale yellow to yellowish white (4A2–4A3) near margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 35–55 mm, low to moderately deep, plane, colonies having a yellowish white to whitish orange color in non-sporulating regions; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose, some loosely funiculose mycelia present; sporulation moderately dense only visible at centre, elsewhere covered by sterile mycelia, conidia en masse greyish green (25E5); exudate absent, soluble pigment brown, reverse pigmentation reddish brown (8D6–8E6) at centre, yellowish white to greyish yellow (4A2–4B3) elsewhere.

CYA, 37 °C, 7d: Colonies 4–12 mm, deep, craterform, having light purple color; margins deep, very narrow (<1 mm), entire; mycelia white; sporulation absent; exudate absent, soluble pigment reddish brown, reverse pigmentation brown to dark brown (7E7–7F7–7F8).

MEA, 25 °C, 7d: Colonies 40–44 mm, low, plane; margins low to subsurface, wide (3–4 mm), entire; mycelia white, especially in older culture, with yellow mycelia also present; texture velutinous with funicles present; sporulation moderately dense, sometimes masked by sterile mycelial overlay, conidia en masse (26D5–27E5); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (7C6) at point of inoculation, fading into greyish red (7B4) centre, greenish grey (27B2) elsewhere.

YES, 25 °C, 7d: Colonies 36–40 mm, moderately deep, plane; margins low, narrow (1 mm), entire; mycelia white; texture mostly floccose, loosely funiculose; sporulation moderately dense, conidia en masse greyish green (27E5–27E6); exudate absent, soluble pigment absent, reverse pigmentation dark brown (8F8) at centre, fading into reddish brown (8E8), with orange white (5A2) margin.

G25N, 25 °C, 7d: Colonies 3–5 mm, sporulation moderate for such a small colony, conidia en masse dull to greyish green (25D4–25D5).

CREA, 25 °C, 7d: Colonies 22–24 mm, strong acid production close to colony periphery.

Micromorphology — Conidiophores biverticillate, subterminal branches present; stipes smooth walled, 85–260 × 2–3 µm; branches 2–3 when present, 15–49 × 2–3 µm; metulae 5–9, appressed to divergent, 27–62° [43±9°], 9.5–15 × 2.5–3.5 [11.6±1.3 × 2.9±0.3] µm; phialides acerose, 3–6 per metula, 10–13 × 2–3 [11.5±0.7 × 2.4±0.2] µm; conidia roughened, sometimes spirally rough walled, ellipsoidal, 2.5–3 (–4.5) × 2–3 [2.7±0.2 × 2.1±0.3] µm, average width/length = 0.74±0.05, n = 51.

Notes — *Talaromyces amestolkiae* prov. nom. is a new species that belong to the taxonomically difficult *T. rubrus* complex. It has been isolated from many sources, including Fynbos soil and house dust within the Fynbos region. This species will be described by Yilmaz *et al.* (2012, unpubl.) and will not be discussed here. The description is only included for the purposes of a complete inventory of Fynbos species isolated in the study. Yilmaz *et al.* (2012) noted that this species produces a unique floccose texture compared to *T. rubrus*, while *T. homine* grows faster on CYA at 37 °C.

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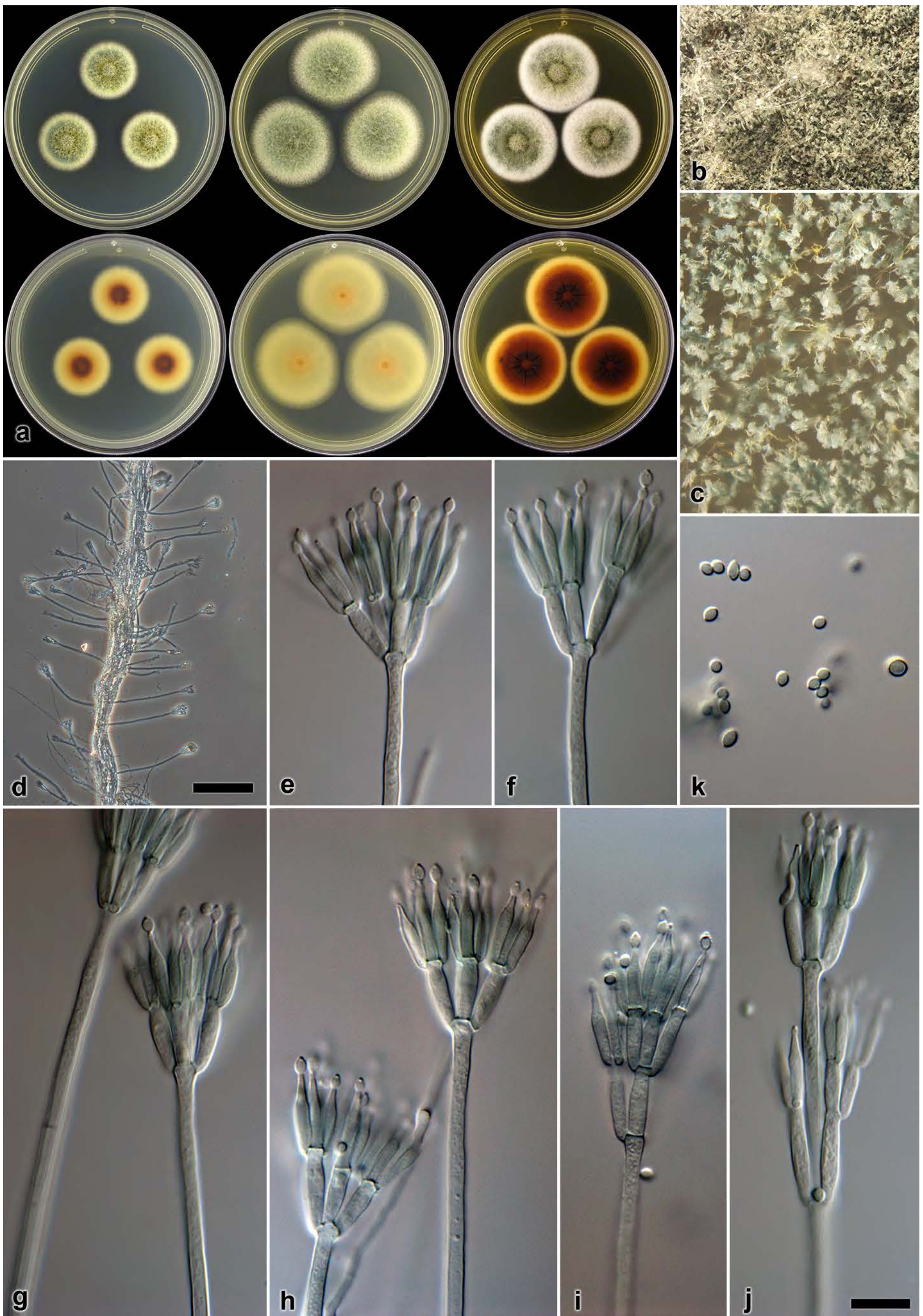

PLATE 1. *Talaromyces amestolkiae*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–c. Texture on MEA. d–j. Conidiophores. k. Conidia (— Scale bar in d = 50 µm; — Scale bar in j = 10 µm, applies to e–k).

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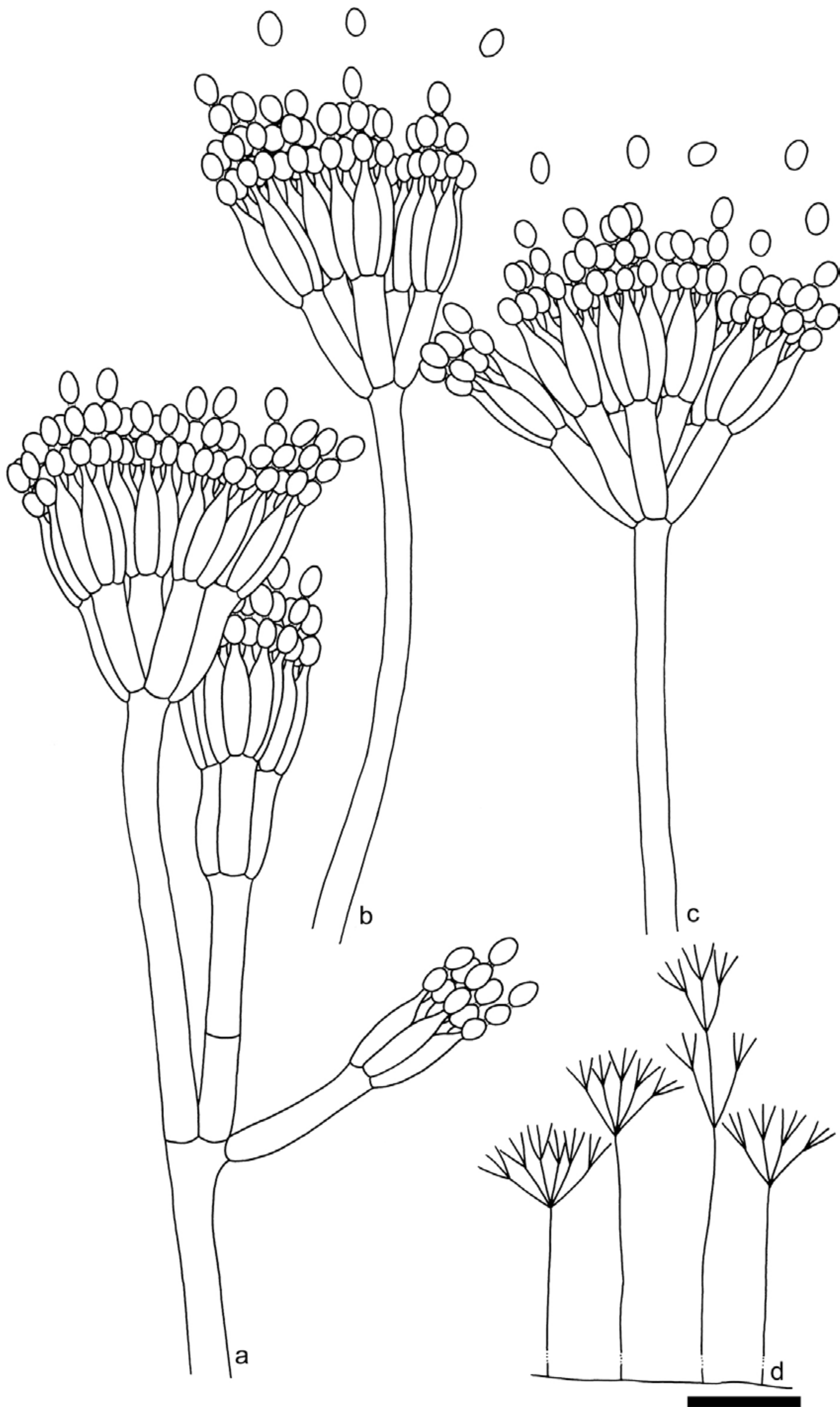

PLATE 2. Line drawing of *Talaromyces amestolkiae*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces chloroloma Visagie & Jacobs PLATES 3, 4, 31b
Persoonia 28: 18. 2012.

MYCOBANK: MB564326
TYPE: PREM60033 (herbarium) = DAOM 241016, CV2802
ISOLATED FROM: Soil, Malmesbury, South Africa
ADDITIONAL SPECIMENS EXAMINED: CV785, CV807, CV1011, CV1114, CV1135, CV1363, CV1366, CV2803

Macromorphology — CYA, 25 °C, 7d: 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; sporulation 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.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 50–55 mm diam, moderately deep, plane; margin low, wide (3–5 mm), entire; mycelia white; texture floccose with funicles present, determinate synnemata produced in incidental sunlight after prolonged incubation; sporulation moderately dense, conidia *en masse* dark green (26F5) at centre fading into greyish green (26D5) near margin; exudate absent, soluble pigment absent, reverse pigmentation olive to olive brown (3D4–4D4) at centre, pale yellow (3A3) elsewhere, fading into greenish grey (1B2) near margin.

CYA, 37 °C, 7d: Colonies 8–12 mm, moderately deep, plane; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia en masse dull green (27E3–27E4), sometimes greyish green (28C3); exudate absent, soluble pigment absent, reverse pigmentation olive yellow to olive (3D6–3E6).

MEA, 25 °C, 7d: Colonies 43–48 mm diam, plane, moderately dense; texture strongly funiculose to floccose; margins subsurface, 4–5 mm, irregular, mycelia white; sporulation 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, 7d: Colonies 39–42 mm diam, plane, moderately dens; texture strongly funiculose; margins wide, 3–5 mm, regular, mycelia white; sporulation medium to heavy, Dull Green to Greyish Green (26E4–26E6); exudate and soluble pigment absent, reverse Greyish Yellow (2A3–2A4).

G25N, 25 °C, 7d: Colonies 6–8 mm, sporulation very sparse.

CREA, 25 °C, 7d: Colonies 14–17 mm, weak to strong acid production close to colony periphery.

Micromorphology — Conidiophores biverticillate and terverticillate, having a olive pigmentation; synnemata on CYA produced after 14–21 days 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; stipes smooth-walled, 12–45 × 3–4 µm when borne on funicles, 700–1200 µm when borne in synnema; branches 2–4 when present, 11–15 × 3–3.5 µm; metulae 3–5, appressed, 16–50° [33.5±9°], 7.5–10.5 × 2–3 [8.7±0.8 × 2.5±0.3] µm; phialides acerose, 4–5 per metula, 7–8.5 × 1.5–2.5 [7.8±0.5 × 2±0.2] µm; conidia smooth, ellipsoidal, 2.5–3.5 × 1.5–2 [3±0.2 × 1.6±0.2] µm, sparse larger conidia present 4.5–5 (–6) × 2–2.5 µm, average width/length = 0.55±0.06, n = 60.

Notes — This species has previously been described from Fynbos soil (Visagie & Jacobs 2012). *Talaromyces chloroloma* belong to CLADE 2 together with species that typically produce synnemata after prolonged incubation. It has *T. coalescens* and *T. cecidicola* as its closest relatives. However, *Talaromyces chloroloma* produces faster growing colonies on CYA at 25 °C and olive brown to light green conidia in contrast to its close relatives growing slower on CYA, as well as *T. cecidicola* that produces turquoise-green conidia and *T. coalescens* dark green conidia. In addition, *T. chloroloma* have, in general, longer synnemata than *T. cecidicola* (250–1250 µm), but shorter than *T. coalescens* (1–2(–5) mm). Based on these morphological characters, it can be distinguished from its close relatives.

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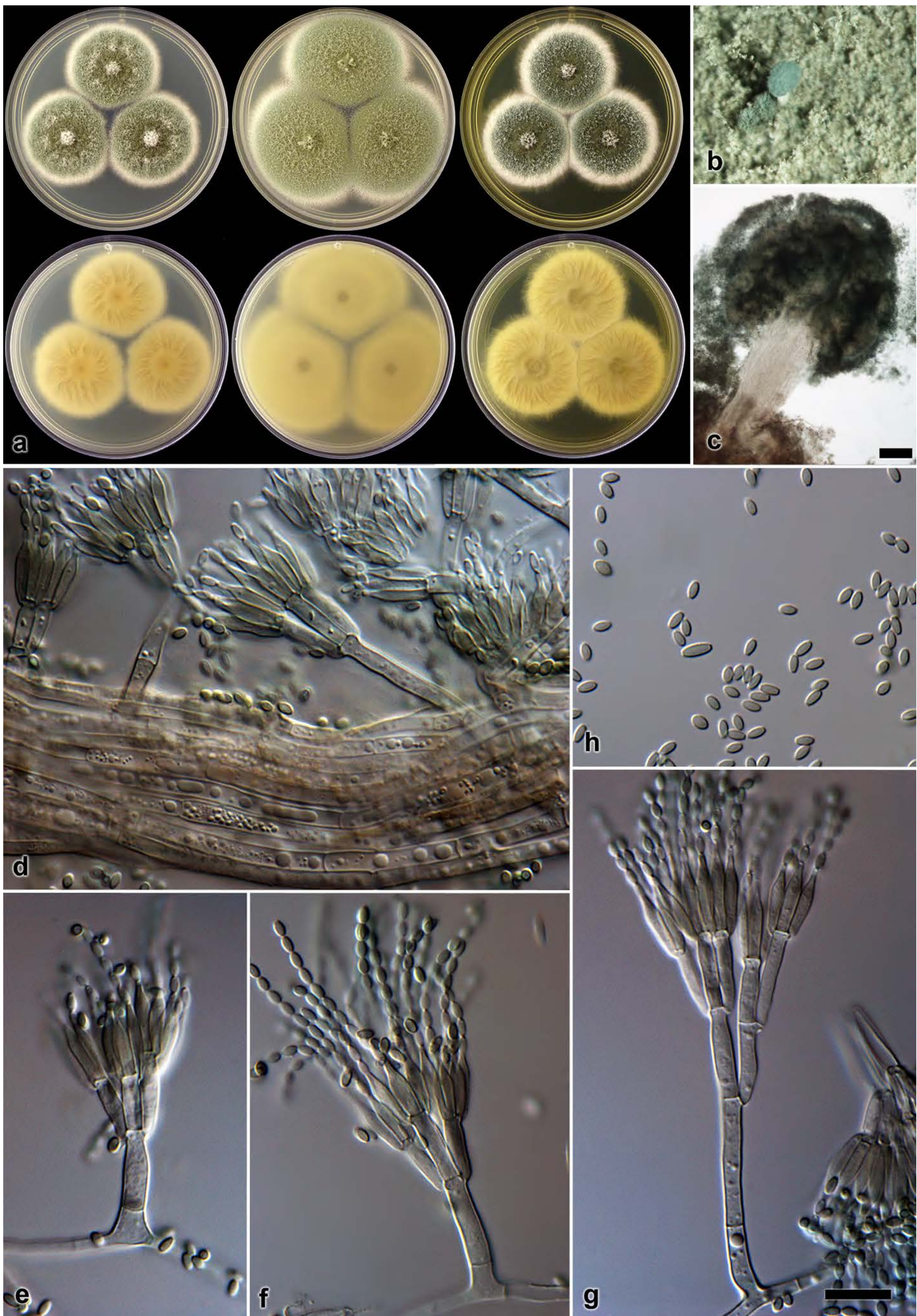

PLATE 3. *Talaromyces chloroloma*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–c. Synnema produced on MEA after prolonged incubation. d–g. Conidiophores. h. Conidia (— Scale bar in c = 50 µm; — Scale bar in g = 10 µm, applies to d–h). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 17.

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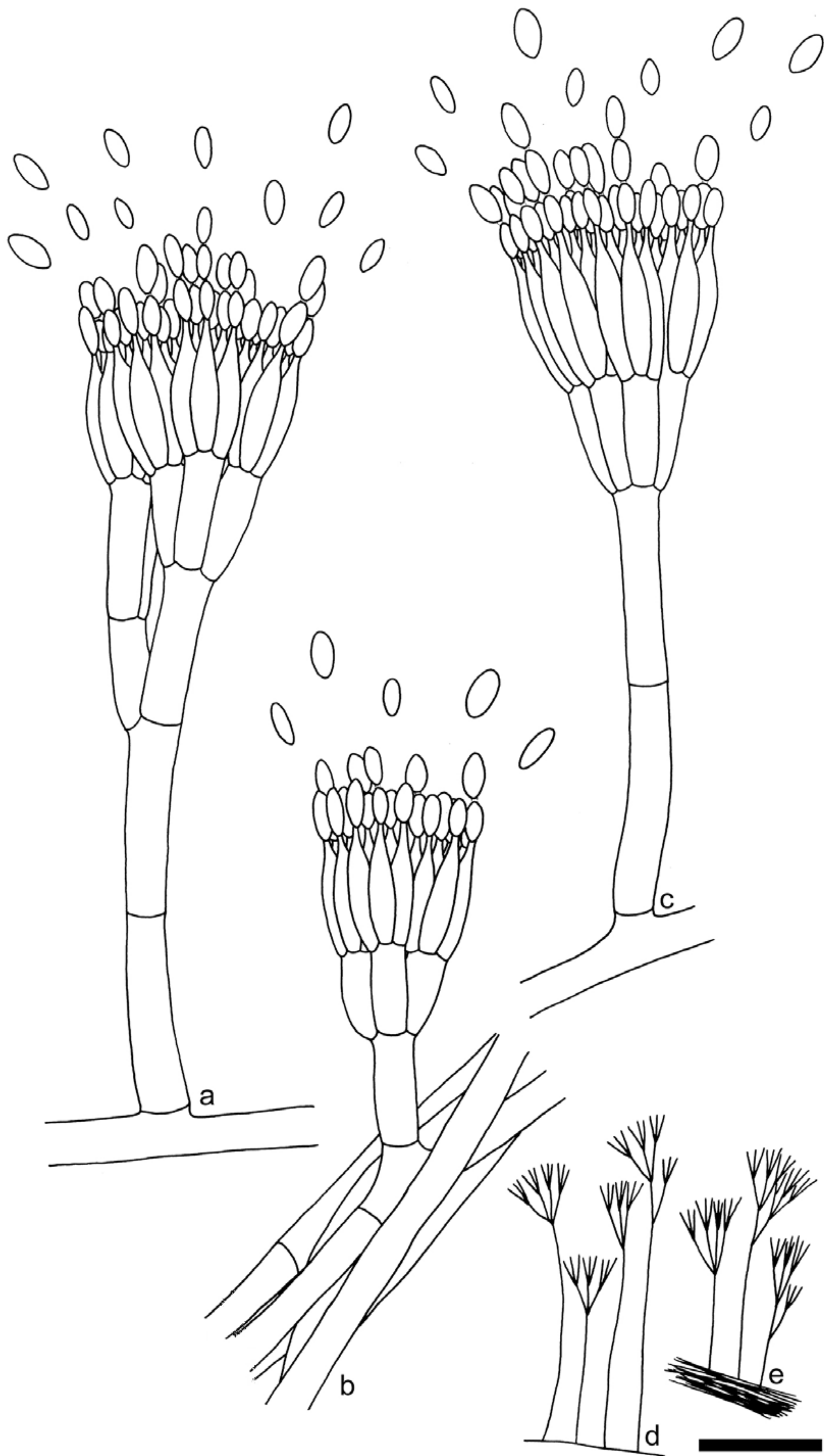

PLATE 4. Line drawing of *Talaromyces chloroloma*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 18.

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Talaromyces crassa Visagie **prov. nom.** PLATES 5, 6, 31c

ETYMOLOGY: Latin, *crassa*: meaning thick, in reference to the thick deep colonies producesd
EX-TYPE: CV131 = DTO181B2 = KAS 3997 = DAOM 241027
TYPE ISOLATED FROM: Mite, *Protea repens* infructescence, Stellenbosch, South Africa, S33°56'47; E18°52'49
ADDITIONAL SPECIMENS EXAMINED: CV123, CV131, CV196

Macromorphology — CYA, 25 °C, 7d: Colonies 11–15 mm, moderately deep, very lightly radially sulcate; margins low, very narrow (<1 mm), entire; mycelia white and yellow; texture velutinous and floccose; sporulation moderately dense at centre, conidia en masse greyish green (25E5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4C5–4C6), with yellowish white (4A2) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 18–20 mm, low, somewhat craterform, radially sulcate; margins low, narrow (1–2 mm), entire; mycelia yellow and white present; texture floccose; sporulation moderately dense, conidia en masse greyish turquoise to greyish green (24D3–24D4–25D4–25D3); exudate minute clear droplets, soluble pigment light yellow, reverse pigmentation olive (2D4) at centre, greyish yellow (3B7) surrounding centre, fading into greyish yellow (1B4) near yellowish white (1A2) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 17–24 mm, moderately deep, plane, bright yellow mycelia dominating colony appearance and masking conidiogenous areas; margins low, very narrow (<1 mm), entire; mycelia bright yellow; texture floccose; sporulation sparse, conidia en masse deep green (28D8–28E8); exudate absent, although a sticky substance visible when removing mycelia overlay to reveal conidiogenous areas, soluble pigment absent, reverse pigmentation (1C3–1C4) at point of inoculation, light yellow (2A4) fading into yellowish white (1A2) margin.

YES, 25 °C, 7d: Colonies 16–20 mm, low, lightly radially sulcate, centrally slightly raised, orange color near centre; margins low, narrow (1 mm), entire; mycelia white, yellow near margin, orange near centre; texture floccose; sporulation sparse to moderately dense in specific regions, conidia en masse greyish green (26D5); exudate absent, soluble pigment absent, reverse pigmentation light to greyish yellow (4A5–4B5), with yellowish white (4A2) margin.

G25N, 25 °C, 7d: Colonies 5–7 mm, sporulation moderately dense, conidia en masse greyish turquoise (24B3), reverse pigmentation dark green (29F7).

CREA, 25 °C, 7d: Colonies 8–10 mm, weak acid production at colony centre.

Micromorphology — Conidiophores biverticillate, sometimes terverticillate and subterminal branches present; stipes smooth walled, 130–390 × 2.5–3.5 µm; branches 2–3, 13–17 × 2.5–3.5 µm, metulae 3–6, somewhat divergent, 28–65° [42.3±10°], 9.5–14 × 2.5–3 [12.1±1.2 × 2.8±0.2] µm; phialides acerose, number per metula, 8.5–11.5 × 1.5–2.5 [10.2±0.8 × 2.1±0.2] µm; conidia smooth walled, ellipsoidal, 2–3 × 1.5–2.5 [2.5±0.2 × 1.9±0.2] µm, average width/length = 0.79±0.05, n = 98.

Notes — *Talaromyces crassa* characteristically shows restricted growth, especially on CYA. It is closely related to the *T. rugulosus* species complex, with *T. phialosporus* (CBS233.60) its closest relative (FIGURES 1, 4). The fluffy floccose deep yellow colonies, longer phialides and smooth walled conidia, distinguish the Fynbos species from the *T. rugulosus* complex. Similar to *T. rugulosus*, *T. phialosporus* produces velutinous colonies with moderate sporulation. This easily distinguishes it from *T. crassa*. Phylogenetically, *T. phialosporus* has *T. rotundus* (CBS369.48) as a close relative. The latter species, however, typically produces a sexual state. More important, *T. rotundus* produces conidiophores with short stipes (<100 µm), very long phialides (up to 20 µm) and large conidia (4–5 × 2–2.5 µm). Molecular data confirmed the novelty of the new species.

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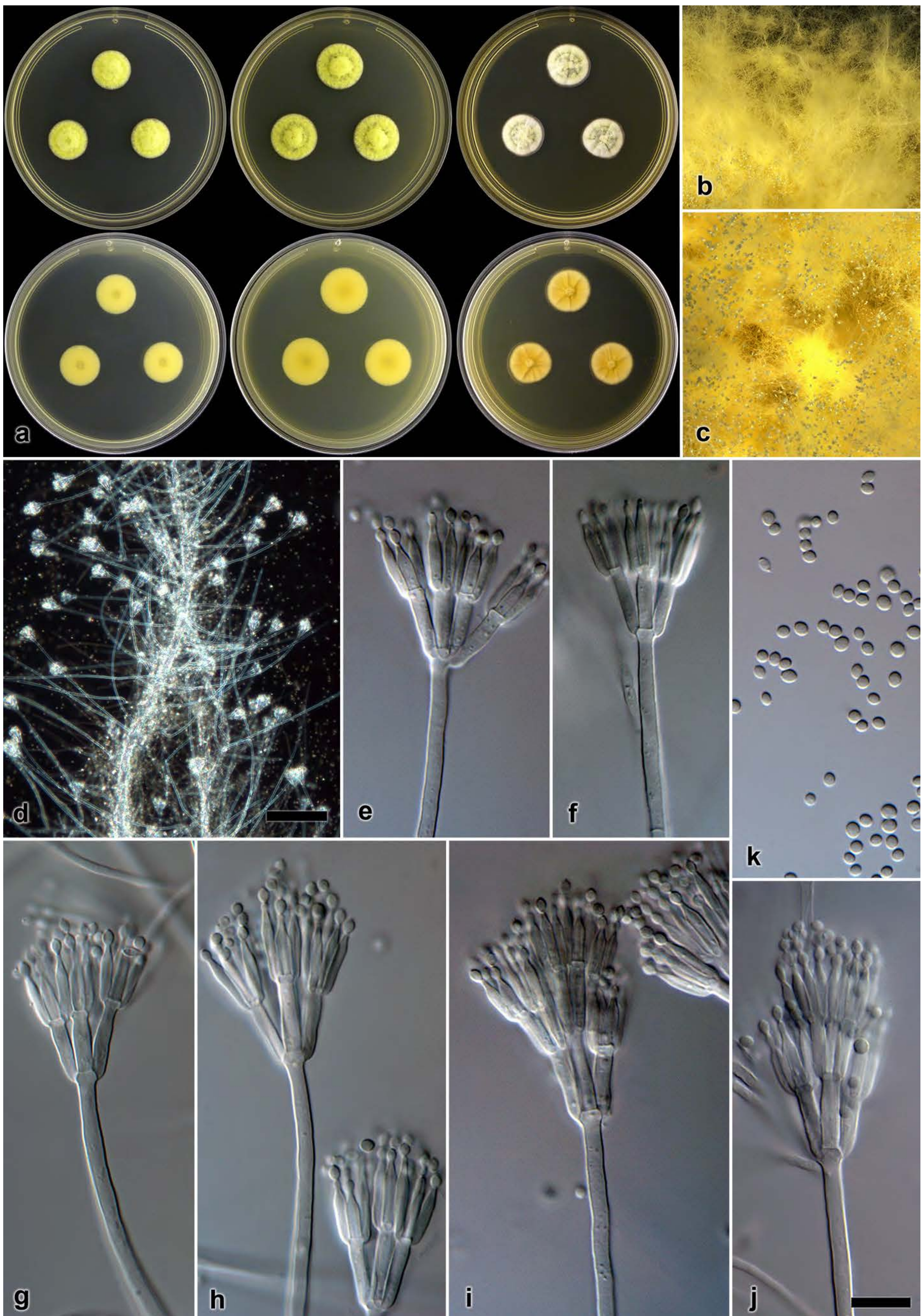

PLATE 5. *Talaromyces crassa*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–j. Conidiophores. k. Conidia (— Scale bar in d = 50 µm; — Scale bar in j = 10 µm, applies to e–k).

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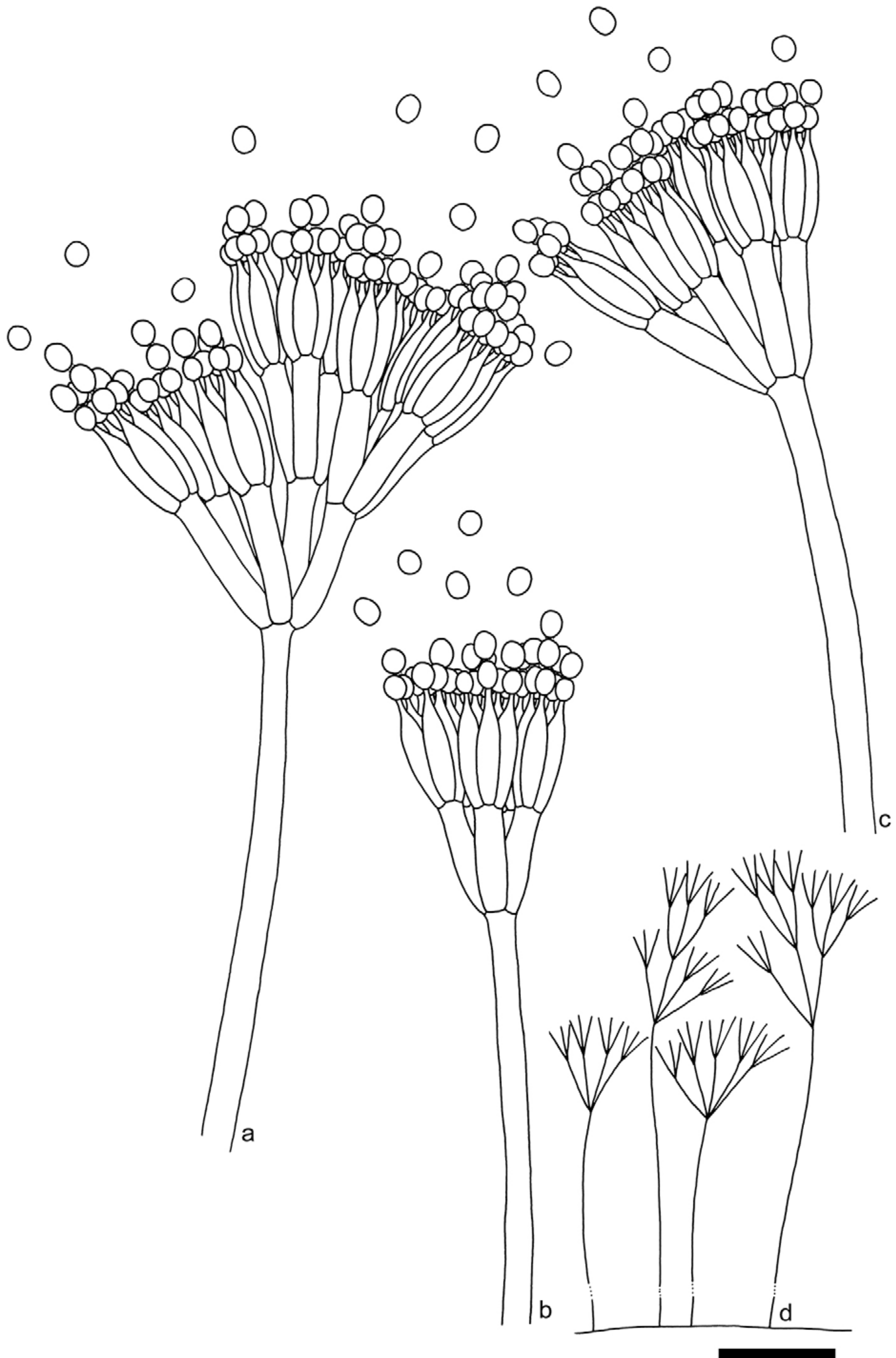

PLATE 6. Line drawing of *Talaromyces crassa*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces dendriticus (Pitt) Samson, Yilmaz, Frisvad & Seifert PLATES 7, 8, 31d

Studies in Mycology 70: 177. 2011.

Mycobank: MB560648
Basionym: *Penicillium dendriticum* Pitt (The genus *Penicillium*: 413. 1979)
Synonyms: *Paecilomyces pascuus* Pitt & Hocking = *Penicillium pascuum* (Pitt & Hocking) Frisvad, Samson & Stolk (CBS253.87); *Penicillium oblatum* Pitt & Hocking (CBS258.87)
Type: CBS660.80 = IMI216897
Type Isolated From: *Eucalyptus* leave litter, Kosciusko National Park, Australia
Specimens Examined: CV2026, KAS899, KAS1190, CBS660.80

Macromorphology — CYA, 25 °C, 7d: Colonies 10–12 mm, sometimes up to 18 mm, low, plane; margins low, narrow (1 mm), entire; mycelia white to conspicuously yellow; texture velutinous, developing into determinate synnemata with yellow stalks after 14 d; sporulation moderately dense, conidia en masse dull green (27E4–27E5); exudate absent, soluble pigment yellow, reverse pigmentation light brown (5D7) at point of inoculation, greyish yellow to greyish green (1C5–1C6, 1B5–1B6) elsewhere.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 17–25 mm, low, plane; margins low, narrow (1–2 mm), entire; mycelia white and yellow present; texture velutinous, intial development of synnema present, as well as overlaying funiculose mycelia; sporulation moderately dense, conidia en masse dark green (26F8–27F8); exudate absent, soluble pigment absent, reverse pigmentation dark brown (6F8) at centre, although less pronounced in some isolates, greyish yellow to brownish orange (4C5–5C5) fading into greyish yellow (1B6) or greyish green (29C4) near yellowish white margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 30–36 mm, low, plane; margins low to subsurface, narrow (2 mm), entire; mycelia white; texture velutinous, sometimes funicles present, developing determinate synnema with yellow stalks after 14 d; sporulation dense, conidia en masse dark green (27F8); exudate absent, soluble pigment absent, reverse pigmentation light orange (5A4) at point of inoculation, becoming dull green (27D3) near margins.

YES, 25 °C, 7d: Colonies 19–21 mm, low, plane; margins subsurface, narrow, regular; mycelia white to yellow in specific regions; texture velutinous, developing determinate synnema with yellow stalks after 14 d; sporulation moderate to heavy, conidia en masse Dull Green (27D3); exudate absent, soluble pigment absent, reverse pigmentation Brownish Grey (6F8) at centre, Light Yellow (2A5) elsewhere.

G25N, 25 °C, 7d: Microcolonies 1–4 mm, sporulation absent to very sparse.

CREA, 25 °C, 7d: Colonies 7–9 mm, no acid production.

Micromorphology — Conidiophores biverticillate, minor proportion tervertcillate, as well as subterminal branches present; synnemata developing after 2 weeks of growth, up to 5000 µm, apex width 100–500 µm, stalk width 45–150 µm; stipes smooth walled, 20–200 × 2.5–4 µm, in synnema up to 5000 µm long; branches 2–4, 12–18 × 2.5–4 µm; metulae 4–6, appressed, 16–50° [34±11°], 9.5–16 × 2–3.5 [12.3±1.5 × 2.6±0.2] µm; phialides acerose, 5–8 per metula, 9.5–14 × 2–2.5 [11.8±1.1 × 2.1±0.2] µm; conidia smooth walled, sometimes lightly rough walled in ridges, ellipsoidal, 2.5–3.5 × 2–2.5 [2.8±0.2 × 2±0.1] µm, average width/length = 0.69±0.05, n = 72.

Notes — *Talaromyces dendriticus* is characterized by restricted growth on CYA, as well as very long and slender phialides. The determinate synnemata, with yellow stalks as long as 5 mm, produced after prolonged incubation is, however, its most striking feature. This species resolved in CLADE 2 (FIGURES 1, 3) together with other synnemata producing species. Its characteristic morphological features make this species easily recognizable.

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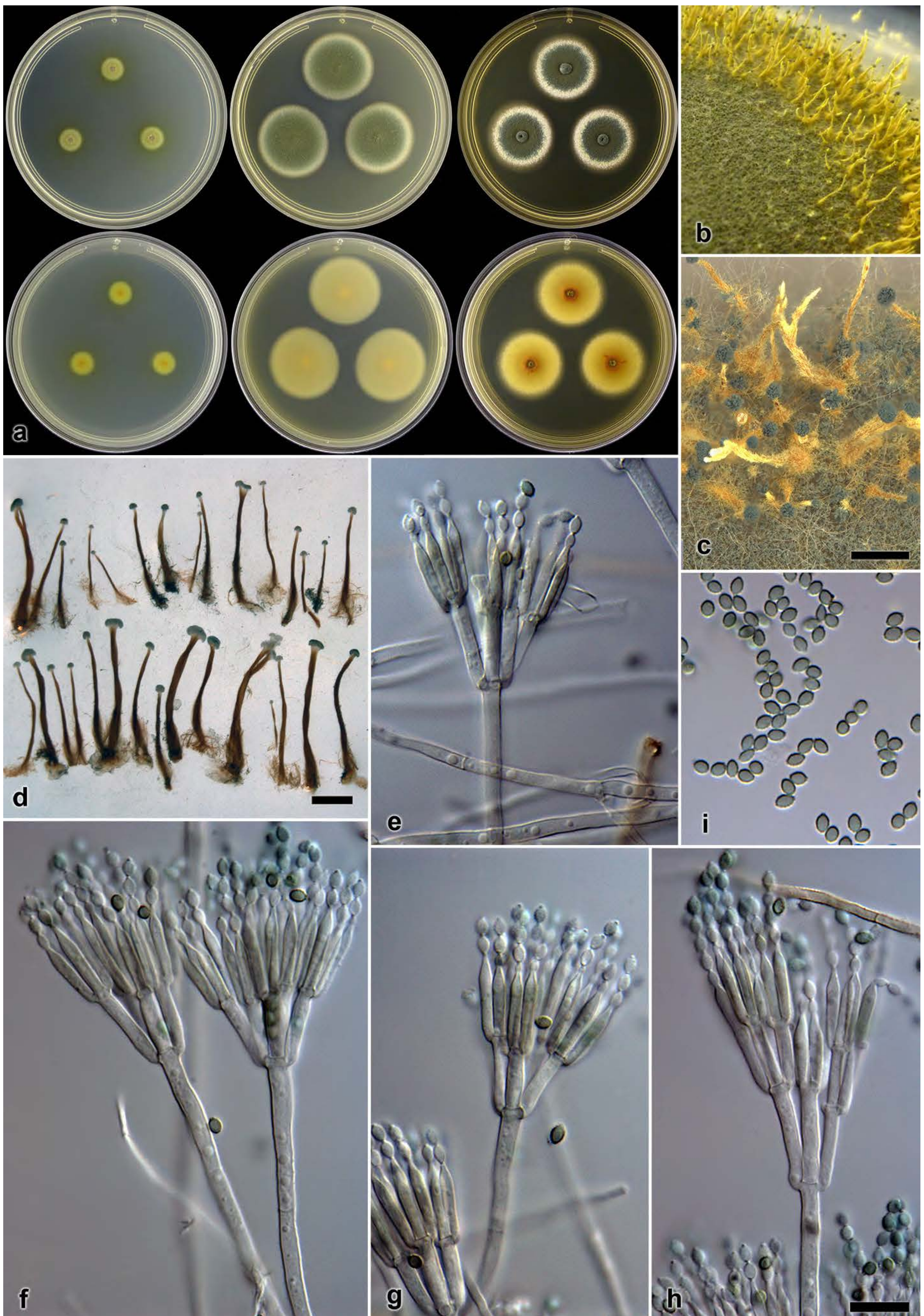

PLATE 7. *Talaromyces dendriticus*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–d. Synnema produced on MEA after prolonged incubation. e–h. Conidiophores. i. Conidia (— Scale bar in c, d = 1 mm; — Scale bar in h = 10 µm, applies to e–i).

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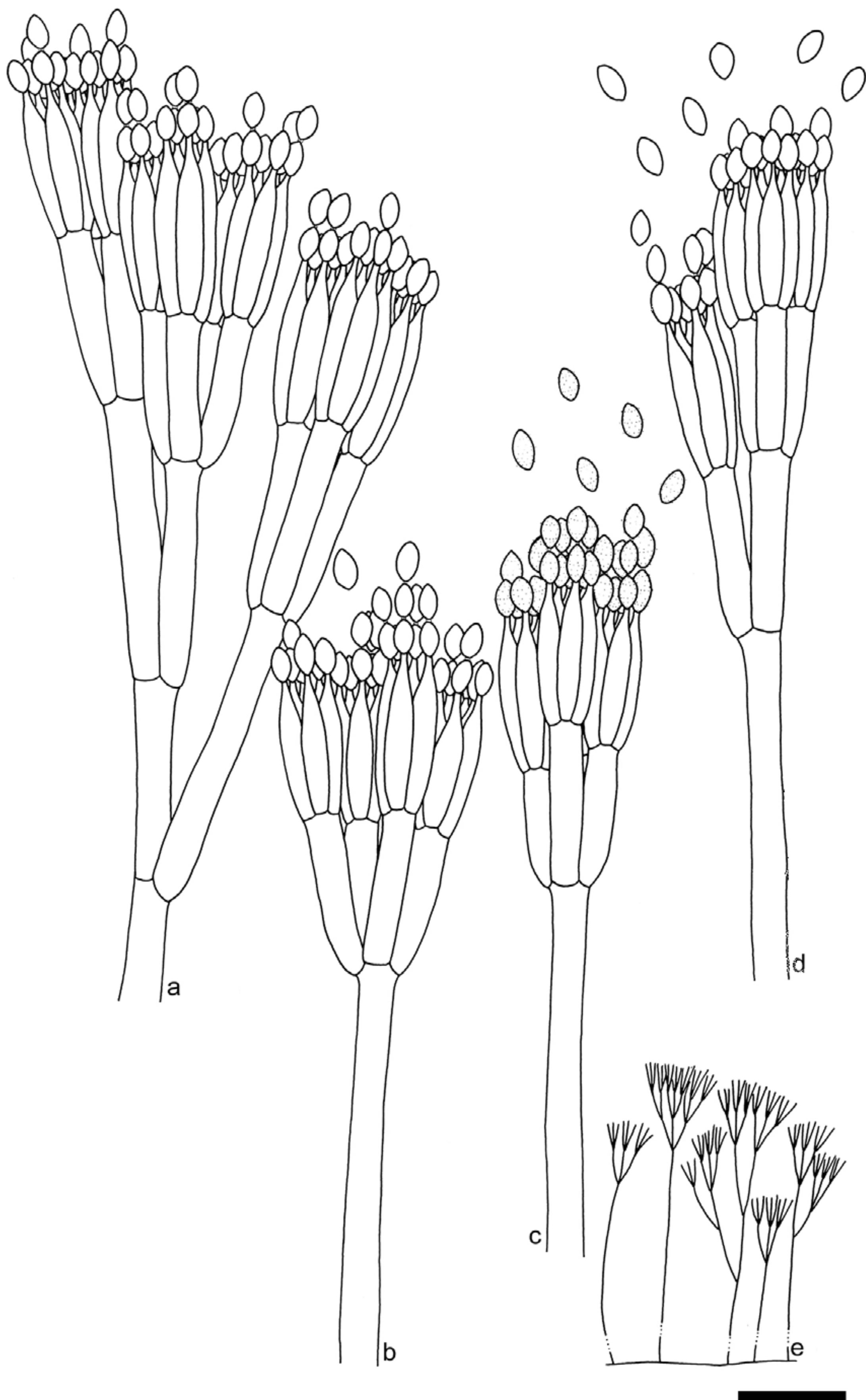

PLATE 8. Line drawing of *Talaromyces dendriticus*. a–d. Conidiophores (— Scale bar = 10 µm). e. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces infrolivacea Visagie **prov. nom.** PLATES 9, 10, 31e

ETYMOLOGY: Latin, *infrolivacea*: meaning below olive, in reference to the olive reverse pigmentation of colonies
EX-TYPE: CV1251 = DTO182I2 = KAS 3988 = DAOM 241024
TYPE ISOLATED FROM: Mite, *Protea repens* infructescence, Malmesbury, South Africa, S33°49'46; E18°35
ADDITIONAL SPECIMENS EXAMINED: CV1742, CV1861, CV2070

Macromorphology — CYA, 25 °C, 7d: Colonies 11–14 mm, low to moderately deep, plane, sunken in at centre; margins low, narrow (1 mm), entire; mycelia yellow; texture velutinous and floccose; sporulation moderately dense, conidia en masse greyish green (25E7–26E6); exudate absent, soluble pigment absent, reverse pigmentation olive (1F8) at centre, olive (1E8) near margin fading into pale yellow (1A3) at margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 12–15 mm, low to moderately deep, lightly radially sulcate; margins low, narrow (1–2 mm), entire, having a yellowish to olive color; mycelia yellow; texture floccose and velutinous; sporulation moderately dense, conidia en masse dull green (25E4) near centre, fading into greyish green (25D5); exudate absent, soluble pigment absent, reverse pigmentation olive (1F8), with greyish yellow (1B3) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 14–17 mm, moderately deep, plane; margins low, narrow (1 mm), entire; mycelia deep yellow, white near margin; texture floccose, overlaying funiculose mycelia present; sporulation moderately dense, only at colony centre, conidia en masse greyish green (26D6–26E6–27E6); exudate absent, soluble pigment absent, reverse pigmentation olive (3F7) at point of inoculation, fading into olive (3E8) to yellow (3A6) near margin, pastel yellow (2A4) margin.

YES, 25 °C, 7d: Colonies 16–20 mm, deep, craterform; margins low, narrow (1–2 mm), entire; mycelia a darkish, almost orange, yellow, white at margin; texture floccose to funiculose in specific regions, velutinous also present; sporulation moderately dense, conidia en masse greyish green (25D4–25D5); exudate absent, soluble pigment absent, reverse pigmentation olive to olive brown (3F8–4F8) at centre, olive (2E8) elsewhere, with pale yellow (2A3) margin.

G25N, 25 °C, 7d: Colonies 4–6 mm; mycelia yellow; sporulation moderate, conidia en masse greyish green (30D5), reverse pigmentation olive (3F8).

CREA, 25 °C, 7d: Colonies 5–6 mm, no acid production.

Micromorphology — Conidiophores biverticillate, with minor proportion terverticillate and subterminal branching; stipes smooth walled, 12–100 × 2–3 µm, branches 2–3 when present, 11–15 × 2–3 um; Metulae 4–6, appressed to divergent, 22–65° [39.7±8.2°], 7.5–11.5 × 2–3 [9.1±0.9 × 2.4±0.2] µm; phialides acerose-ampulliform, 3–4 per metula, 7–10 × 1.5–2.5 [8.4±0.7 × 2.2±0.2] µm; conidia rough walled, sometimes in ridges, ellipsoidal, 2.5–4 × 1.5–3 [2.7±0.2 × 2.1±0.2] µm, average width/length = 0.77±0.1, n = 82.

Notes — This species belongs to the larger *T. rugulosus* species complex, which is currently under review. Morphological differences include more lightly colored conidia *en masse* and MEA colonies that are more floccose in *T. infrolivacea* than the velutinous colonies of the *T. rugulosus* species complex. It has another species, *T. vermiculicola*, as close relative that is described in this paper. Its faster growing colonies on CYA, the dark olive colored reverse pigmentation and funicles absent on all media distinguish *T. infrolivacea* from its closely related Fynbos species. Phylogenetically the two new species do not match any strains that have previously been identified to belong in this clade and are thus considered to represent a novel species (FIGURES 1, 4).

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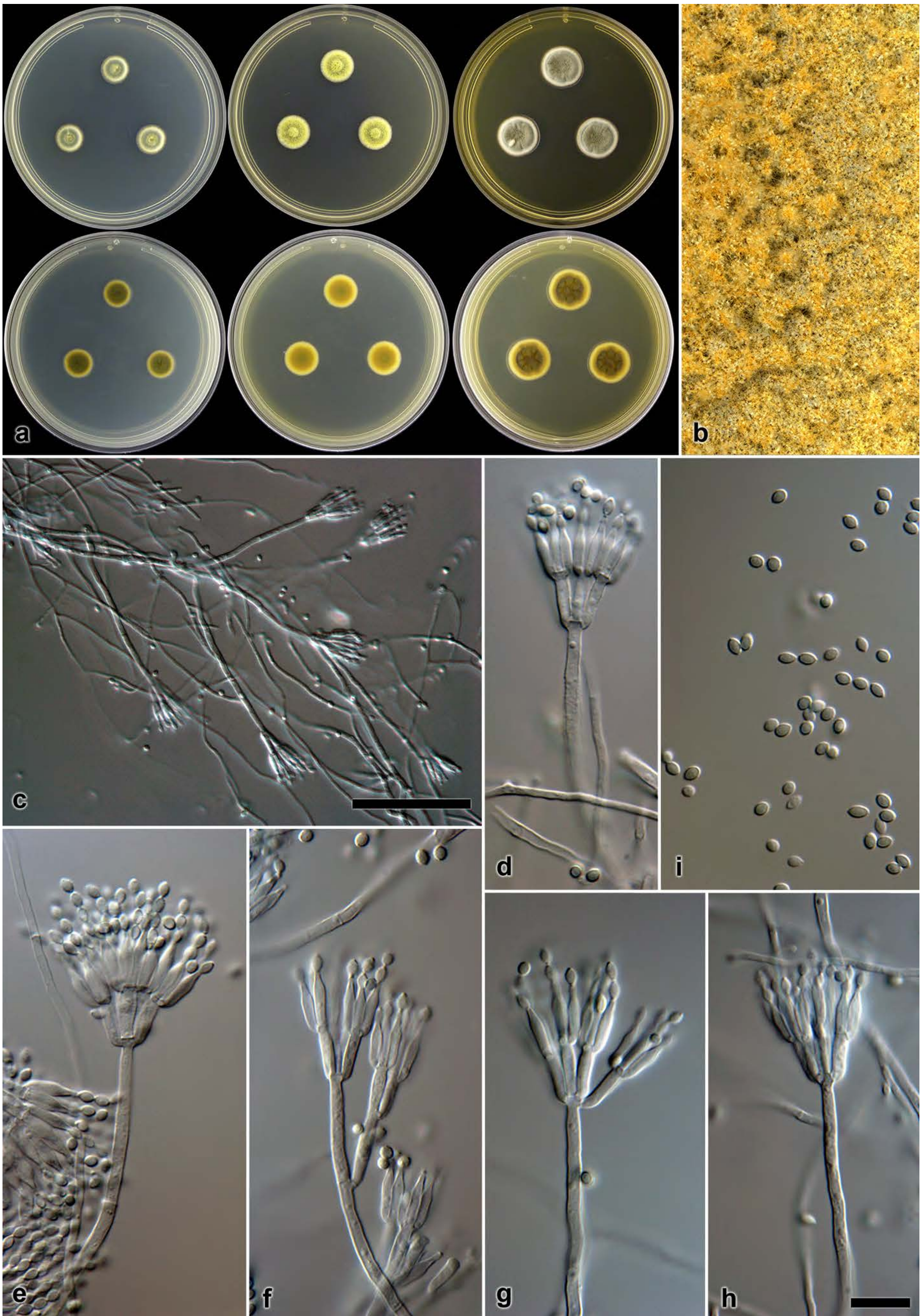

PLATE 9. *Talaromyces infrolivacea*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–h. Conidiophores. i. Conidia (— Scale bar in c = 50 µm; — Scale bar in h = 10 µm, applies to d–i).

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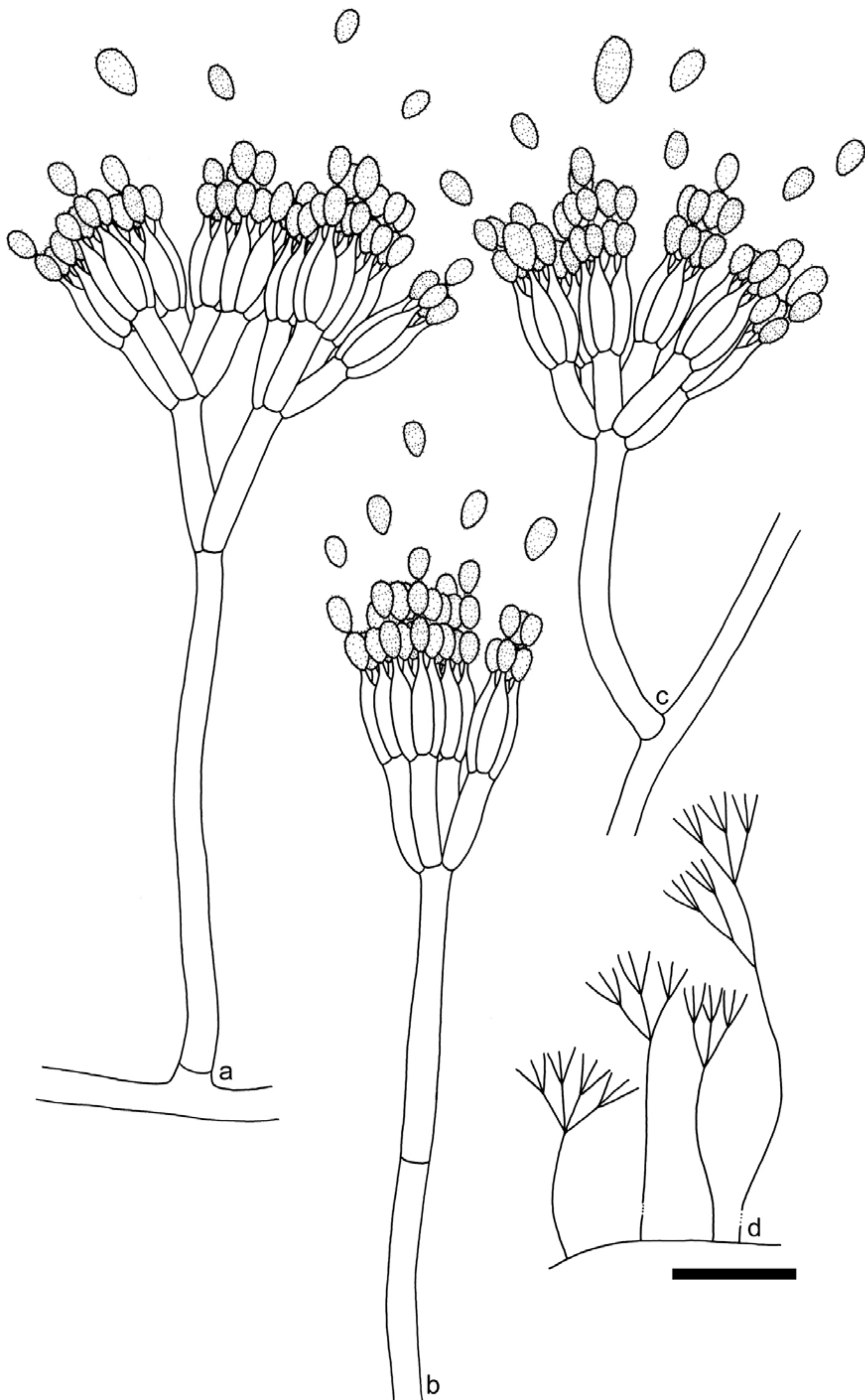

PLATE 10. Line drawing of *Talaromyces infrolivacea*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces minioluteus (Dierckx) Samson, Yilmaz, Frisvad & Seifert PLATES 11, 12, 31f
Studies in Mycology 70: 176. 2011.

MYCOBANK: MB560657
BASIONYM: *Penicillium minioluteum* Dierckx (Ann. Soc. Scie. Bruxelles 25: 87. 1901)
TYPE: CBS642.68 = IMI089377 = MUCL28666
TYPE ISOLATED FROM: Unknown source
SPECIMENS EXAMINED: CV383, KAS3859, MUCL28666.

Macromorphology — CYA, 25 °C, 7d: Colonies 8–12 mm, low, raised centrally, plane; margins low, narrow (1–2 mm); mycelia white and yellow; texture velutinous and floccose; sporulation greyish to dark green (25E5–26F5), conidia en masse moderately dense; exudate absent, soluble pigment absent, reverse pigmentation violet brown (11F8) at point of inoculation, fading into a light violet brown (10E8) and then light orange (5A4) near yellowish white (4A2) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 12–20 mm, low, plane, slightly raised at centre; margins low, very narrow to narrow (0.2–2 mm), entire; mycelia yellow, some isolates white; texture floccose; sporulation moderately dense, conidia en masse dark green (28F8–25F8); exudate yellow, absent in some isolates, soluble pigment absent, reverse pigmentation dark brown (6F8) at centre, light brown (6D6) and orange (6A6) elsewhere, with yellowish white (3A2) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 16–20 mm, low, plane; margins low, narrow (1 mm), irregular; mycelia yellow and white; texture velutinous; sporulation dense, conidia en masse dark green (25F8–26F8) in some isolates, greyish green (29E5–29E7) in others; exudate absent, soluble pigment absent, reverse pigmentation light yellow (4A5) at centre, dull yellow (3B4–3B5) elsewhere, with yellowish white (2A2) margin.

YES, 25 °C, 7d: Colonies 18–23 mm, low, raised at centre; margins low, narrow (1–2 mm), entire; mycelia yellow, some isolates only white; texture velutinous and floccose; sporulation moderately dense, conidia en masse dark green (25F6–26F6); exudate orange to clear, some isolates absent, soluble pigment absent, reverse pigmentation light orange (5A5) and brown to reddish brown (7E8–8E8), pale yellow (4A3) margin.

G25N, 25 °C, 7d: Microcolonies of 2–3 mm, sporulation very sparse.

CREA, 25 °C, 7d: Colonies 3–8 mm, no acid production to sometimes very weak production close to colony periphery.

Micromorphology — Conidiophores biverticillate; stipes 75–250 × 2.5–3.5 µm; metulae 5–8, appressed, 15–37° [25.3±5.6°], 10–14 × 3–3.5 [11.6±0.9 × 3.2±0.2] µm; phialides acerose, 3–5 per metula, 9.5–13 × 2–3 [11.4±0.9 × 2.6±0.2] µm; conidia smooth walled, ellipsoidal, 2.5–3.5 × 1.5–2.5 [2.8±0.2 × 2±0.1] µm, average width/length = 0.73±0.06, n = 88.

Notes — *Talaromyces minioluteus* is characterized by slow growing colonies on both CYA and MEA, often with red pigments. It produces dark green conidia on MEA, and conidiophores are strictly biverticillate, with metulae almost parallel to each other, which distinguishes it from its close relatives (FIGURES 1, 2).

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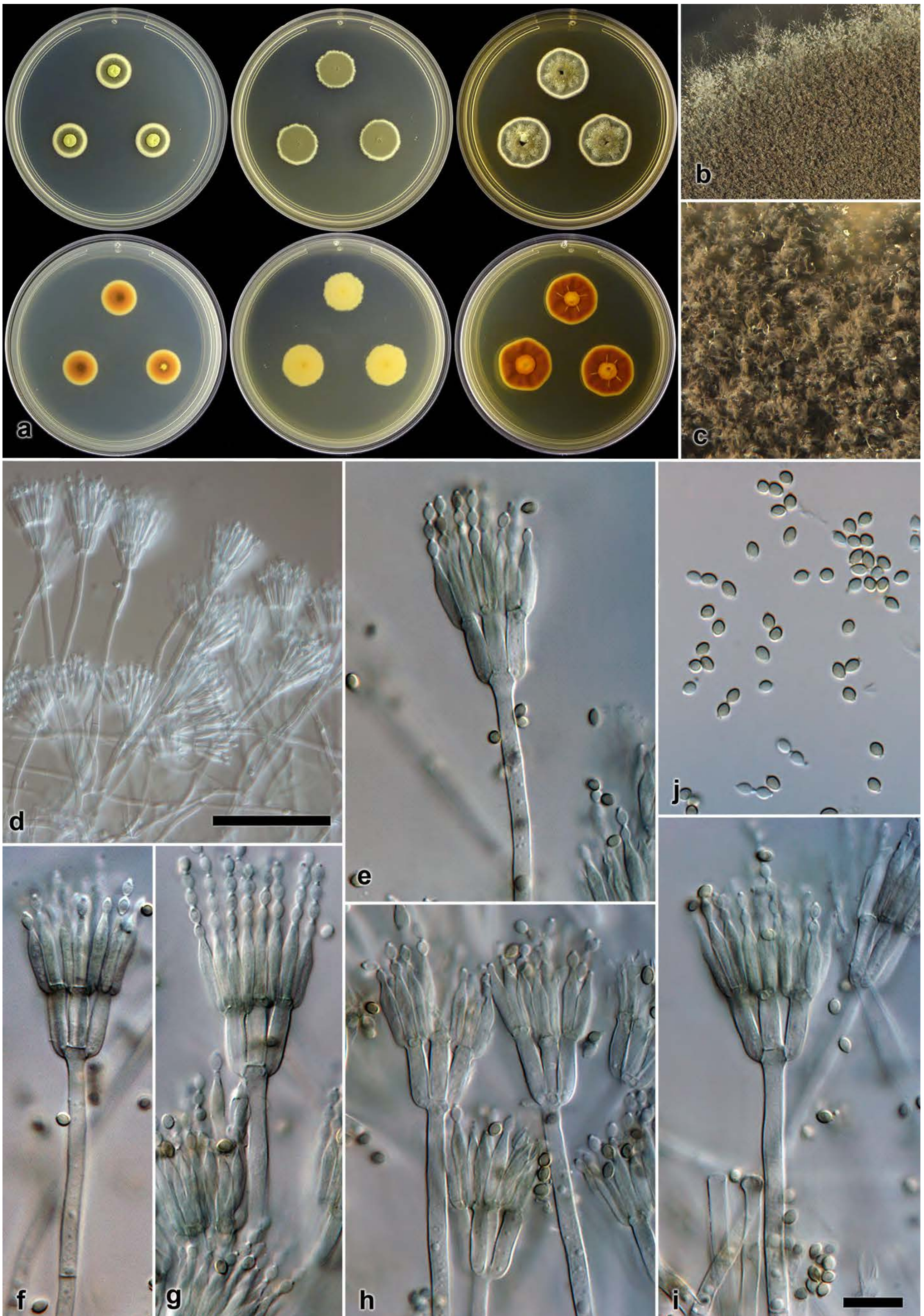

PLATE 11. *Talaromyces minioluteus*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 50 μm; — Scale bar in i = 10 μm, applies to e–j).

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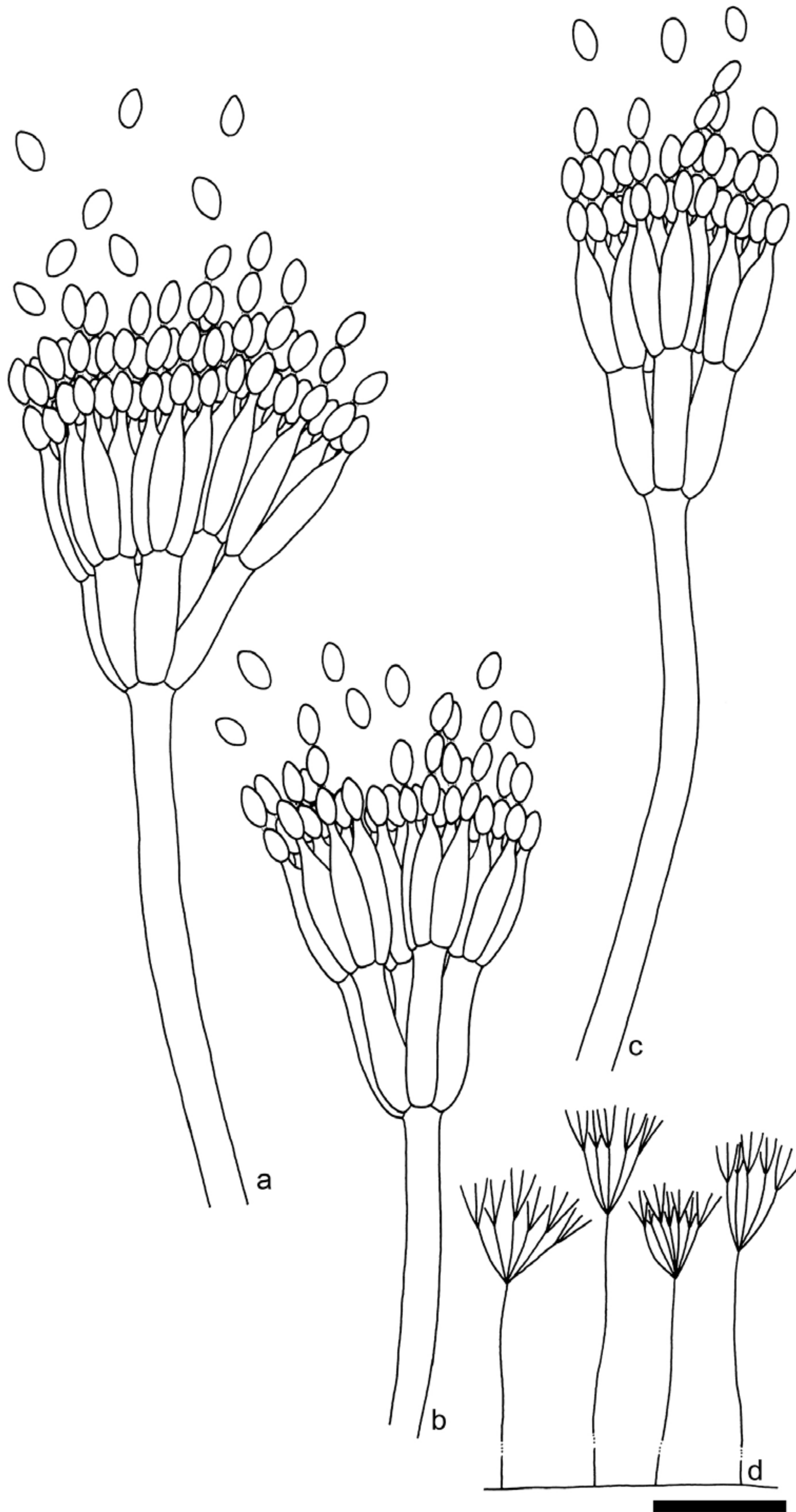

PLATE 12. Line drawing of *Talaromyces minioluteus*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces parvaurantica Visagie **prov. nom.** PLATES 13, 14, 31g

ETYMOLOGY: Latin, *parvaurantica*: meaning small orange, in reference to the slow growth rate and orange mycelia produced
EX-TYPE: CV549 = DTO181I2 = KAS 4154 = DAOM 241020
TYPE ISOLATED FROM: Soil, Stellenbosch, South Africa, S33°56'47; E18°52'49, 1 March 2009
ADDITIONAL SPECIMENS EXAMINED: This species is based on a single specimen.

Macromorphology — CYA, 25 °C, 7d: Colonies 8–10 mm, low, plane; margins low, very narrow (1 mm), entire; mycelia white; texture velutinous near margin, floccose elsewhere; sporulation moderately dense, conidia en masse greyish green (25E5–26E5); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4C4) at centre, greyish yellow (1B3–2B3) elsewhere, with yellowish white (2A2) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 13–15 mm, deep, plane, at centre covered with sterile mycelia; margins low, narrow (1 mm), entire; mycelia white; texture velutinous with some floccose regions; sporulation moderately dense, conidia en masse (24E5–25E5); exudate absent, soluble pigment absent, reverse pigmentation olive brown (4F8) and greyish yellow (4C6), with yellowish white margin (1A2).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 15–16 mm, moderately deep, plane; margins low to subsurface, wide (2 mm), entire to somewhat irregular because of overlaying mycelia above subsurface margin; mycelia white, orange and yellow; texture velutinous with some floccose mycelia present; sporulation moderately dense, conidia en masse greyish green (25E5–25E6); exudate absent, soluble pigment absent, reverse pigmentation brownish yellow (5C7) and yellow (3A6).

YES, 25 °C, 7d: Colonies 14–17 mm, moderately deep, plane, raised centrally, sterile mycelia covering central areas; margins low, narrow (1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia en masse greyish green (25D6–25E6–25E7); exudate absent, soluble pigment absent, reverse pigmentation olive (1F3) at centre, yellowish brown (5E8) near centre, surrounded by greyish yellow (2B3), with yellowish white (2A2).

G25N, 25 °C, 7d: Colonies 3–5 mm, sporulation moderately dense greyish green (25D5–25D5).

CREA, 25 °C, 7d: Colonies 5–7 mm, no acid production.

Micromorphology — Conidiophores biverticillate; stipes smooth walled, 50–285 × 2.5–3.5 µm; metulae number, somewhat appressed to mostly divergent, 32–90° [57±16]°, 9–13 × 2–3.5 [11±0.9 × 2.9±0.3] µm; phialides acerose, number per metula, 8.5–11 × 2–3 [9.7±0.6 × 2.4±0.2] µm; conidia finely rough walled, ellipsoidal, 2–3 × 2–2.5 [2.6±0.1 × 2.1±0.1] µm, average width/length = 0.8±0.05, n = 110.

Notes — *Talaromyces parvaurantica*, similar to other species from CLADE 5, produces restricted growth on CYA. Morphologically it is similar to the *T. rugulosus* complex. However, the orange mycelia and less compacted colonies compared to *T. rugulosus* complex, distinguishes this as a new species. In general, *T. parvaurantica* also produces phialides that is more slender and longer than other species from this clade. Phylogenetically it is closely related to *T. allabahadensis* (based on ITS) (FIGURE 1) and *T. variabilis* (based on β-tubulin) (FIGURES 4). However, *T. allabahadensis* produces fast growing colonies on CYA and MEA (Pitt 1979, Ramirez 1982), something rather unique for species from this clade. *Talaromyces variabilis*, also isolated in this study, characteristically produces very large conidia (2.5–4(–9) × 1.5–2.5(–3.5) µm) and divergent conidiophores, compared to the small conidia and appressed conidiophores of *T. parvaurantica*.

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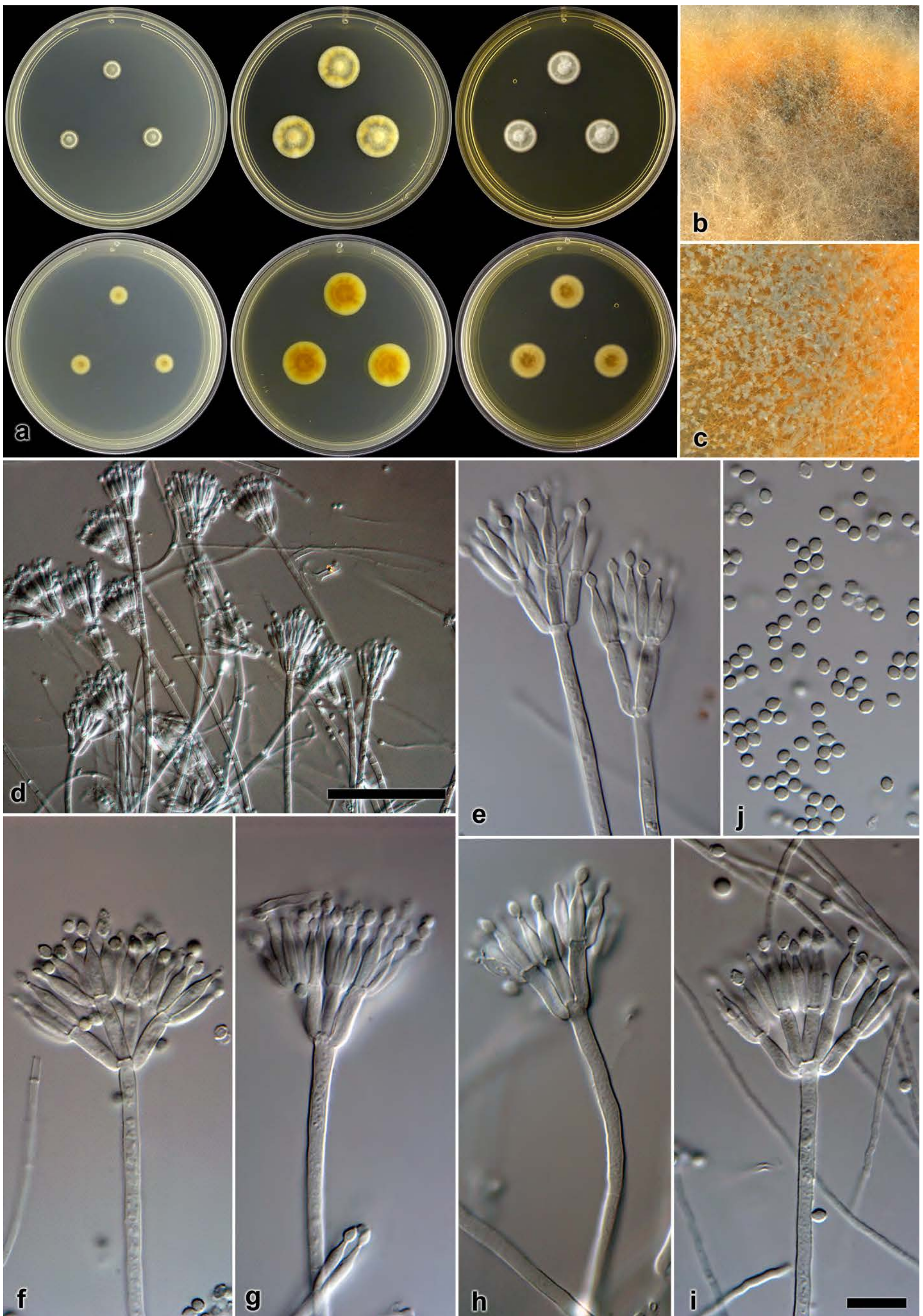

PLATE 13. *Talaromyces parvaurantica*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on CYA. c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 50 µm; — Scale bar in i = 10 µm, applies to e–j).

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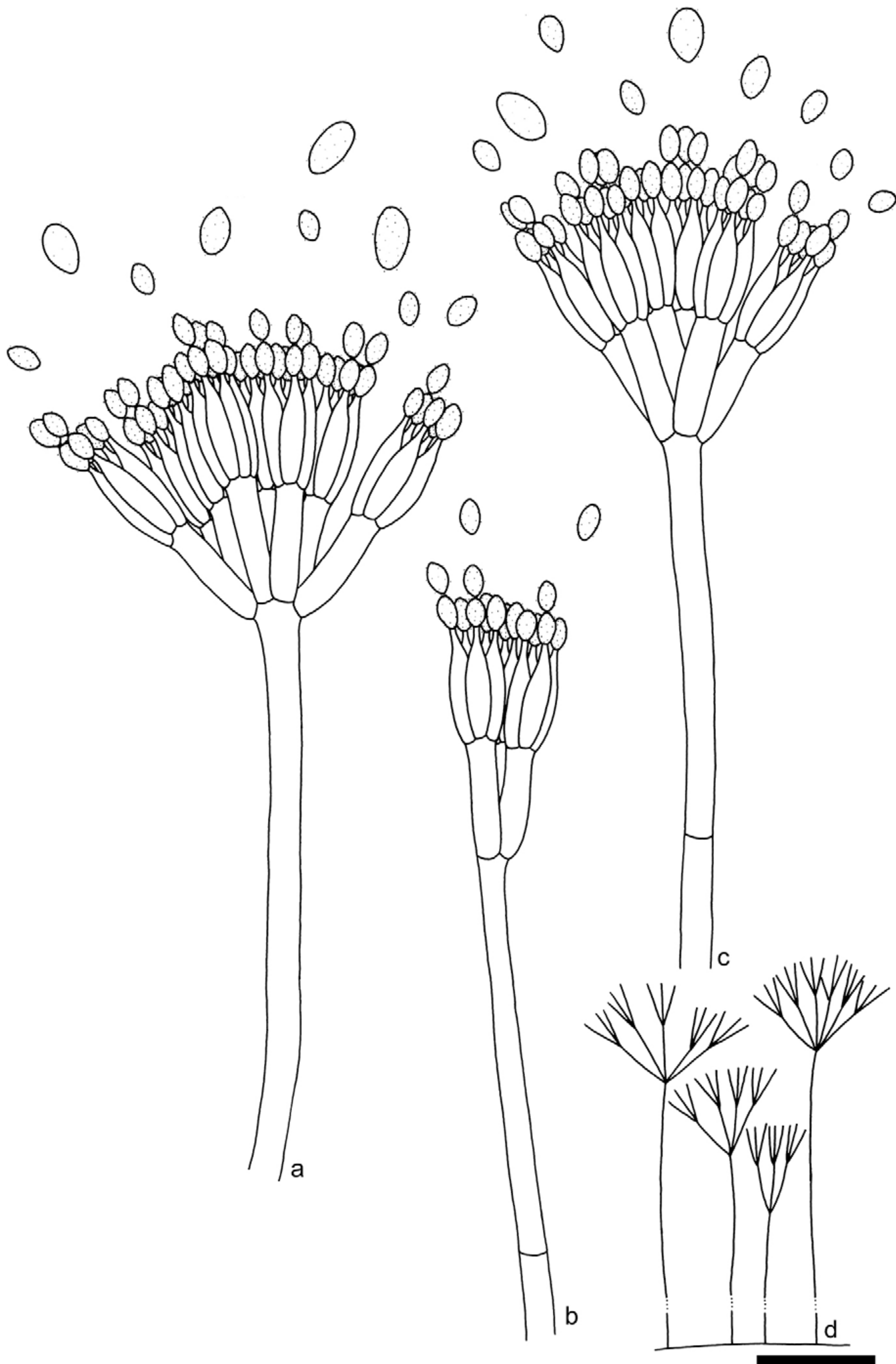

PLATE 14. Line drawing of *Talaromyces parvaurantica*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces pinophilus (Hedgcock) Samson, Yilmaz, Frisvad & Seifert PLATES 15, 16, 31h
Studies in Mycology 70: 176. 2011.

MYCOBANK: MB560662
BASIONYM: *Penicillium pinophilum* Hedgcock (Bull. Bur. Anim. Ind. US. Dept. Agric. 118: 37. 1910)
SYNONYM: *Penicillium korosum* Rai (CBS762.86)
TYPE: CBS631.66 = ATCC36839 = IMI114933
TYPE ISOLATED FROM: Polyvinyl Chloride Plastic, France
SPECIMENS EXAMINED: CV2460, CBS631.66

Macromorphology — CYA, 25 °C, 7d: Colonies 24–26 mm, low, plane, white sterile mycelial overlay at centre; margins subsurface, narrow (2 mm), entire; mycelia white and yellow; texture floccose; sporulation moderately dense, conidia en masse greyish green (28D6–28E6); exudate absent, soluble pigment absent, reverse pigmentation greyish yellow (4B5) and reddish brown (8D6), with pale yellow (3A3) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 40–42 mm, low, slightly raised at centre, lightly radially sulcate, white sterile mycelia covering central regions; margins low, wide (3 mm), entire; mycelia yellow, with white present; texture mostly floccose, velutinous present; sporulation moderately dense, conidia en masse dark green (28F8) and greyish green (28D6); exudate absent, soluble pigment absent, reverse pigmentation greyish orange (5B5) at centre, fading into greyish yellow (4B5) into more green greyish yellow (1B3) near yellowish white (2A2) margin.

CYA, 37 °C, 7d: Colonies 32–35 mm, low, lightly radially and concentrically sulcate, having a yellowish color in non sporulating regions; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous, with some floccose as well as funicles covering colonies; sporulation moderately dense, condia en masse greyish green (25E5–26E5); exudate clear, soluble pigment yellowish halo surrounding colonies followed by brownish orange further away, reverse pigmentation orange yellow (4B8), with pale to dull yellow (3A4–3B4) margin.

MEA, 25 °C, 7d: Colonies 35–40 mm, low, plane; margins low to subsurface, wide (2–4 mm), entire; mycelia white and yellow; texture floccose; sporulation moderately dense, conidia en masse greyish green (29C7–29D7); exudate absent, soluble pigment absent, reverse pigmentation brown (6D7) at centre, fading into olive yellow (3C7) near pastel yellow (3A4) margin.

YES, 25 °C, 7d: Colonies 28–31 mm, low, lightly radially sulcate, sterile mycelia covering parts of colonies; margins low to subsurface, wide (2–3 mm); mycelia yellow, with white also present; texture floccose; sporulation moderately dense, conidia en masse dull to greyish green (27E4–27E7); exudate absent, soluble pigment absent, reverse pigmentation greyish to brownish yellow (4C7–5C7) at centre, greyish yellow (4B6) elsewhere, with light yellow (4A4) margin.

G25N, 25 °C, 7d: Microcolonies 2–3 mm, no sporulation.

CREA, 25 °C, 7d: Colonies 8–13 mm, strong acid production only close to colony periphery.

Micromorphology — Conidiophores biverticillate; stipes smooth walled, often very short, 30–200 × 2–3 µm; metulae 5–10, some appressed, mostly divergent, 37–112° [62.4±21°], 10–11 × 2.5–3 [11.2±1.3 × 2.8±0.2] µm; phialides acerose, number per metula, 8.5–12 × 2–3 [10.3±0.8 × 2.5±0.2] µm; conidia smooth walled, subspheroidal to ellipsoidal, 2–3 × 2–3 [2.4±0.2 × 2.2±0.2] µm, average width/length = 0.87±0.1, n = 80.

Notes — *Talaromyces pinophilus* is characterized by colonies that have bright yellow mycelia on CYA and MEA. Conidiophores are typically biverticillate with acerose phialides and produces subspheroid conidia that is smooth to finely rough walled. It is closely related to *Talaromyces verruculosus*, *T. aculeatus* and *T. stellenbossiensis*. *Talaromyces pinophilus* resemble these species based on colony morphology, but the smooth conidia in contrast to the heavy rough walled conidia produced by *T. verruculosus, T. aculeatus* and *T. stellenbossiensis* makes it distinct. Pitt (1979) considered *P. purpurogenum* var. *rubisclerotium* (CBS270.35), *T. allahabadensis* (= *P. allahabadense*) (CBS453.93) and *P. korosum* (CBS762. 68) as synonyms based on morphological similarities. However, based on the Samson *et al*. (2011) phylogeny, only *P. korosum* can be considered a synonym, which is confirmed by the β-tubulin and Calmodulin phylogeny presented here (FIGURE 2). The other species previously considered as synonyms (Pitt 1979), resolved in distinct clades from *T. pinophilus* (FIGURES 1, 2).

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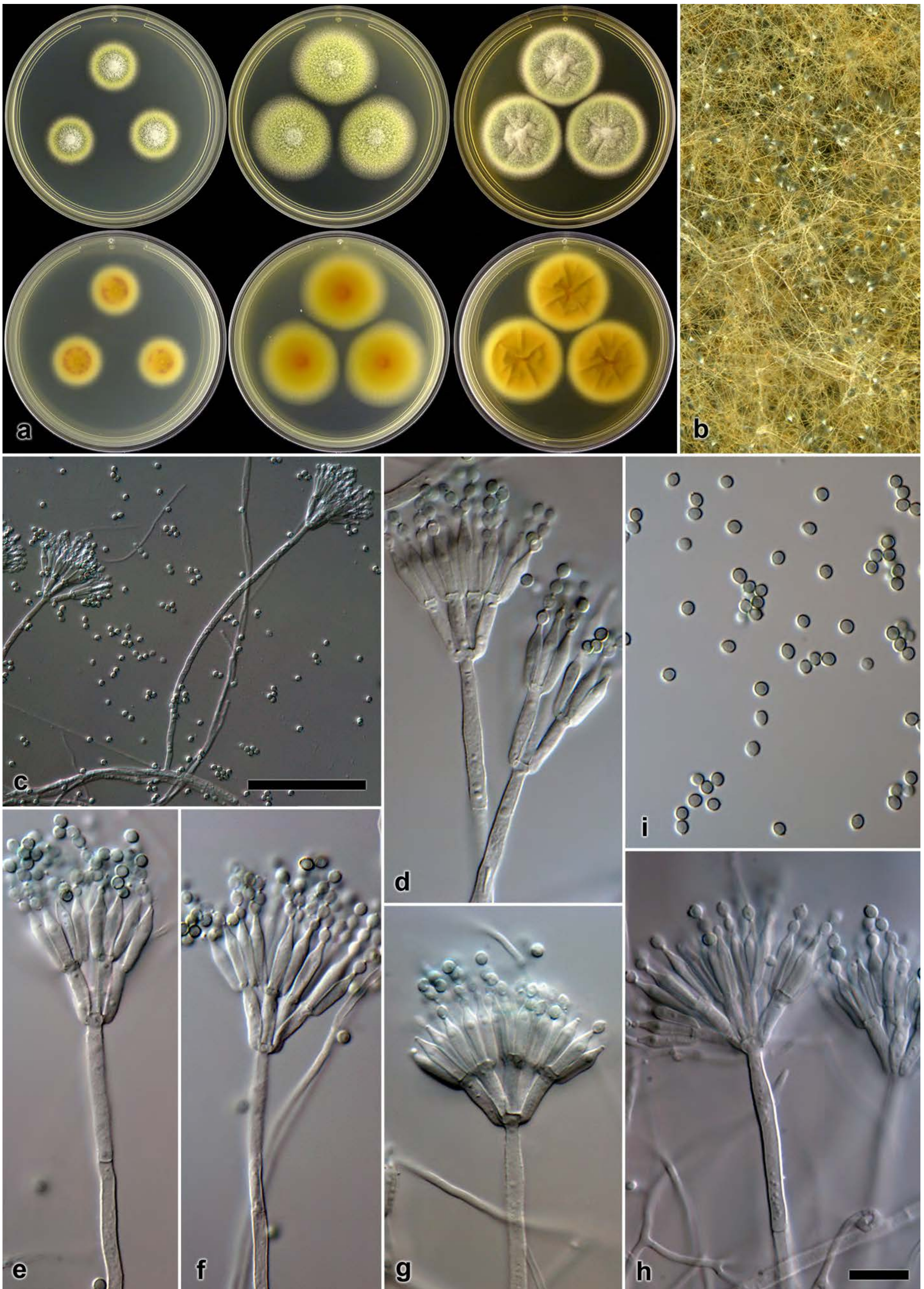

PLATE 15. *Talaromyces pinophilus*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–h. Conidiophores. i. Conidia (— Scale bar in c = 50 µm; — Scale bar in h = 10 µm, applies to d–i).

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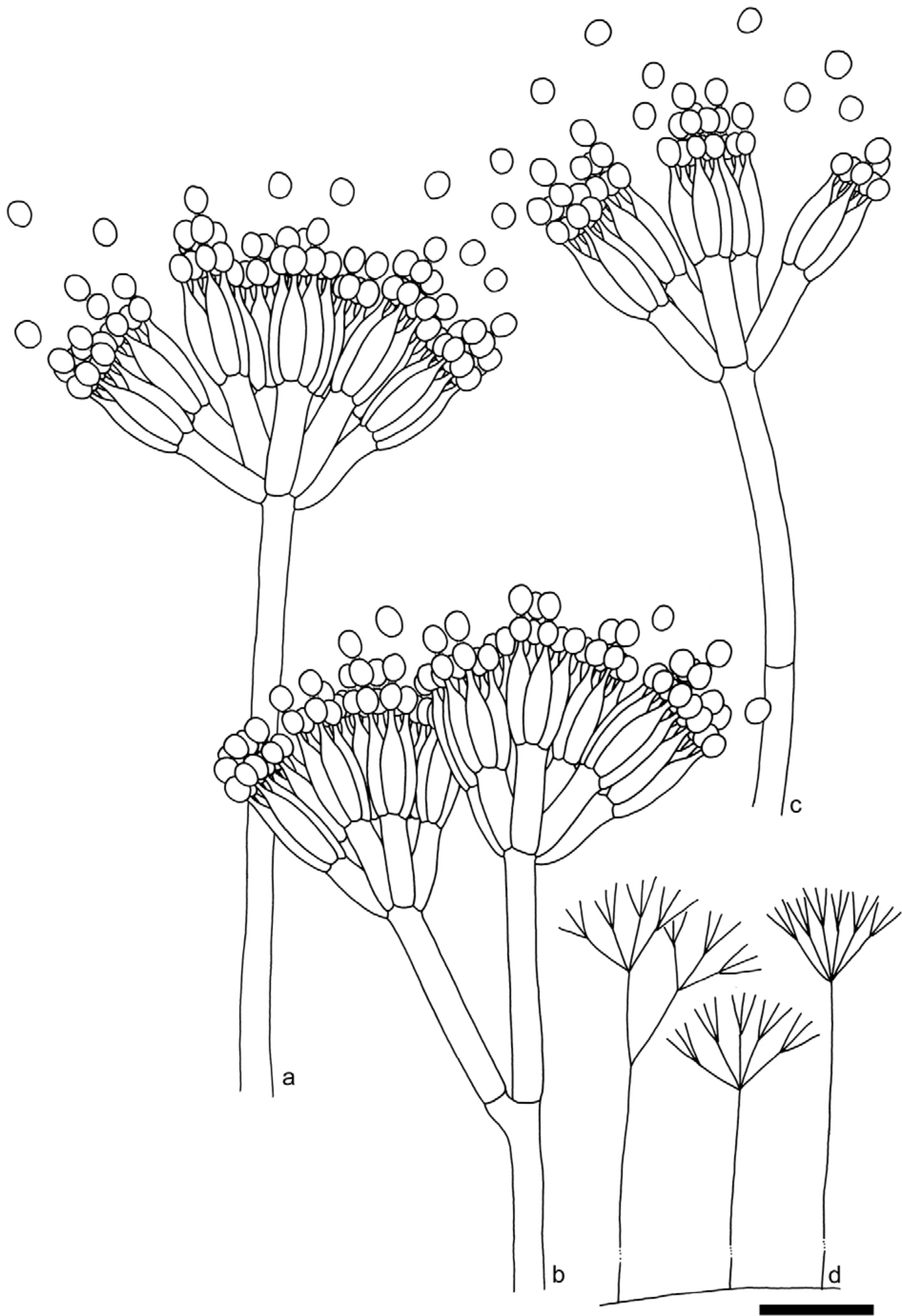

PLATE 16. Line drawing of *Talaromyces pinophilus*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm)

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Talaromyces ptychonidium Visagie & Jacobs PLATES 17, 18, 31i

Persoonia 28: 18. 2012.

MYCOBANK: MB564327
TYPE: PREM60041 (herbarium) = CV2808 = DAOM241017
TYPE ISOLATED FROM: Soil, Malmesbury, South Africa
SPECIMENS EXAMINED: CV2808, CV2807, CV2806

Macromorphology — CYA, 25 °C, 7d: Colonies 8–12 mm diam, plane, loose to moderately dense; texture floccose; margins subsurface, 3–4 mm wide, regular, mycelia white, becoming yellow with age; sporulation 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).

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 17–22 mm, craterform, having a greyish beige (4C2); margin low, narrow (1–2 mm), entire; mycelia white; sporulation absent; exudate brownish yellow and clear, soluble pigment absent, reverse pigmentation blackish brown and brown (5F4–5F5) at centre, brown (5E6) and pale yellow (3A3) elsewhere.

CYA, 37 °C, 7d: Colonies 8–9 mm, low to deep, plane, having a greyish beige (4C2) color; texture velutinous; white mycelia; sporulation sparse, Brownish Orange (6c3–6c5); exudate and soluble pigment absent, reverse pale (6b2).

MEA, 25 °C, 7d: Colonies 15–21 mm, plane, loose, sometimes having a somewhat pinkish colour; texture loosely funiculose; margins subsurface, narrow, irregular, mycelia yellow; sporulation sparse to moderate, Dark Green (28f4); clear to pale slimy exudate produced, soluble pigment absent, reverse Raw Umber (5f8).

YES, 25 °C, 7d: Colonies 20–25 mm, moderately sulcate to umbonate; margins low, mycelia white, margins wide; sporulation sparse to moderate, conidida en masse Spinach Green to Dull Green (29e6–29e3); 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, 7d: Colonies 4–5 mm diam, low, plane, consisting out of white mycelia.

CREA, 25 °C, 7d: Colonies 9–12 mm, no acid production.

Micromorphology — Conidiophores strictly biverticillate, having a green pigment; stipes smooth walled, 38–93 × 2.5–3.5 µm; metulae whorl of 3–7, appressed, 20–65° [37±13°], 10–12.5 × 2–3 [11±0.58 × 2.7±0.3] µm; phialides acerose, 3–4 per metula, 10–12 × 2–2.5 [10.9±0.4 × 2.2±0.2] µm; conidia spirally rough walled, ellipsoidal, some apiculate, 3–4.5(–5) × 2–3 [3.5±0.5 × 2.3±0.2] µm, average width/length = 0.7±0.05, n = 115.

Notes — A species previously described from Fynbos soil (Visagie & Jacobs 2012). This species typically produces slow growing colonies on CYA and MEA, and grows faster at higher temperatures. Colonies are typically covered by sticky exudates. Conidiophores are pigmented and it produces large, spirally rough walled ellipsoid conidia. It resolved in a clade with only *T. purpureus* (CBS475.71) as its close relative (FIGURES 1, 3). However, the latter species typically has vesiculated stipes, as well as red-pigmented mycelia.

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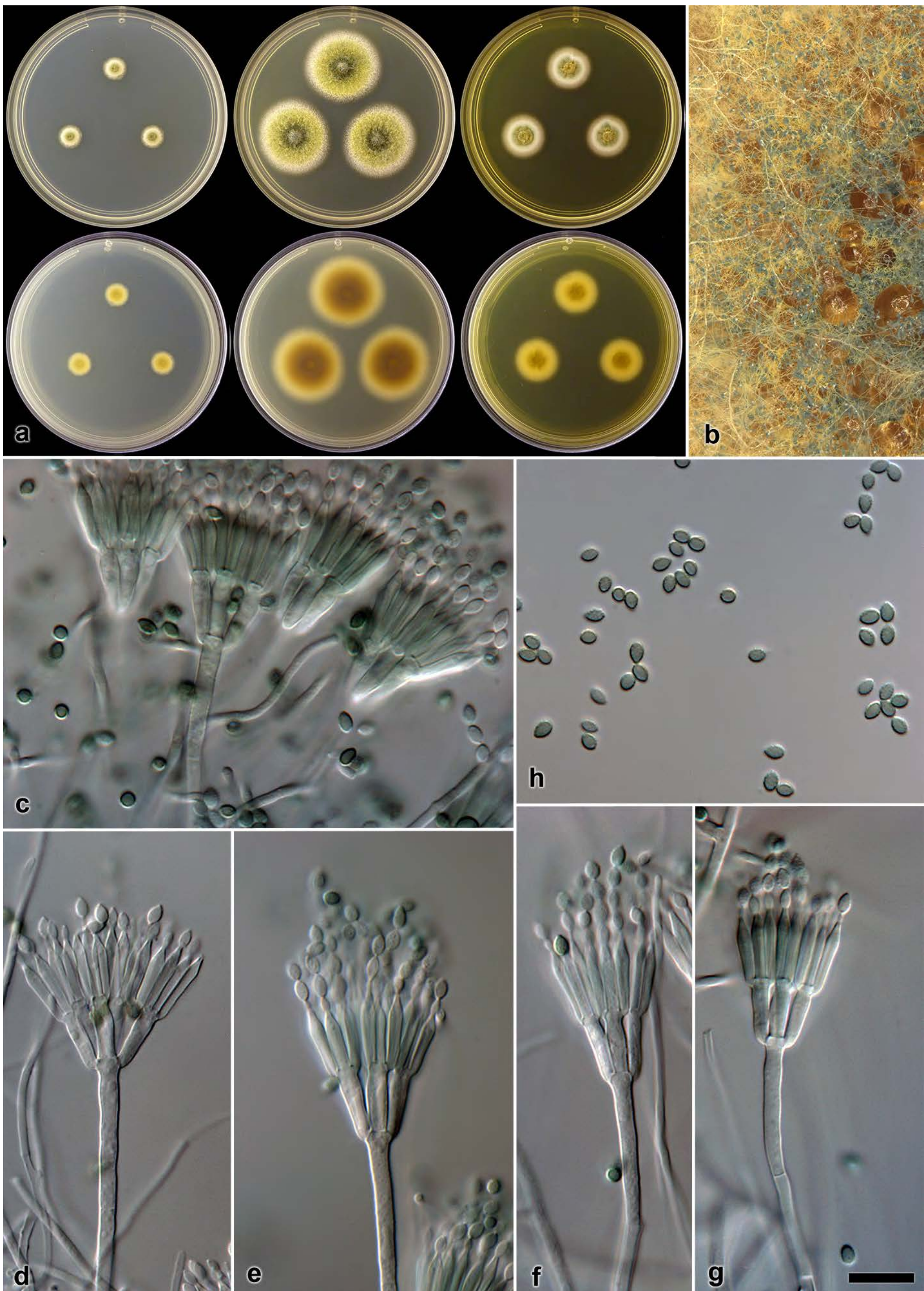

PLATE 17. *Talaromyces ptychoconidium*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–g. Conidiophores. h. Conidia (— Scale bar in g = 10 µm, applies to c–h). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 19.

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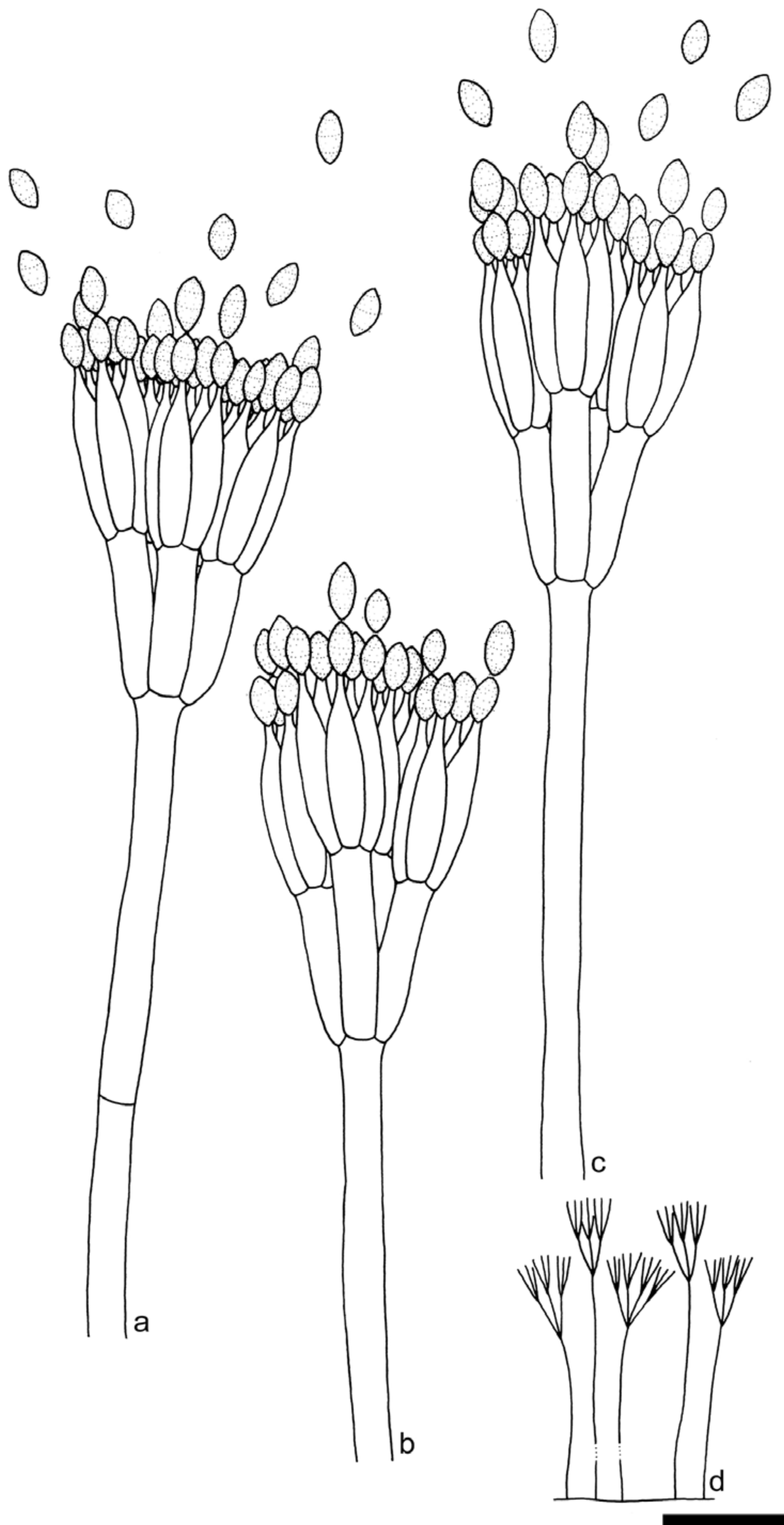

PLATE 18. Line drawing of *Talaromyces ptychoconidium*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 20.

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Talaromyces radicus (Hocking & Whitelaw) Samson, Yilmaz, Frisvad & Seifert PLATES 19, 20, 31j
Studies in Mycology 70: 177. 2011.

MYCOBANK: MB560669
BASIONYM: *Penicillium radicum* Hocking & Whitelaw (Mycological Research 102: 802. 1998)
TYPE: CBS100489, FRR4718
TYPE ISOLATED FROM: Root of *Triticum aestivum*, Wagga Wagga, Australia
SPECIMENS EXAMINED: CV245, CV247, CV253, CV279

Macromorphology — CYA, 25 °C, 7d: Colonies 11–16 mm, deep, plane, sterile mycelia masking conidial regions; margins low, very narrow (<1 mm), entire; mycelia white near margins, a deep yellow elsewhere; texture seems to be floccose; sporulation mostly sparse, conidia en masse greyish green (29E7); exudate mostly absent, clear when present, soluble pigment absent, reverse pigmentation brownish orange (6C8), pale yellow (3A3) margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 22–25 mm, low, lightly radially sulcate; margins low, narrow (1 mm), entire; mycelia bright yellow, white at margin; texture floccose; sporulation sparse to moderately dense, conidia en masse greyish green (28C6–29C6); exudate orange, absent in some isolates, soluble pigment absent, reverse pigmentation brown (6E8) at centre, fading into orange (6B7) at yellowish white (3A2) margin.

CYA, 37 °C, 7d: Colonies 9–16 mm, deep, lightly radially sulcate; margins low, narrow (1–2 mm), entire; mycelia yellow; texture floccose; sporulation sparse to moderately dense in some areas, conidia *en masse* dull green (27D4–28D4); exudate yellow, soluble pigment absent, reverse pigmentation olive (3F3) at centre, light brown (5D4) and greyish yellow (4B5) elsewhere, with yellowish white (3A2) margin.

MEA, 25 °C, 7d: Colonies 13–20 mm, very deep, plane, colonies mostly consisting out of dense sponge-like mycelial mass; margins low, very narrow (<1 mm), entire; mycelia white near margins, yellow elsewhere, deep yellow inside colony; texture floccose; sporulation sparse to absent in some isolates, conidia en masse dark green (29F6); exudate absent, soluble pigment very light yellow, reverse pigmentation brownish yellow (5C8) at point of inoculation, orange (5B8) elsewhere, with pastel yellow (3A4) margin.

YES, 25 °C, 7d: Colonies 13–30 mm, low to moderately deep, plane, raised at centre; margins low, narrow to wide (1–3 mm), entire; mycelia yellow, white at margin; texture floccose, synnema starting to develop near margins; sporulation moderately dense, conidia en masse dark green (28F8); exudate absent, soluble pigment absent, reverse pigmentation dark brown (6F6) at centre becoming brownish orange (6B8–6D8) and deep orange (5A8) near yellowish white (2A2) margin.

G25N, 25 °C, 7d: Colonies 3–6 mm; mycelia inconspicuously yellow; sporulation moderately dense to absent, conidia en masse dull green (25D4).

CREA, 25 °C, 7d: Colonies 5–6 mm, no acid production.

Micromorphology — Conidiophores biverticillate, small proportion terverticillate; stipes smooth walled, 80–270 × 2–2.5 µm, branches 2 when present, 14–15 × 2–2.5 µm; metulae number, appressed to divergent, 20–60° [38±11.5°], 9–12 × 2–3 [10.4±0.9 × 2.5±0.2] µm; phialides acerose, number per metula, 8–11 × 2–2.5 [9.2±0.6 × 2.2±0.2] µm; conidia lightly rough walled in ridges, subspheroidal to ellipsoidal, 2–3 × 2–2.5 [2.6±0.17 × 2.1±0.12] µm, average width/length = 0.8±0.05, n = 80.

Notes — *Talaromyces radicus* is characterized by restricted growth on CYA and MEA, making it similar to species in CLADE 5. Colonies are deep and consist of bright yellow mycelia that form a fluffy texture, with the production of funicles on MEA (Hocking *et al*. (1998). It also has the ability to grow at 37 °C, a character not common in CLADE 5. The ITS phylogeny (FIGURE 1) place *T. parvaurantica* and *T. allahabadensis* as close relatives. β-tubulin confirms *T. allahabadensis* as a close relative (FIGURE 4); however, the latter species grows faster on CYA and MEA, which distinguishes these species. *Talaromyces radicus* also grows faster than T. parvaurantica on CYA at 25 °C and can grow at 37 °C.

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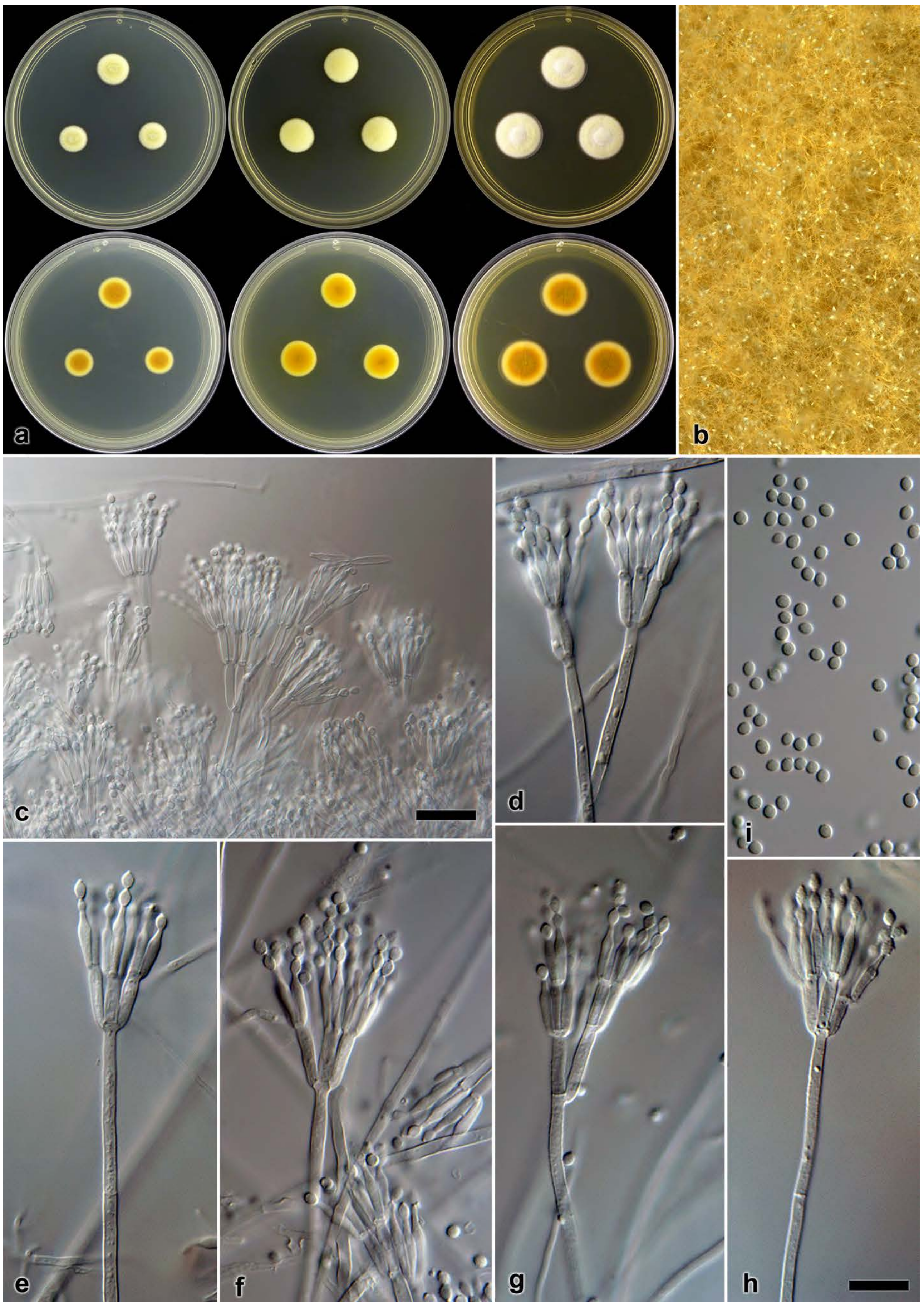

PLATE 19. *Talaromyces radicus*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–h. Conidiophores. i. Conidia (— Scale bar in c = 50 µm; — Scale bar in h = 10 µm, applies to d–i).

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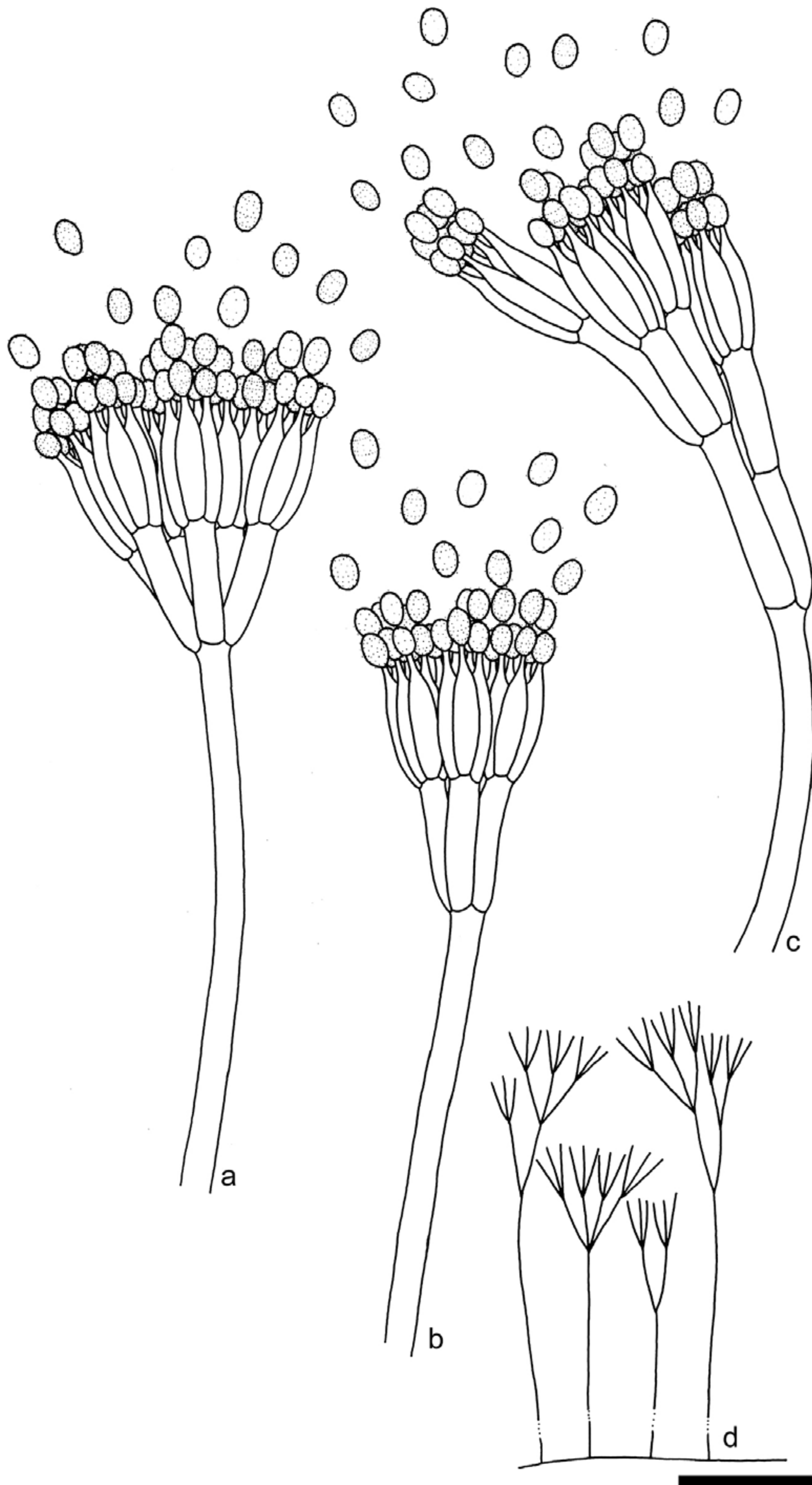

PLATE 20. Line drawing of *Talaromyces radicus*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces ramulosus (Visagie & Jacobs) Samson, Yilmaz, Frisvad & Seifert PLATES 21, 22, 31k
Studies in Mycology 70: 177. 2011.

MYCOBANK: MB560670
BASIONYM: *Penicillium ramulosum* Visagie & Jacobs (Mycologia 101: 890. 2009)
TYPE: PREM59947 (herbarium) = CV2837 = DAOM241160
TYPE ISOLATED FROM: Soil, Malmesbury, South Africa
SPECIMENS EXAMINED: CV2837, CV1426, CV2130, CV2282, CV2332, CV314, CV316, CV328, CV394, CV735, CV787

Macromorphology — CYA, 25 °C, 7d: Colonies 35–45 mm, low to moderately deep, plane, colonies often having overlaying sterile mycelia that range from white to yellow to pink depending on isolate; margins low, sometimes subsurface, wide (3–5 mm), entire; mycelia white; texture strongly funiculose, covering velutinous areas; sporulation sparse to moderately dense, conidia en masse dull to greyish green (26E4–26E6) lighter when covered by sterile mycelia; exudate clear slimy exudate produced, soluble pigment absent, reverse pigmentation ranging from dark brown to brown (6F8) to (6D8) at centre, to light orange (6A5) to pale yellow (4A3) to yellowish white to pale yellow (2A2–2A3) to brownish orange (7C6).

CYA, 5 °C, 7d: No germination, sometimes germination.

CYA, 30 °C, 7d: Colonies 39–50 mm, low, plane, colonies having pinkish to yellowish colors in colonies; margins low and subsurface, varying from narrow to very wide, entire; mycelia white; texture velutinous, colony however dominated by funiculose mycelia; sporulation ranging from moderately dense to absent, conidia en masse greyish green (26D4–26D5) to greyish green (25D5) in some isolates; exudate clear to glaucus, very sticky, soluble pigment absent, reverse pigmentation showing great variation from isolate to isolate, colony centre ranging from dark brown (7F8) to brownish orange (6C4) to greyish orange (6B6), elsewhere greyish yellow (4B3), yellowish white (2A2), greenish grey (1B2), greyish orange (6B6).

CYA, 37 °C, 7d: Colonies varying, sometimes no growth, sometimes colonies up to 12 mm, consisting of white mycelia.

MEA, 25 °C, 7d: Colonies 36–50 mm, low to moderately deep, plane; margins low to subsurface, wide (3–5 mm), entire; mycelia white; texture strongly funiculose, velutinous areas also present, synnemata produced after prolonged incubation in incidental sunlight, synnemata can be induced by scraping away colony and incubating plate again; sporulation moderately dense, conidia en masse sometimes pink (11A5) at centre, greyish green (27B6) elsewhere; clear slimy exudate produced, soluble pigment absent, reverse pigmentation greyish orange to light brown (5B5–5D5), greyish green to greyish yellow (1C3–2C3) and yellowish grey (2B2) elsewhere.

YES, 25 °C, 7d: Colonies 33–50 mm, low to moderately deep, plane, sterile white and yellowish aerial mycelia present; margins low to subsurface, wide (3–5 mm), entire; mycelia white; texture strongly funiculose, velutinous elsewhere; sporulation moderately dense, conidia en masse dark to dull green (25F4–25E4–26E4); clear and yellowish slimy exudate produced, soluble pigment absent, reverse pigmentation dark brown (6F6–6F8) at centre in some isolates, elsewhere ranging from olive to olive brown (2D4–4D4) to greyish orange to light brown (6B6–6D6) to dull yellow (3B4) depending on isolate.

G25N, 25 °C, 7d: Colonies 3–7 mm, sporulation very sparse to absent.

CREA, 25 °C, 7d: Colonies 15–17 mm, strong acid production close to colony periphery.

Micromorphology — Conidiophores biverticillate, terverticillate to quaterverticillate, often also with subterminal branches, having a green pigment; synnemata developing after 2 weeks of growth in incidental sunlight, stalk white to reddish brown, 100–150 (–250) × 130–190 (–210) µm, apex width 50–250 µm; stipes smooth walled, 10–60 × 2.5–3.5 µm; branches 10–18 × 2.5–3.5 µm; metulae whorl of 3–7, appressed, 10–45° [30±8.4°], 8–11 × 2–3 [9.5±0.9 × 2.6±0.2] µm; phialides acerose, 3–4 per metula, 8.5–11 × 2–2.5 [9.6±0.8 × 2.2±0.2] µm; conidia smooth, subspheroid to ellipsoid, 2–3 × 1.5–2.5 [2.5±0.2 × 1.9±0.2] µm, average width/length = 0.77±0.06, n = 106.

Notes — A species previously described from Fynbos soil (Visagie *et al*. 2009). It is characterized by good growth on CYA and MEA, with strongly funiculose colonies often having yellow and pink encrusted mycelia. Colonies also typically produce a slimy exudate. It resolved in CLADE 2 with other synnemata producing species (FIGURES 1, 3), although it typically produces short synnemata. Interestingly, synnemata production was induced in this species by scraping away colonies with a needle, thus leaving bare patches of media. After an additional week of incubation in incidental sunlight, synnemata was observed in these bare patches (FIGURE 25d).

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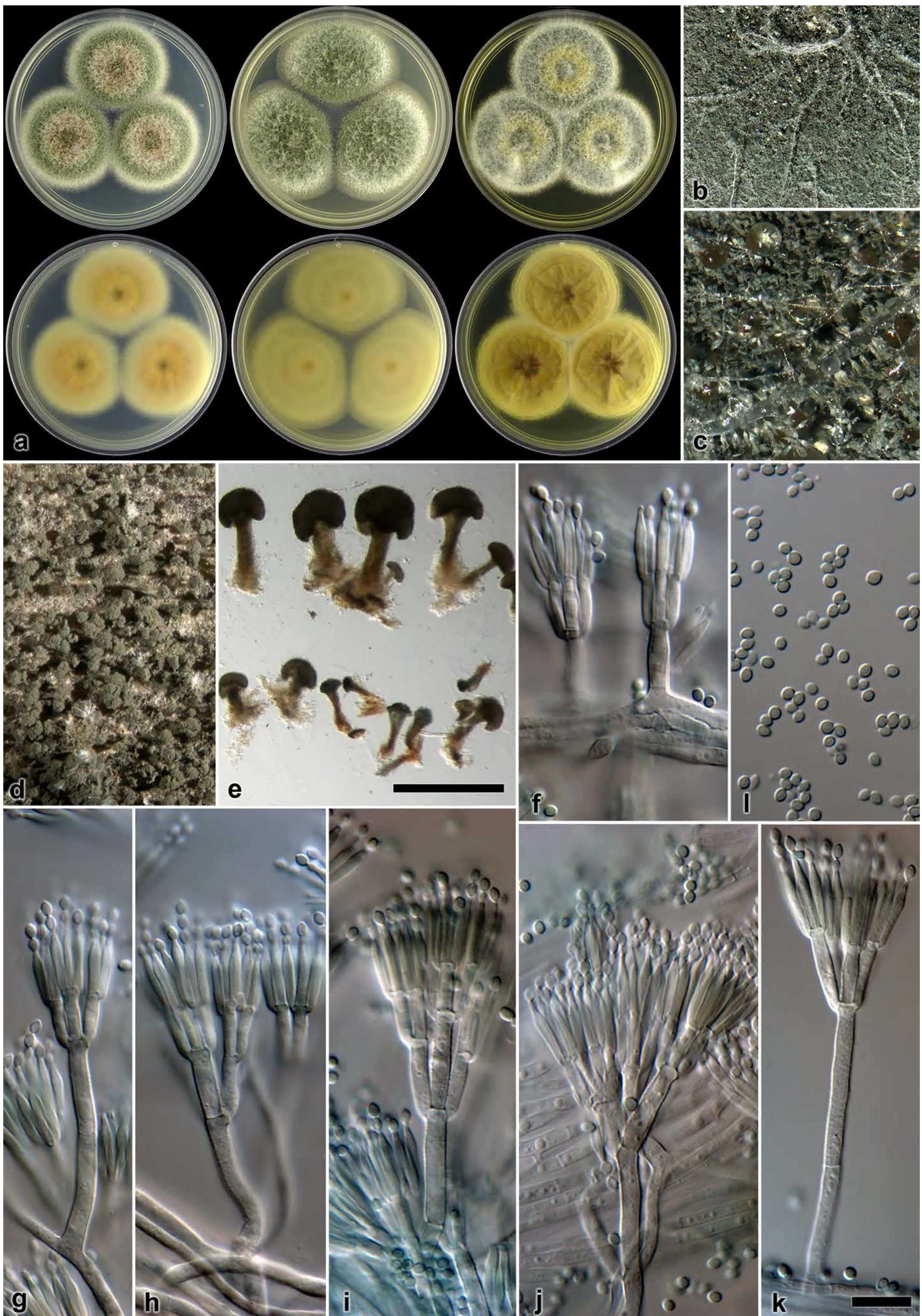

PLATE 21. *Talaromyces ramulosus*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b, c Funicles on MEA. d, e Synnemata produced on MEA after prolonged incubation. f–k. Conidiophores. l. Conidia (— Scale bar in e = 500 µm; — Scale bar in k = 10 µm, applies to f–l).

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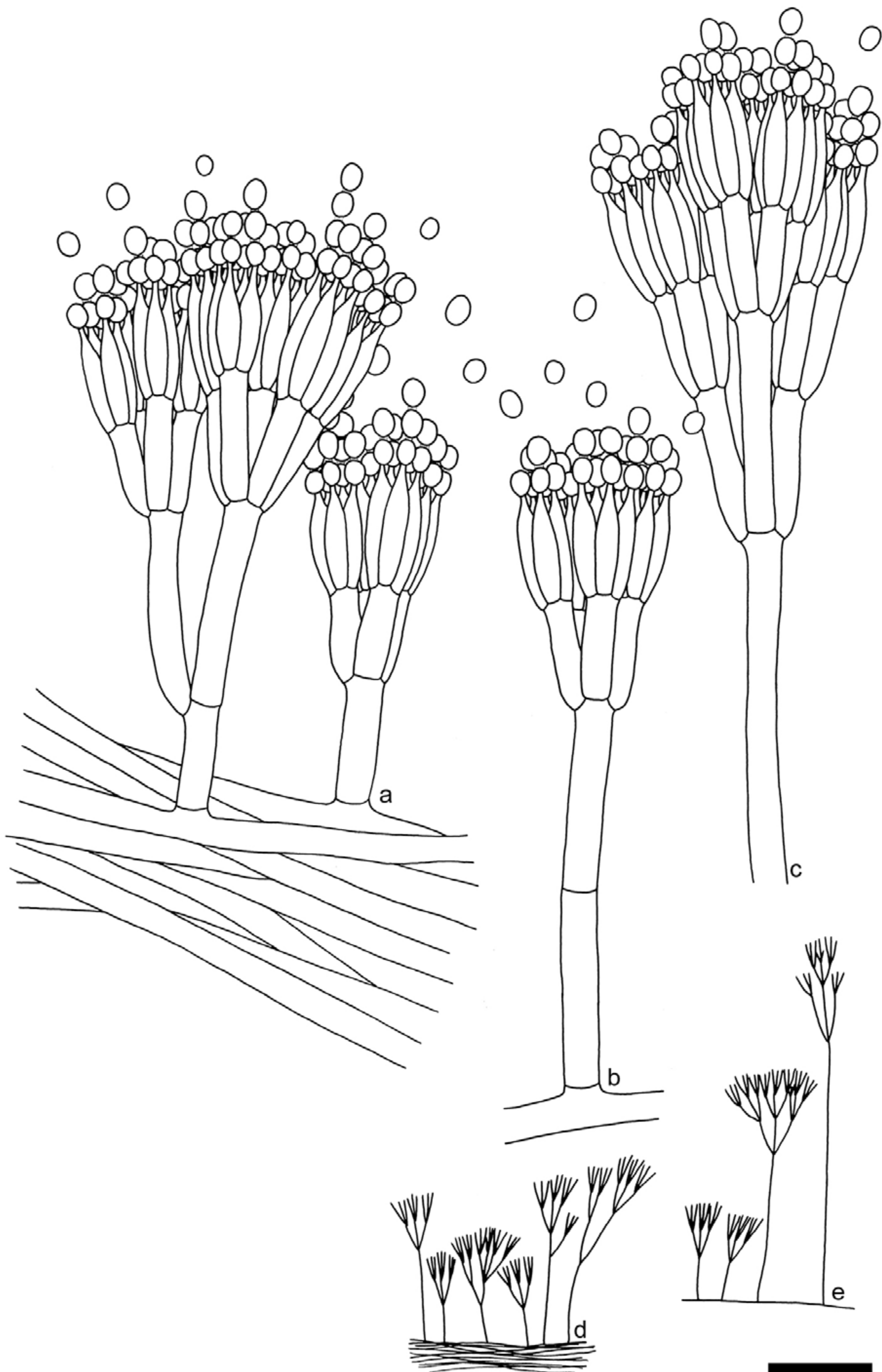

PLATE 22. Line drawing of *Talaromyces ramulosus*. a–c. Conidiophores (— Scale bar = 10 μm). d, e. Conidiophore branching (— Scale bar = 50 μm).

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Talaromyces solicola Visagie & Jacobs

PLATES 23, 24, 31l

Persoonia 28: 19. 2012.

MYCOBANK: MB564328
TYPE: PREM60037 (herbarium) = CV2800 = DAOM241015
TYPE ISOLATED FROM: Soil, Malmesbury, South Africa
SPECIMENS EXAMINED: CV2800, CV2801

Macromorphology — CYA, 25 °C, 7d: Colonies 3 mm diam, sometimes only germination, low, plane, loose; texture floccose; margins low, irregular, mycelia white; sporulation absent to sparse, conidia en masse white when present; clear to orange exudate produced, soluble pigment absent, reverse pale white.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 4–6 mm, low, plain, having a greyish yellow (4B4) color, no sporulation; exudate absent, soluble pigment absent, reverse pigmentation pale yellow (4A3).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: 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; sporulation 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, 7d: Colonies 8–10 mm diam, dense; texture floccose; margins low, irregular, mycelia white; sporulation 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, 7d: No germination.

CREA, 25 °C, 7d: Colonies 2–3 mm, no acid production.

Micromorphology — Conidiophores biverticillate; stipes smooth walled, 90–230 × 2.5–3.5 µm; metulae 5–8, sometimes up to 10, appressed, 25–65° [34±9.3°], 8.5–11 × 2.5–3.5 [9.7±0.1 × 3±0.1] µm; phialides acerose, 3–4 per metula, 9–11 × 2–2.5 [10.1±0.4 × 2.4±0.2] µm; conidia verrucose, subspheroidal, 2–3.5 × 2–2.5 [2.9±0.2 × 2.4±0.2] µm, average width/length = 0.83±0.07, n = 50.

Notes — A species previously described from Fynbos soil (Visagie & Jacobs 2012). *Talaromyces solicola* typically struggles to grow on CYA. On MEA though, it produces colonies with dark green-pigmented conidia. It is closely related to other species producing red colors in mycelia or colony reverses (FIGURES 1, 2). It resolved in a clade together with *T. erythromellis* and *T. albobiverticillius* as closest relatives. The verrucose, subspheroid conidia of the Fynbos species distinguish it from the latter two, which produce smooth walled conidia.

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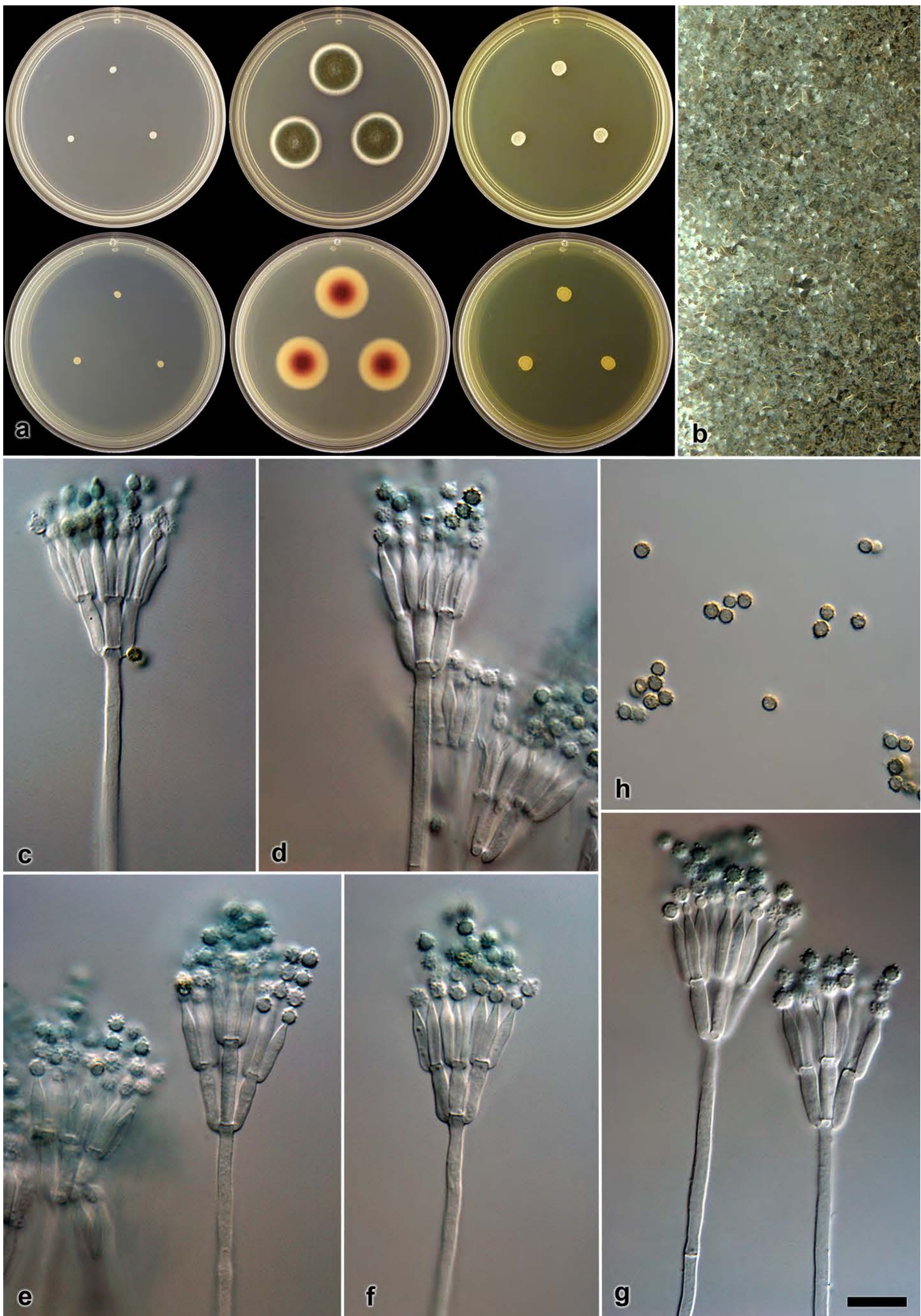

PLATE 23. *Talaromyces solicola*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–g. Conidiophores. h. Conidia (— Scale bar in g = 10 µm, applies to c–h). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 21.

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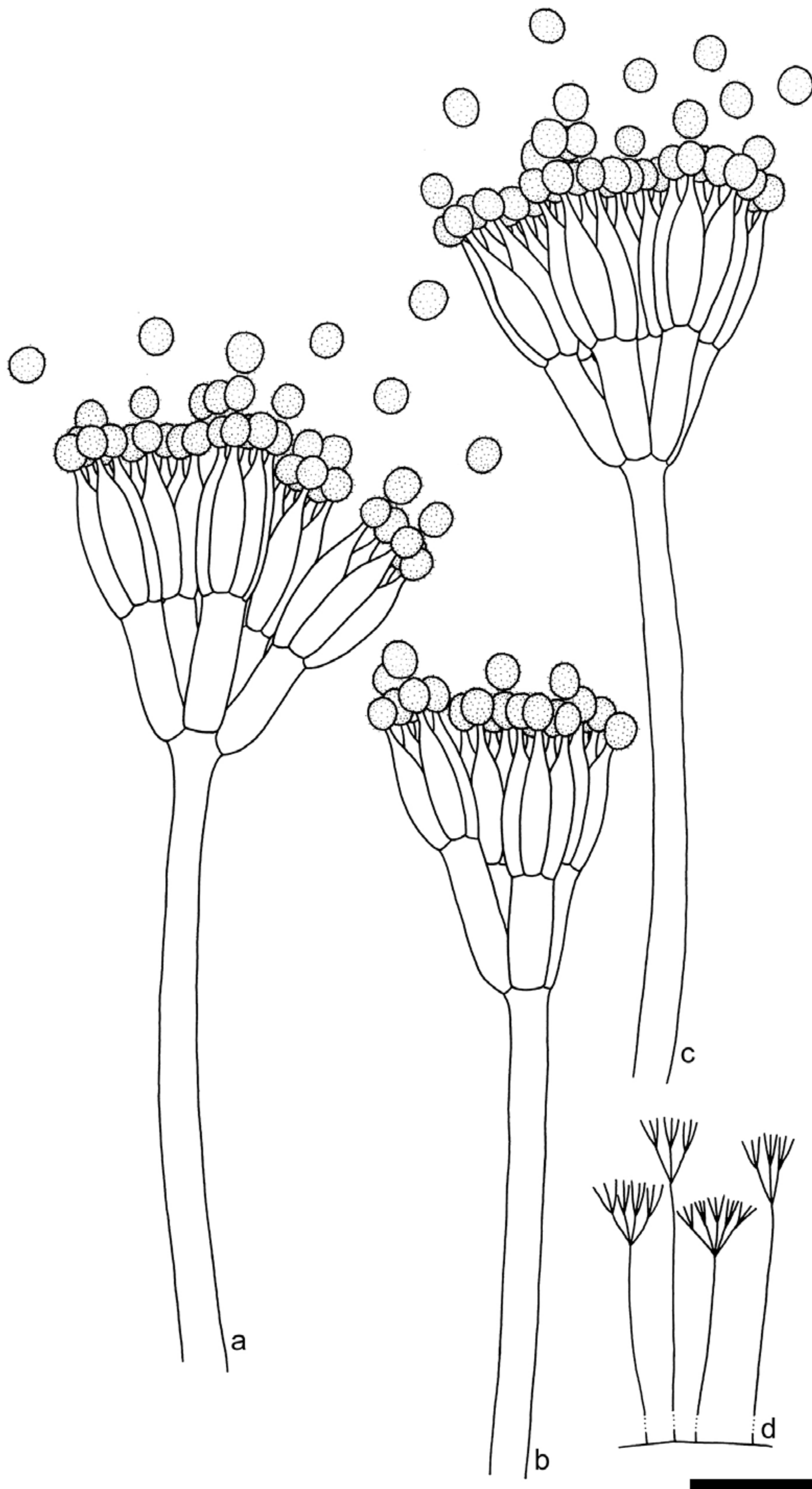

PLATE 24. Line drawing of *Talaromyces solicola*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm). Plate reproduced from Visagie & Jacobs, 2012. Persoonia 28: 20.

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Talaromyces stellenbossiensis Visagie **prov. nom.** PLATES 25, 26, 31m

ETYMOLOGY: Latin: *stellenbossiensis*: referring to the Stellenbosch collection site from where the species was isolated.
EX-TYPE: CV104 = DTO181A2 = KAS 3966 = DAOM 241021
TYPE ISOLATED FROM: Soil, Stellenbosch, South Africa, S33°56'47; E18°52'49
ADDITIONAL SPECIMENS EXAMINED: CV103, CV2809, CV2810

Macromorphology — CYA, 25 °C, 7d: Colonies 37–41 mm, low to moderately deep, plane, colonies often having greyish red (7B4); margins low, narrow (1–2 mm), entire; mycelia white and yellow; texture velutinous and floccose, funicles present; sporulation moderately dense at centre, sparse elsewhere, conidia en masse (28F8–29F8); exudate absent, soluble pigment absent, reverse pigmentation greyish red (9C5) and dark brown (9F6) and pale orange to orange grey (6A3–6B3) and dull yellow (3B4) areas.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 42–52 mm, low, lightly radially sulcate, having colors ranging from greyish purple to greyish red to greyish orange depending on isolate in non-sporulating regions; margins low, wide (3–4 mm), entire; mycelia yellow in some regions, but mostly white; texture velutinous, floccose and funiculose; sporulation sparse to moderately dense, conidia en masse greyish to dark green (28D7–28F7); exudate minute clear, soluble pigment absent, reverse pigmentation showing isolate to isolate variation, centrally brown (5E8) to yellowish brown (5D5), elsewhere orange white (5A2), brownish orange (5C6), greyish yellow (4C5), with some pastel red to greyish red (9A4–9C6) regions.

CYA, 37 °C, 7d: Colonies 30–40 mm, moderately deep, concentrically sulcate; margins low, narrow (1–2 mm), entire; mycelia white; texture floccose; sporulation sparse to moderately dense at colony centre, absent elsewhere, conidia en masse dull green (27E4–28E4); exudate absent, soluble pigment absent, reverse pigmentation dark grey (1F1) at centre, greyish yellow (4B4) and pale yellow (4A3) elsewhere, to yellowish brown (5D5) in some isolates.

MEA, 25 °C, 7d: Colonies 34–38 mm, low, plane; margins subsurface, wide (4–5 mm), entire; mycelia mostly yellow, with some white; texture some velutinous conidiophores near margin, elsewhere floccose with numerous overlaying funicles overlaying colony; sporulation moderately dense, conidia en masse (27E8–27F8–28F8); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (5C3–5C4) at centre, greyish green (1C3) elsewhere, with pale yellow (2A3) margin.

YES, 25 °C, 7d: Colonies 40–43 mm, moderately deep, lightly radially sulcate, colonies having a reddish pink color in non sporulating regions; margins low, narrow (1 mm), entire; mycelia yellow, white at margin; texture velutinous and floccose present, with overlaying funicles, numerous sterile mycelia also present; sporulation sparse to moderately dense, absent in some regions, conidia en masse dark green (28F8) at centre, greyish to deep green (28E7–28E8) elsewhere; exudate absent, soluble pigment absent, reverse pigmentation regions of violet brown (10F8) near centre, dull yellow (3B4) to yellowish white (2A2) elsewhere.

G25N, 25 °C, 7d: Colonies 3–5 mm, mostly no sporulation, sometimes sparse, conidia en masse dull green (25D4).

CREA, 25 °C, 7d: Colonies 15–20 mm, acid producesd at colony periphery.

Micromorphology — Conidiophores strictly biverticillate; stipes smooth walled, 150–350 × 3–4 µm; metulae 4–8, divergent, 61–150° [97±19.7°], 8–12 × 3–4.5 [9.3±0.8 × 3.7±0.24] µm; phialides ampulliform, tapering into very narrow neck, 3–5 per metula, 7–10 × 3–4 [8.6±0.6 × 3.3±0.2] µm; conidia heavily rough walled, spheroid, 2.5–3.5 × 2.5–3.5 [2.9±0.2 × 2.9±0.2] µm, average width/length = 0.95, n = 60.

Notes — Morphologically this species is closely related to *T. aculeatus* (CBS289.48) and *T. verruculosus* (CBS388.48). These species all produces ampulliform-like phialides that taper into very fine apical pores and produces heavy rough walled spheroid conidia. This phialide shape is rather unique for the species and is also observed in its morphological close relatives. The fast growth rate of *T. stellenbossiensis* on CYA at 25 °C (37–41 mm), however, distinguishes it from both *T. aculeatus* (15–25 mm) and *T. verruculosus* (20–30 mm). Pitt (1979) placed both *T. apiculatus* and *T. proteolyticus* as synonyms of *T. verruculosus*. Although morphologically similar, phylogenetically they are distinct species based on the Samson *et al.* (2011) phylogeny. Most notably, the faster growth rate of *T. stellenbossiensis* easily distinguishes it from related species.

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PLATE 25. *Talaromyces stellenbossiensis*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–g. Conidiophores. h. Conidia (— Scale bar in g = 10 µm, applies to c–h).

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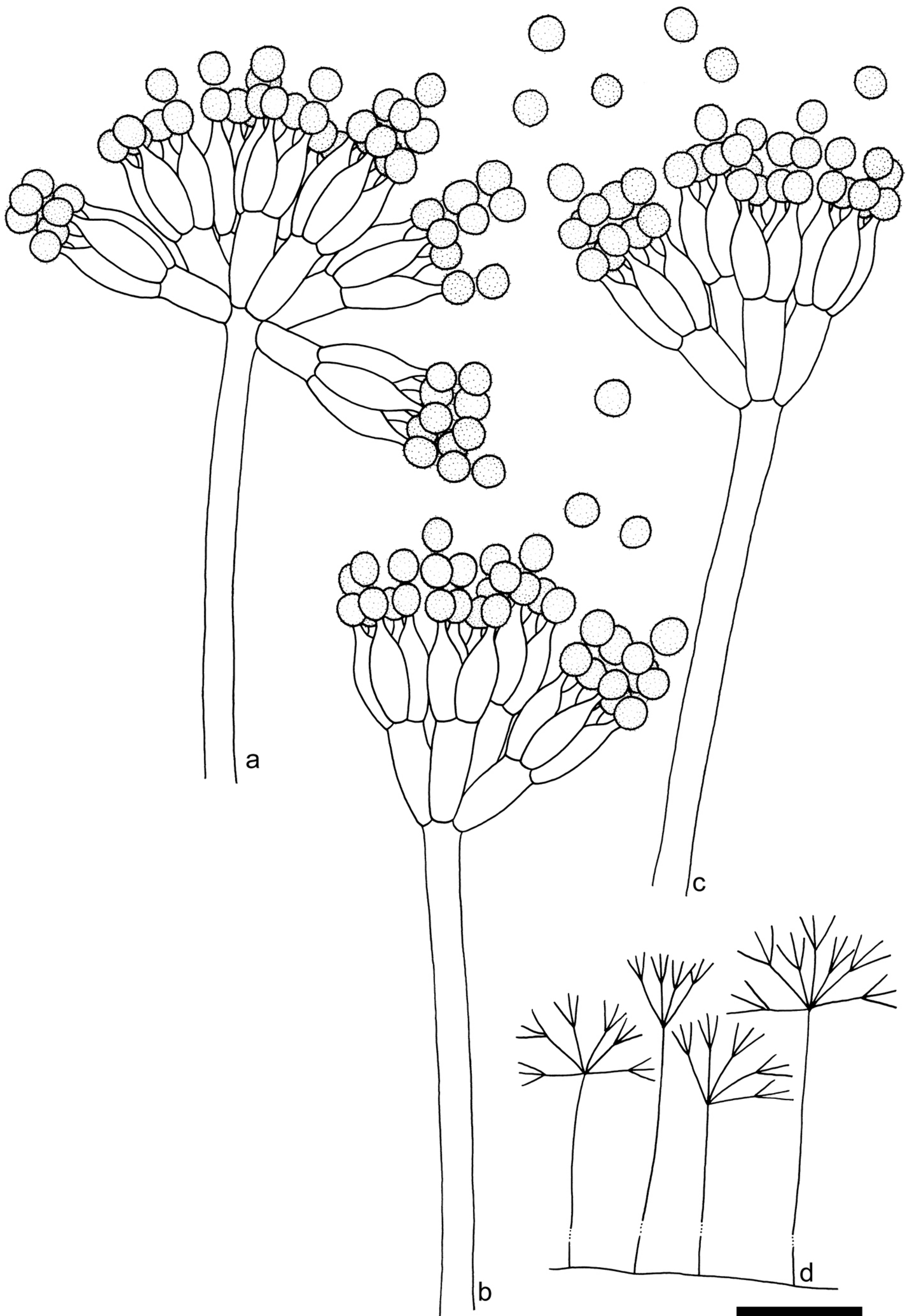

PLATE 26. Line drawing of *Talaromyces stellenbossiensis*. a–c. Conidiophores (— Scale bar = 10 μm). d. Conidiophore branching (— Scale bar = 50 μm).

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Talaromyces variabilis (Sopp) Samson, Yilmaz, Frisvad & Seifert PLATES 27, 28, 31n

Studies in Mycology 70: 177. 2011.

MYCOBANK: MB560676
BASIONYM: *Penicillium variabile* Sopp (Skr. Vidensk.-Selsk. Christinia, Math.-Naturrvidensk. KL 11: 169: 1912)
TYPE: CBS385.48 = ATCC10508 = IMI040040 = NRRL1048
TYPE ISOLATED FROM: Cocos fibre, Johannesburg, South Africa
SPECIMENS EXAMINED: CV175, CV507, CV603

Macromorphology — CYA, 25 °C, 7d: Colonies 17–23 mm, low, plane, slightly raised at centre; margins low, narrow (1–2 mm), entire; mycelia yellow near centre, white elsewhere; texture velutinous; sporulation moderately dense, conidia en masse dark green (26F8); exudate absent, soluble pigment yellowish not diffusing far off the margin, reverse pigmentation dark brown (6F8), fading into orange yellow (4B8), greyish green (29B4) near white margin.

CYA, 5 °C, 7d: No germination.

CYA, 30 °C, 7d: Colonies 23–28 mm, low, raised at centre, plane; margin low, narrow (1–2 mm), entire; mycelia yellow, white at margin; texture velutinous; sporulation dense, conidia en masse dull green (28D3) near centre, fading into greyish green (27E5–27E6) near margin; exudate absent, soluble pigment absent, reverse pigmentation olive (2E7) at centre, fading into brown (6D8), into greyish green (29B3–29B6).

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 20–25 mm, low, plane; margins low to somewhat subsurface, narrow (1–2 mm), entire; mycelia white; texture velutinous; sporulation dense, conidia en masse dark green (26F8); exudate absent, soluble pigment absent, reverse pigmentation greyish to dull green (29C3–29D3–30D5), with greenish grey (28B2) margin.

YES, 25 °C, 7d: Colonies 23–29 mm, low, raised at colony centre; margins low, narrow (1–2 mm), entire; mycelia mostly white, with yellow present in between; texture velutinous; sporulation dense, breaking of in crusts, conidia en masse dull to greyish green (25D3–25D5); exudate absent, soluble pigment absent, reverse pigmentation brown (7E8) at centre, fading into orange (6A7–6B7), then into pale green (25A3).

G25N, 25 °C, 7d: Colonies 6–9 mm, sporulation moderately dense, conidia en masse greyish green (26E5–26E6); reverse pigmentation greyish green (1C4) at centre, greyish green (27D6).

CREA, 25 °C, 7d: Colonies 12–15 mm, weak acid production close to colony periphery.

Micromorphology — Conidiophores biverticillate, minor proportion terverticillate and subterminal branches;

stipes smooth, 35–265 × 2.5–4 µm, branches 15–20 × 2.5–4 µm; metulae 6–9, divergent and appressed, 20–100° [50.4±22°], 9.5–15 × 3–4 [12.3±1.3 × 3.4±0.3] µm; phialides acerose, 3–5 per metula, 9.5–13 × 2–3.5 [10.9±0.8 × 2.9±0.3] µm; conidia strongly ellipsoidal, smooth walled, 2.5–4 × 1.5–2.5 [3.3±0.3 × 1.9±0.2] µm, very large conidia also present, then 6–9 × 3–3.5 µm, average width/length = 0.57±0.07, n = 100.

Notes — *Talaromyces variabilis* produces typical velutinous colonies dominated by dull to greyish green conidia on CYA and MEA and also grows better at 30 °C. Its most striking features are the commonly divergent conidiophores and strongly ellipsoid conidia that are 2.5–4(–9) µm in length. It is closely related to *T. sublevisporus*. However, this species is phylogenetically distinct from *T. sublevisporus* and was not compared morphologically (FIGURES 1, 4). Interestingly, ITS and RPB1 sequences (Samson *et al*. 2011) are identical between *T. variabilis* and *T. wortmannii* (CBS391.48). β-tubulin and Calmodulin (FIGURE 4) also suggest synonymy between the two species. Samson *et al*. (2011) also showed that *Penicillium concavorugulosum* is a possible synonym in this clade. This will form part of a future study.

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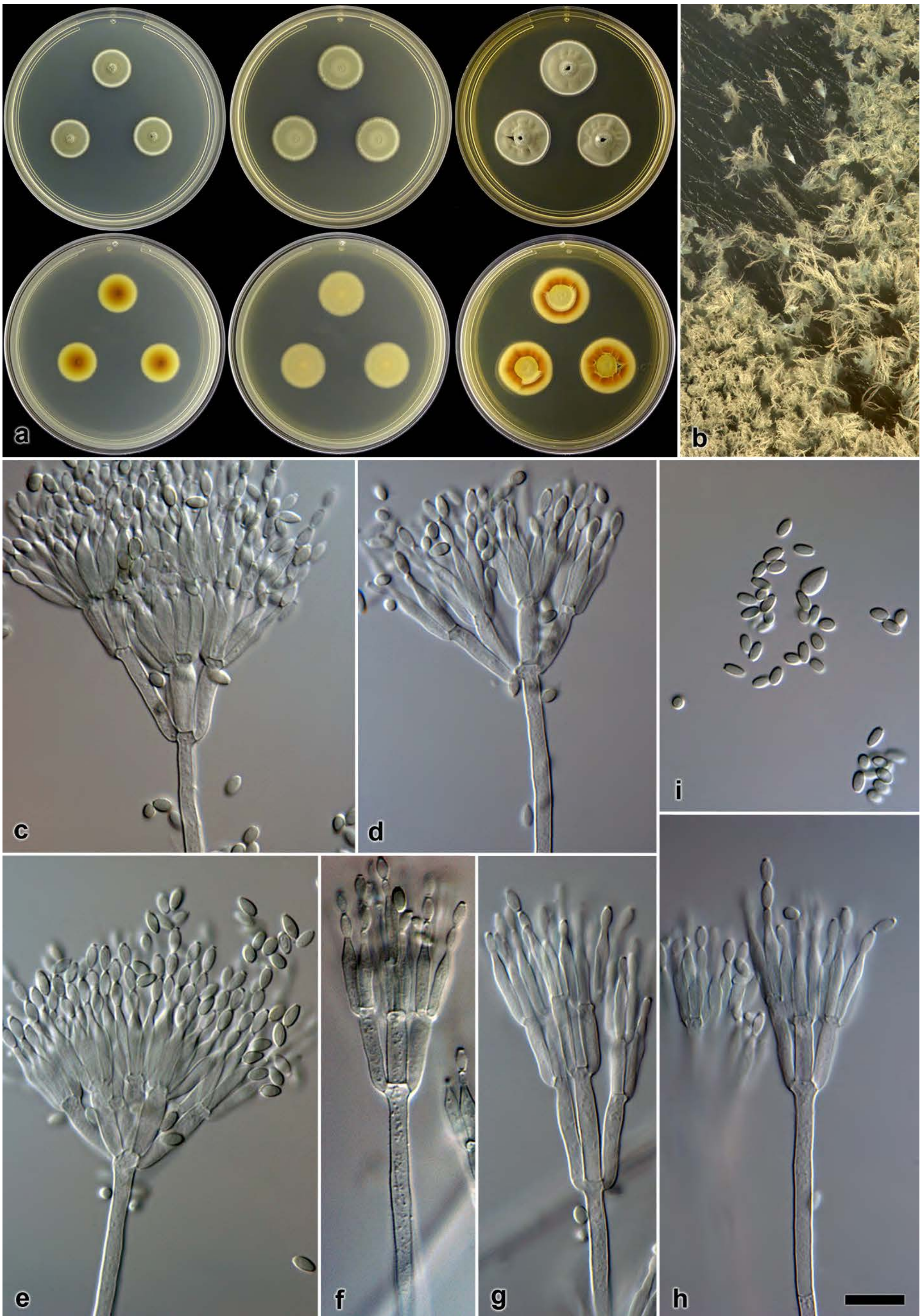

PLATE 27. *Talaromyces variabilis*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b. Texture on MEA. c–h. Conidiophores. i. Conidia (— Scale bar in h = 10 µm, applies to c–i).

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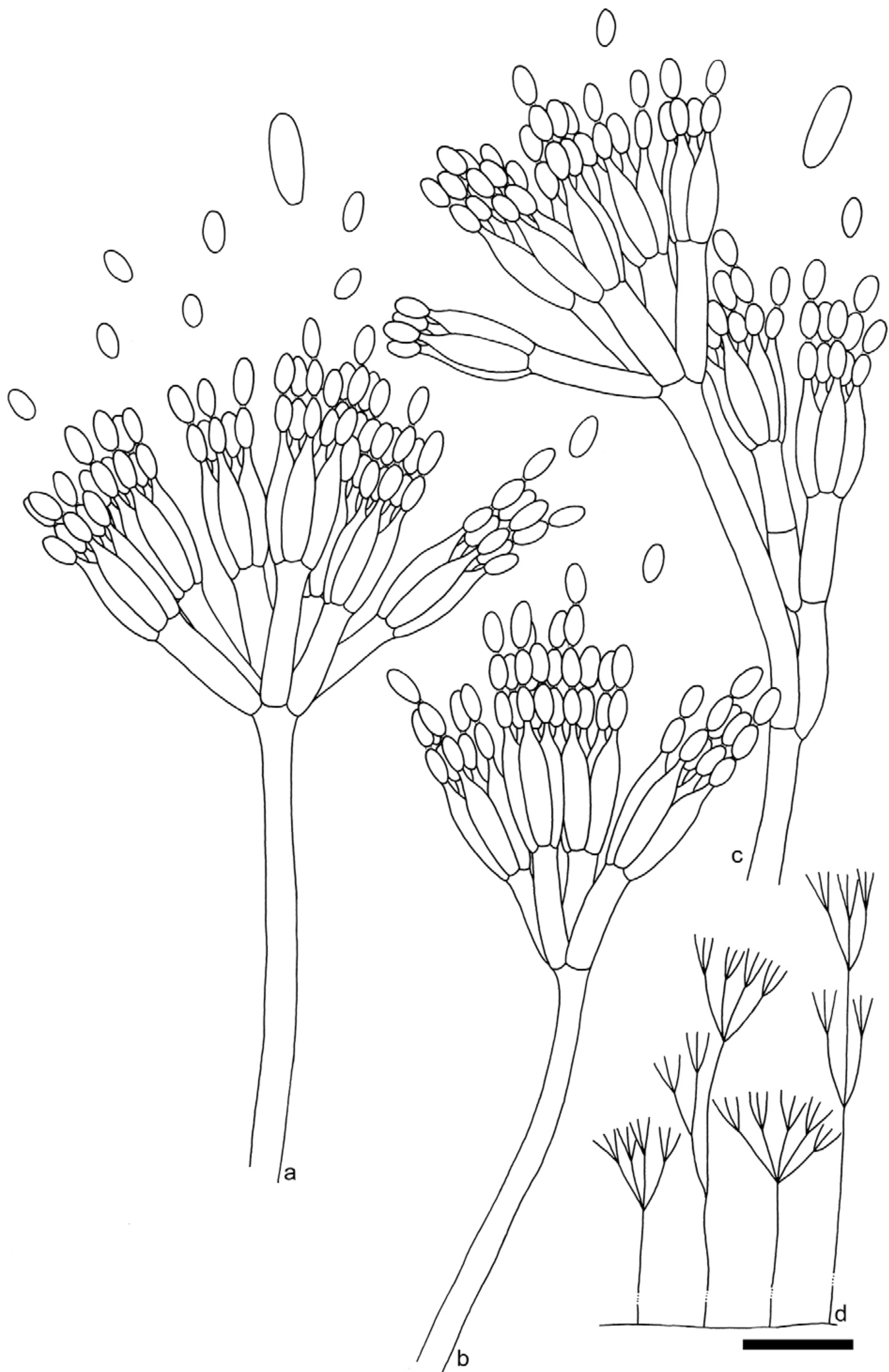

PLATE 28. Line drawing of *Talaromyces variabilis*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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Talaromyces vermiculicola Visagie **prov. nom.** PLATES 29, 30, 31o

ETYMOLOGY: Latin, *vermiculicola*: meaning resident on mites, in reference to strains only isolated from mites
EX-TYPE: CV1467 = DTO183B3 = KAS 4011 = DAOM 241025
ISOLATED FROM: Mite, *Protea repens* infructescence, Malmesbury, South Africa, S33°49'46; E18°35
ADDITIONAL SPECIMENS EXAMINED: CV1469, CV1503, CV1825, CV2466

Macromorphology — CYA, 25 °C, 7d: Colonies 6–9 mm, low, plane; margins low, very narrow (<1 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia en masse greyish green (25E5–25E6–25D6); exudate clear, soluble pigment absent, reverse pigmentation greyish yellow (3C4–3C6) at centre, yellowish white (3A2) near margin.

CYA, 5 °C, 7d: Germination.

CYA, 30 °C, 7d: Colonies 5–8 mm, low, plane; margins low, very narrow (<1 mm), entire; mycelia white; texture floccose and velutinous; sporulation moderately dense, absent in some isolates, conidia en masse (26F5) near centre fading into (26D5) near margin; exudate clear, soluble pigment absent, reverse pigmentation olive brown (4E8) near centre, fading into yellowish grey (4B2) margin.

CYA, 37 °C, 7d: No germination.

MEA, 25 °C, 7d: Colonies 12–16 mm, low, plane; margins low, narrow (1–2 mm), entire, consisting of sterile bright yellow mycelia, white also present; mycelia bright yellow; texture floccose; sporulation moderately dense, conidia en masse dark green (26F6–27F6), greyish green (27E5) in some isolates; exudate mostly absent, but clear when produced, soluble pigment absent, reverse pigmentation orange yellow to dark yellow (4B8–4C8) at centre, fading into greyish yellow (4B5) to pale yellow (4A3) at margin.

YES, 25 °C, 7d: Colonies 10–14 mm, low to slightly raised at centre, craterform; margins low, narrow (1–2 mm), entire; mycelia white; texture velutinous and floccose; sporulation moderately dense, conidia en masse greyish green (25E5–25E6–25D6); exudate clear, soluble pigment absent, reverse pigmentation greyish yellow (3C4–3C6) at centre, yellowish white (3A2) near margin.

G25N, 25 °C, 7d: Colonies 5–8 mm; mycelia inconspicuously yellow; mostly moderate sporulation, sometimes no sporulation, conidia en masse greyish green (25F5), reverse pigmentation dull green (29D4).

CREA, 25 °C, 7d: Colonies 4–6 mm, no acid production.

Micromorphology — Conidiophores biverticillate, with minor proportion; stipes smooth walled, 40–160 × 2–3 µm, branches 2–3, 14–22 × 2–3 µm; metulae 3–5, divergent, 30–100° [58±17.6°], 7.5–12 × 2–3 [9.5±1.2 × 2.6±0.2] µm; phialides acerose-ampulliform, 3–5 per metula, 6.5–9.5 × 2–3 [8.34±0.6 × 2.4±0.2] µm; conidia rough walled, sometimes forming ridges, ellipsoidal, ranging in size from 2.5–5.5 × 2–3 [3±0.5 × 2.2±0.2] µm, average width/length = 0.74±0.1, n = 87.

Notes — Similar to *T. infrolivacea*, this species belongs to the larger *T. rugulosus* species complex, which is currently under review. Morphological differences include lightly colored conidia and floccose colonies produced on MEA by *T. infrolivacea*, compared to the dark green conidia and velutinous colonies produced by the *T. rugulosus* species complex. *Talaromyces vermiculicola* has *P. infrolivacea*, described in this paper, as its closest relative (FIGURES 1, 4). Its more restricted growth on CYA, the lack of dark olive colored reverse pigmentation and funicles produced on all media distinguish *T. vermiculicola* from *T. infrolivacea*. Phylogenetically the two new species do not match any strains that have previously been identified to belong in this clade and are thus considered to represent a novel species.

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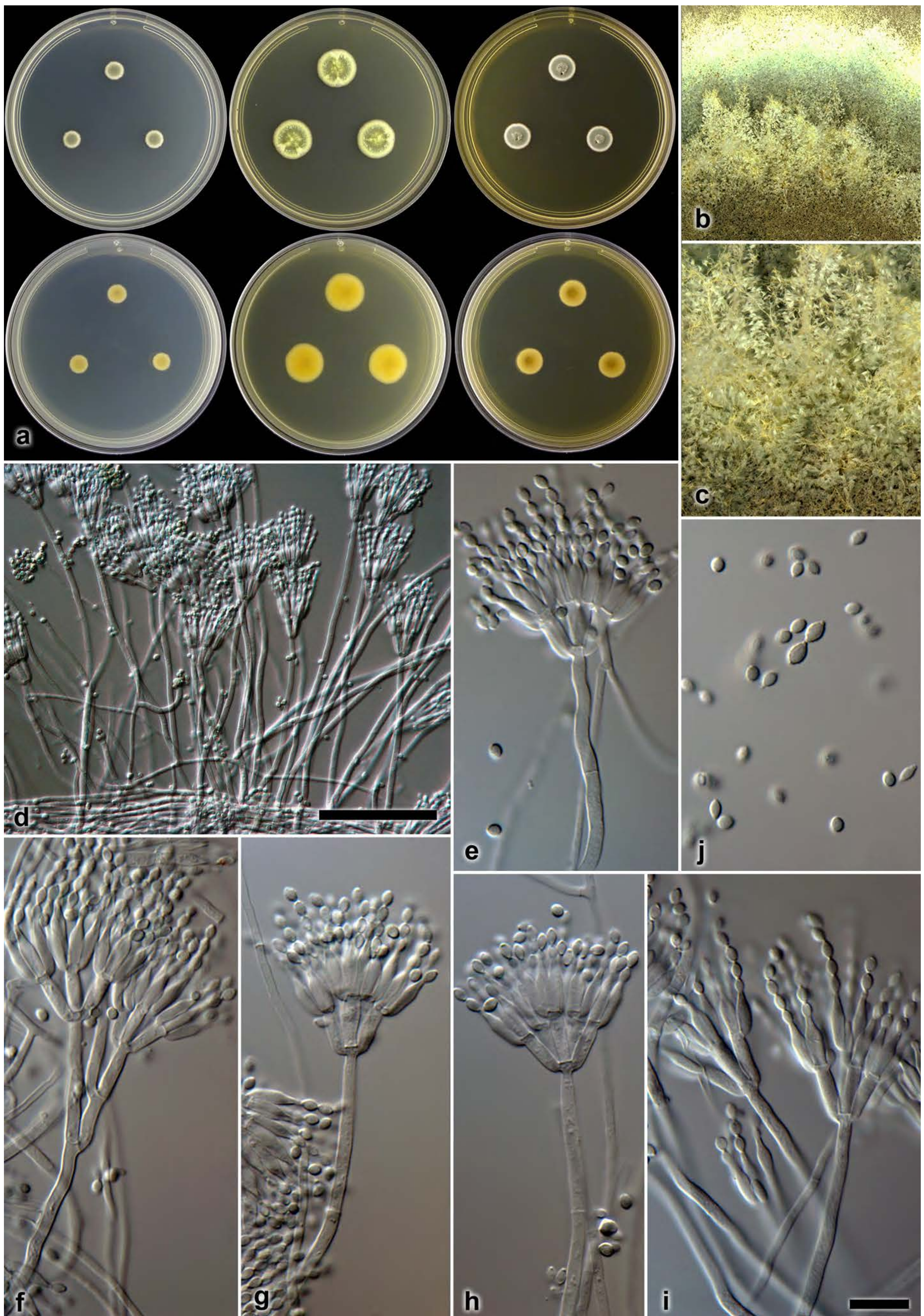

PLATE 29. *Talaromyces vermiculicola*. a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse). b–c. Texture on MEA. d–i. Conidiophores. j. Conidia (— Scale bar in d = 50 µm; — Scale bar in i = 10 µm, applies to e–j).

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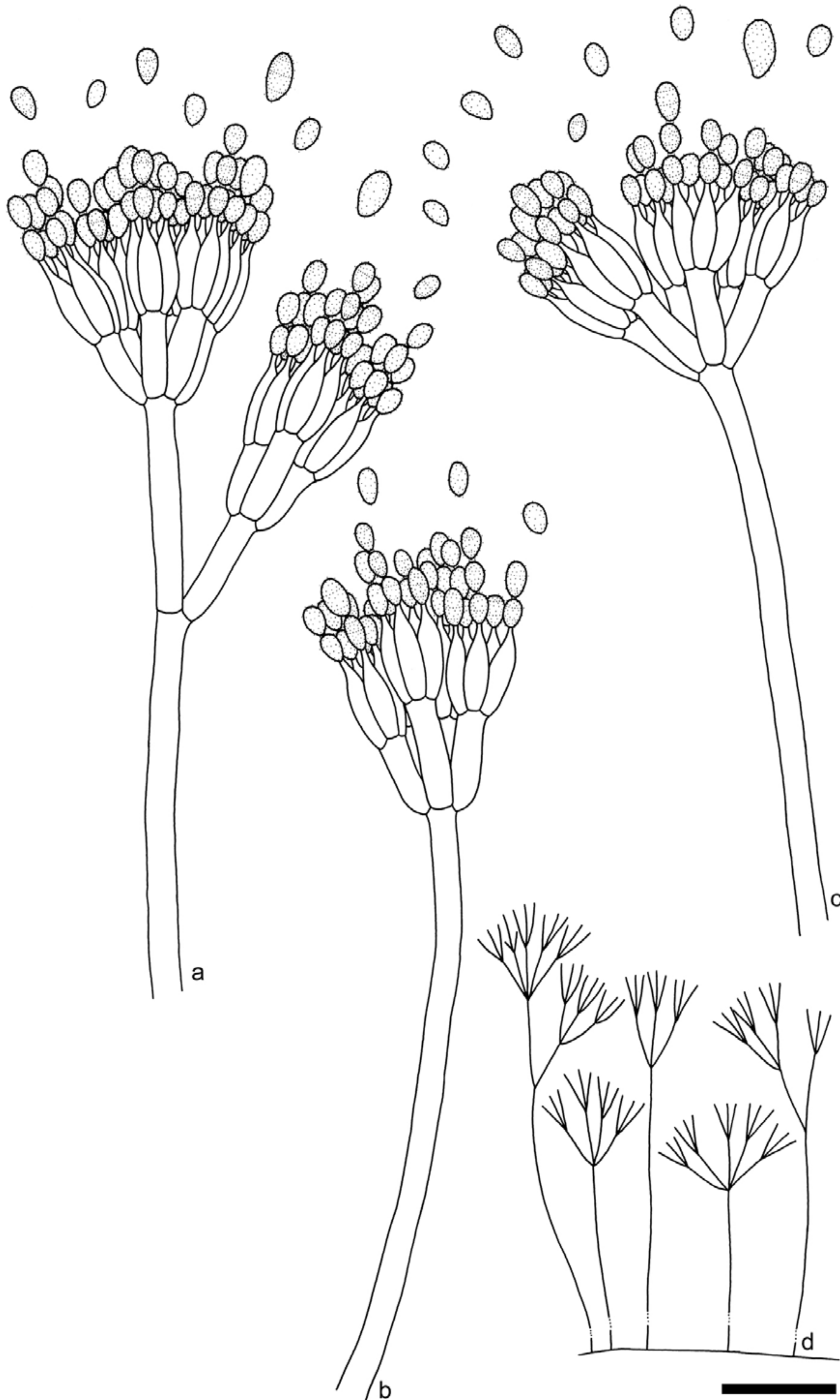

PLATE 30. Line drawing of *Talaromyces vermiculicola*. a–c. Conidiophores (— Scale bar = 10 µm). d. Conidiophore branching (— Scale bar = 50 µm).

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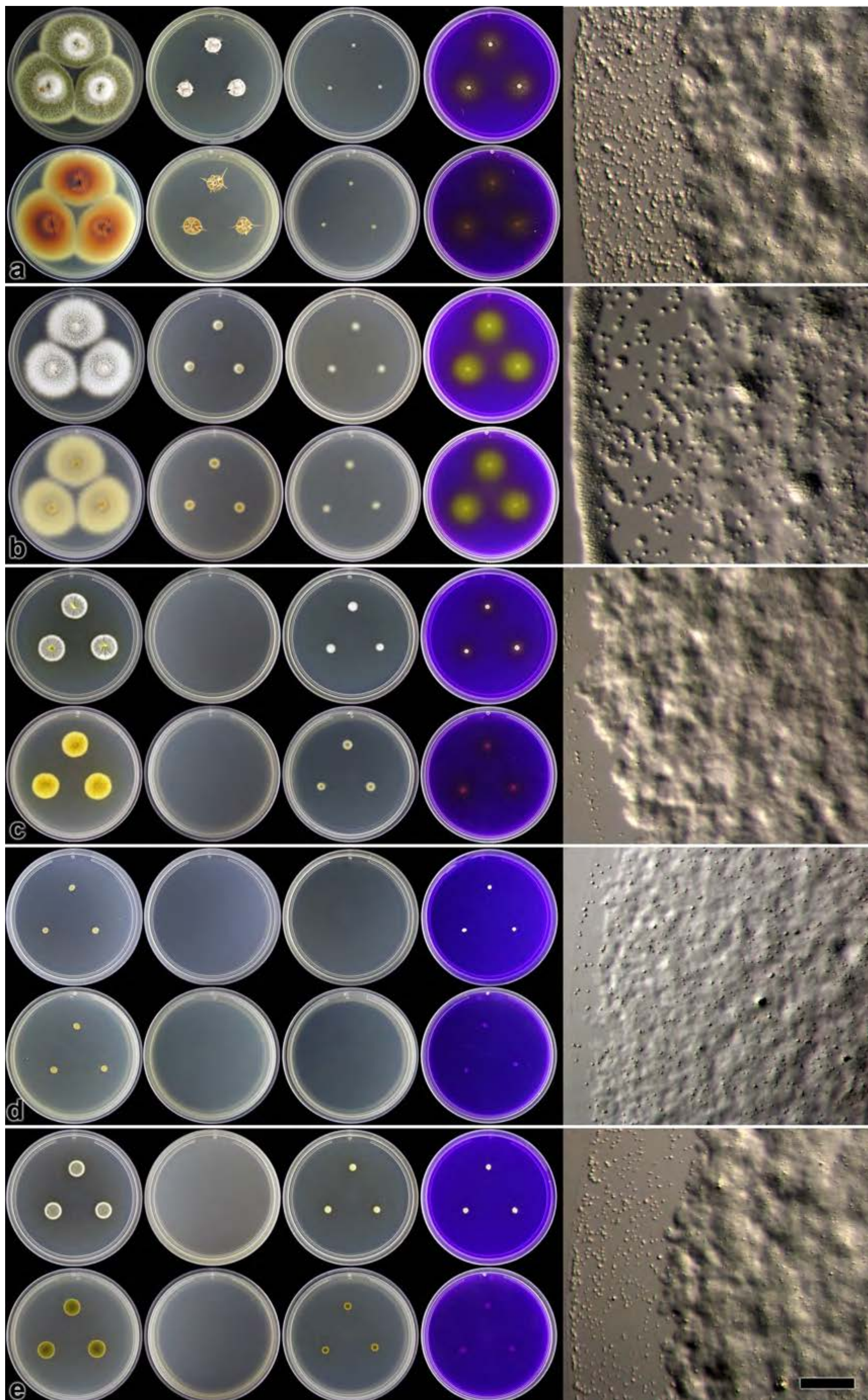

PLATE 31A. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). a. *T. amestolkiae*. b. *T. chloroloma*. c. *T. crassa*. d. *T. dendriticus*. e. *T. infrolivacea*.

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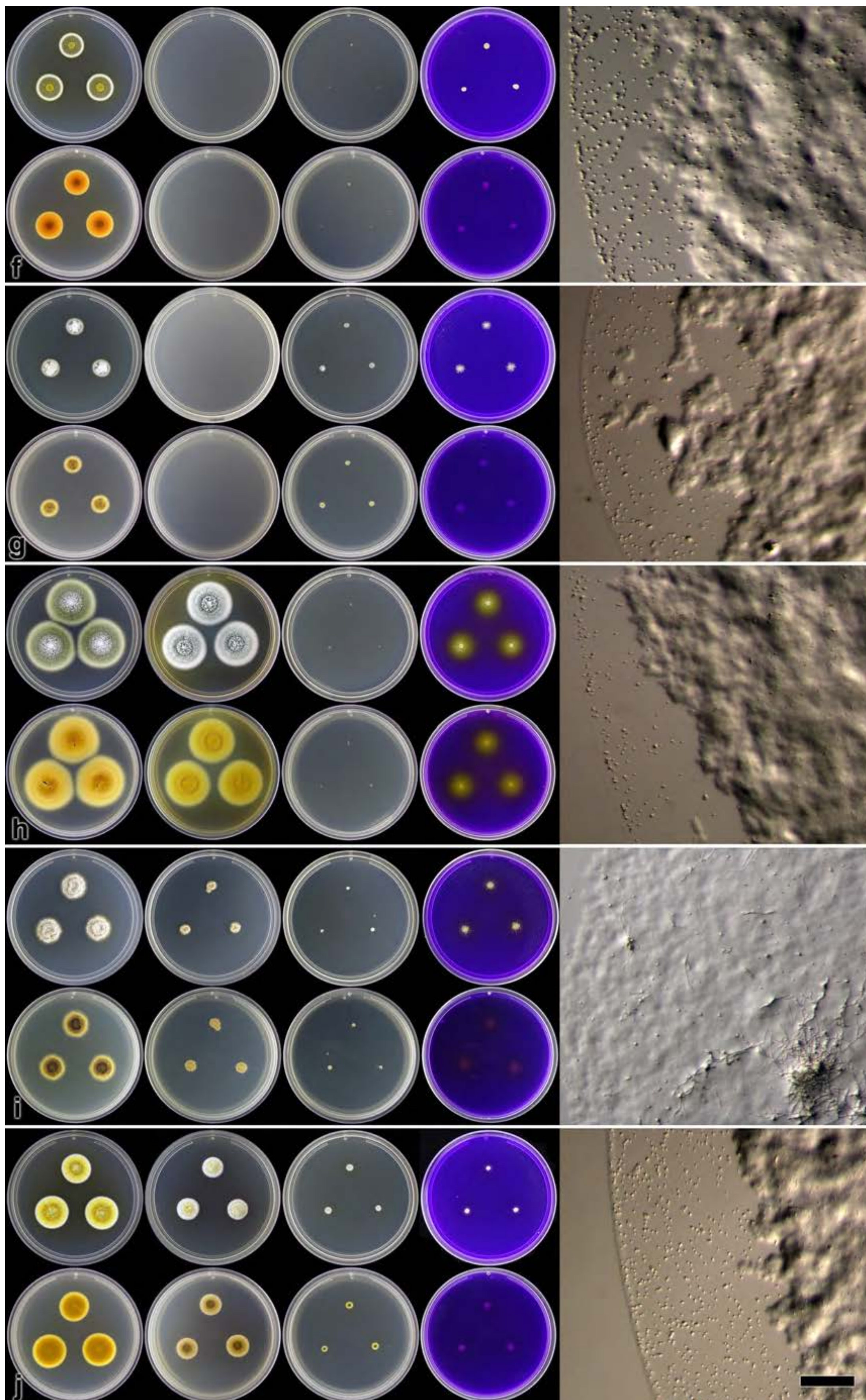

PLATE 31B. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). f. *T. minioluteus*. g. *T. parvaurantica*. h. *T. pinophilus* i. *T. ptychoconidium*. j. *T. radicus*.

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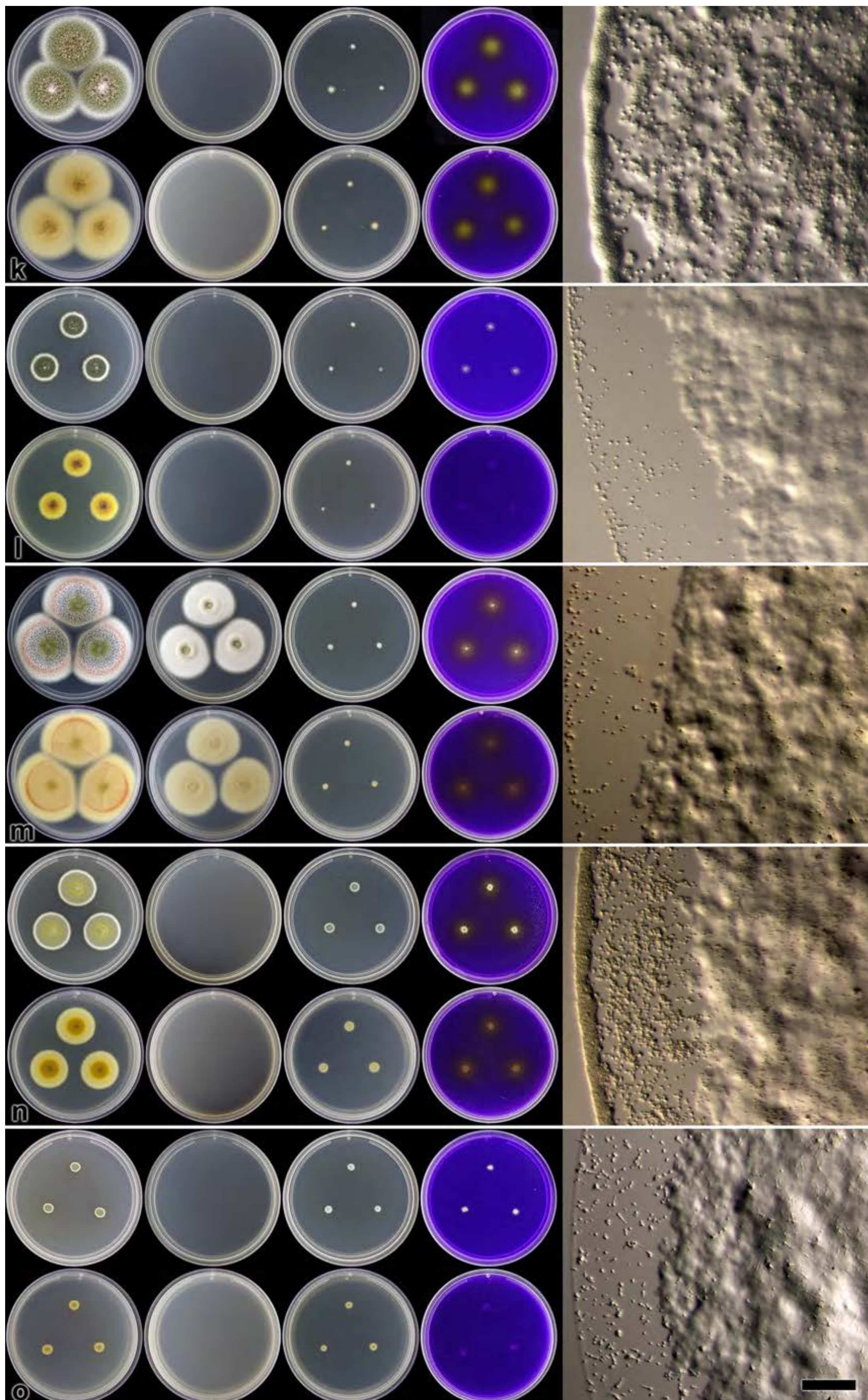

PLATE 31C. Additional macromorphological characters, from left to right (top row = obverse, bottom row = reverse): CYA at 30 °C, CYA at 37 °C, G25N, CREA, CYA at 5 °C (— Scale bar = 100 µm). k. *T. ramulosus*. l. *T. solicola*. m. *T. stellenbossiensis*. n. *T. variabilis*. o. *T. vermiculicola*.

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Key to *Talaromyces* species occurring in the Fynbos region

1. Colonies CYA (25°C) <20mm .... 2.
1. Colonies CYA (25°C) >20mm .... 11.
2. Colonies MEA >30mm .... ***T. dendriticus***
2. Colonies MEA <30mm .... 3.
3. Colonies CYA (25°C) <6mm .... ***T. solicola***
3. Colonies CYA (25°C) >6mm .... 4.
4. Growth on CYA (37°C) .... 5.
4. No growth on CYA (37°C) .... 6.
5. Conidia 3–5µm; Stipes <100µm; Conidiophores borne close to surface and typically covered by slimy substance .... ***T. ptychoconidium***
5. Conidia 2–3µm; Stipes typically >100µm; Conidiophore borne from aerial hyphae, slime absent and colonies fluffy .... ***T. radicus***
6. Conidia smooth .... 7.
6. Conidia rough .... 9.
7. Conidia strongly ellipsoid, 2.5–4 × 1.5–2.5 µm; Metulae commonly at angles close to 100° .... ***T. variabilis***
7. Conidia and conidiophores not as above .... 8.
8. Metulae almost parallel to each other, strictly biverticillate; red pigmentation in colony reverse on CYA and YES; no acid production on CREA .... ***T. minioluteus***
8. Metulae more divergent, sub-terminal branching common; red pigmentation lacking; weak acid production on CREA .... ***T. crassa***
9. Colonies CYA (30°C) <10mm; overlaying funicles dominate colony appearance .... ***T. vermiculicola***
9. Colonies CYA (30°C) >10mm; funicles absent .... 10.
10. Reverse color dark olive on CYA, MEA, YES .... ***T. infrolivace***
10. Reverse color pale to orange on CYA, MEA, YES .... ***T. parvaurantica***
11. Colonies MEA <30mm .... ***T. variabilis***
11. Colonies MEA >30mm .... 12.
12. Colonies CYA (37°C) >30mm .... 13.
12. Colonies CYA (37°C) <30mm .... 14.
13. Conidia smooth walled, subspheroid to ellipsoid .... ***T. pinophilus***
13. Conidia heavy rough walled, spheroid .... ***T. stellenbossiensis***
14. Colonies CYA (25°C) <30mm .... ***T. amestolkiae***
14. Colonies CYA (25°C) >30mm .... 15.
15. Mycelia white; synnema length >250µm; phialide length <8.5µm .... ***T. chloroloma***
15. Mycelia yellow, pink, white; synnema length <250µm; phialide length >8.5µm .... ***T. ramulosus***

Discussion

The movement towards single name nomenclature (Hawksworth *et al*. 2011, Norvell *et al*. 2011) will necessitate numerous name changes across Kingdom Fungi. These changes were also the catalyst for redefining *Talaromyces* s.str. in order to accommodate species that traditionally belonged to *Penicillium* subgenus *Biverticillium.* Thus *Talaromyces* now includes species that produce soft, yellow to cream to pink cleistothecia (Houbraken & Samson 2011, Samson *et al*. 2011) and have symmetrical biverticillate conidiophores, which ends in typical narrow phialides. However, a couple of species (eg. *T. verruculosus*) do produce ampulliform-like phialides that end in fine apical openings. Also, species often have a metula to phialide ratio of 1–1.2 (Pitt 1979). As a result, Samson *et al*. (2011) transferred most *Penicillium* subgenus *Biverticillium* spp. into *Talaromyces* as new combinations. Samson *et al*. (2011), however, did not transfer species with unresolved taxonomies. These species are still known by their old anamorph names although they should reside in *Talaromyces* (Samson *et al*. 2011). This should be addressed in future studies.

The current Fynbos *Penicillium* project resulted in the isolation of 76 *Penicillium* sensu lato species (Chapter 2). Fifteen of these were found to belong to *Talaromyces*. The recent Samson *et al*. (2011) paper published two gene phylogenies, which included ITS data for almost all ex-type cultures in the genus. This dataset greatly aided identifications, even though ITS often do not have enough variation to distinguish between species. Alternative genes were thus also sequenced in the study. Aligning β-tubulin and Calmodulin datasets across the entire genus, resulted in a large number of gaps in the alignments. This results in the loss of phylogenetic information. Thus, for a stronger phylogeny, the genus was divided into five clades based on the ITS phylogeny.

The ITS phylogeny had a similar tree topology as Samson *et al*. (2012), although they identified only

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three clades. Morphological characters and branch support in the phylogenies, however, support the five clades. Phenotypic characters consistent for species in these clades were difficult to identify. Members of CLADE 1 in general display fast growth with many species that produce yellow floccose mycelia. These characters are, however, not consistent in all species. CLADE 2 contains species that all produce synnemata after prolonged incubation. Interestingly, there are reports that most of these species have in some way an association with insects or mites (Seifert *et al*. 2004, Visagie & Jacobs 2012). Species from CLADE 5 typically shows restricted growth, especially on CYA. These phenotypic characters are, however, only in general and need further investigation.

Based on the five clades, detailed phylogenies of the β-tubulin and Calmodulin regions were included. These resolved Fynbos strains in consistent coherent clades, which in many cases were considered to represent species. The species identified phylogenetically, corresponded well with observed morphological differences. The β-tubulin phylogenies were thus used to confirm morphological observations. The Calmodulin dataset is currently not complete for ex-type cultures. As such, the data presented here will serve as reference for future studies and will aid as barcode-like reference sequences for the Fynbos species. Five new species were isolated and described in this paper. Strains displayed unique morphological characters, not observed in previously described *Talaromyces* species. The novelty of these species was confirmed for all genes studied. ITS, the official DNA barcode for fungi (Schoch *et al*. 2012), was able to distinguish between the Fynbos *Talaromyces* species. However, it is advisable that β-tubulin and Calmodulin be included for confirmation of identifications, when considering species for the rest of the genus.

This paper follow on from previous papers by Visagie *et al*. (2009) and Visagie & Jacobs (2012), which described four new *Talaromyces* species isolated from Fynbos soil. The survey has thus reported on the isolation of nine new species and six previously described ones. Schutte (1992) published a checklist for *Penicillium* s.l., *Eupenicillium* and *Talaromyces* species reported in South African literature. Many of these cultures have been deposited as dried specimens in the PPRI and PREM. With the exception of *T. duclauxii* (= *P. duclauxii*), the identity of these cultures could not be confirmed. This was mainly because the herbarium materials were badly deteriorated and conidiophores often not observed in the specimens.

Misidentifications are not uncommon in *Talaromyces*. Frisvad (1989) reported on misidentification of isolates, which included strains from South Africa. The current study isolated *T. pinophilus, T. dendriticum, T. minioluteum* and *T. variabilis,* which have also previously been recorded in South African literature (Schutte 1992). *Talaromyces radicus* is a new report for South Africa, a species described from wheat roots from Australia (Hocking *et al*. 1998). Species reported in South African literature also include *T. aculeatus* (=*P. aculeatum*), *T. diversus* (=*P. diversum*), *T. funiculosus* (=*P. funiculosum*), *T. islandicus* (=*P. islandicum*), *T. piceus* (= *P. piceum*), *T. purpurogenus* (= *P. purpurogenum*), *T. rugulosus* (=*P. rugulosum*), *T. verruculosus* (=*P. verruculosum*), *T. flavus*, *T. trachyspermus* and *T. wortmannii*. Not being able to confirm these identifications is unfortunate, although ex-neotype material for *T. islandicus* and *T. variabilis* originates from South Africa.

It is, however, worrying how the new species recently described from Fynbos, could easily be misidentified for above-mentioned species. For instance, the identification keys provided by Raper & Thom (1949) and Pitt (1979) will key *Talaromyces ramulosus* as *T. funiculosus, T. stellenbossiensis* as *T. verruculosus*, *T. solicola* as *T. diversus* and both *T. infrolivacea* or *T. vermiculicola* as *T. rugulosus*. This makes comparison of results from this study to previous studies very difficult, since many studies probably had misidentified strains.

Although past identification of *Talaromyces* species have been problematic, the polyphasic approach used in modern taxonomy should provide the basis from where misidentifications are restricted. For instance, many of the Fynbos species were also isolated during other projects that focused on indoor environments (Borchardt, unpublished data) and apple orchards (Van der Walt *et al*. 2011). This paper will thus not only serve as basis for identification of species from the Fynbos, but will also be useful for other studies in the Western Cape area. Also, from this project it has become clear that there are more *Talaromyces* species to be found in the diverse Fynbos region (Chapter 4). Thus far, only three sites have been sampled, each representing a different Fynbos type (Mucina & Rutherford 2006). It is expected that when starting to explore different Fynbos types, even more species will be found.

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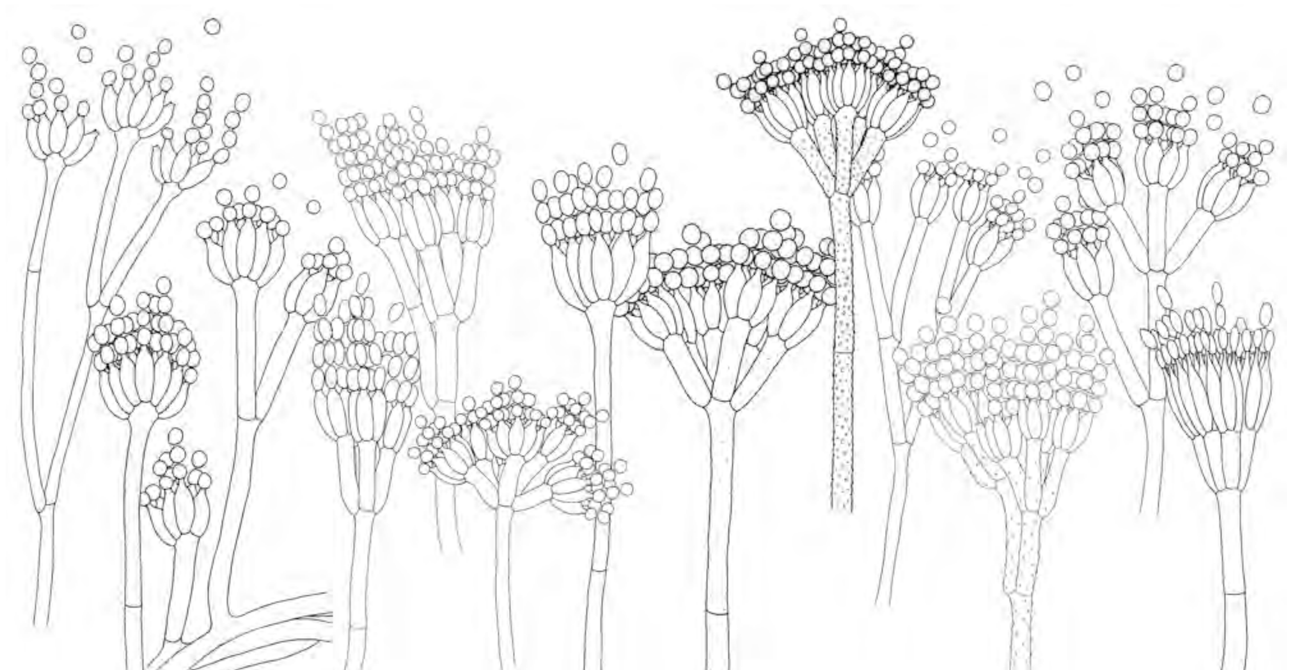
CHAPTER 4

Biological and environmental characters that influence the diversity and distribution of *Penicillium* and *Talaromyces* species from proteoid Fynbos



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Biological and environmental characters that influence the diversity and distribution of *Penicillium* and *Talaromyces* species from proteoid Fynbos

ABSTRACT — *Penicillium* and *Talaromyces* are two of the dominating groups of fungi in the diverse Fynbos. In the present study, we evaluate the diversity of both these genera associated with *Protea repens* infructescences. A total of 61 *Penicillium* and 15 *Talaromyces* species were isolated from *Protea repens* infructescences, its associated mites, soil and air, collected from sampling sites at Malmesbury, Stellenbosch and Struisbaai. These sites represented three unique Fynbos vegetation types. We aim to determine the effect of plant locality and /or infructescence age on the diversity and community composition in this niche, compared to the surrounding soil and air, in order to evaluate the possibility of whether mites and/or air currents may have been involved in the dispersal of these fungi between infructescences. Species richness comparisons showed that communities inside infructescences were not only diverse, but also contained a large proportion of unique species not found in the soil and air samples. It was also observed that a large number of species was only found at specific sites. As a result, Multidimensional scaling (MDS) and permANOVA analysis were used to show that communities are unique between sites and thus that site location has a significant effect on community composition. However, the age of infructescences had no significant effect on these communities. To infer whether mites could act as possible transport vectors of *Penicillium* and *Talaromyces*, communities at each plant individual were compared between mites, *Protea repens* infructescences and combined soil and air samples, using area proportional Venn diagrams and Sørenson's similarity indexes. In general, data showed that communities obtained from mites were more similar to *Protea repens* infructescences, than to the communities from soil and air. We therefore propose that mites might in fact play a role in vectored dispersal of these species. However, an exhaustive sampling effort from a specific sampling site is needed in future studies to statistically test this hypothesis.

KEYWORDS — Ecology, Polyphasic species concept, Infructescences, Inflorescences.

Introduction

Penicillium sensu lato is one of the most ubiquitous fungi in the world and has been reported from almost all terrestrial environments (Thom 1930, Raper & Thom 1949, Pitt 1979, Christensen *et al*. 2000, Frisvad & Samson 2004, Houbraken & Samson 2004). A review of the family *Trichocomaceae* and the movement towards single name nomenclature resulted in *Penicillium* subgenus *Biverticillium* combined into its previously associated teleomorph genus *Talaromyces* (Norvell 2011, Houbraken & Samson 2011, Samson *et al*. 2011). For the purpose of this study, the ecology of these two genera will be considered together. The ecologically diverse nature of *Penicillium* s.l. is exemplified by the ability of many constituent species to grow at diverse temperature, pH and water-potential ranges (Frisvad & Samson 2004). Their preferred/native habitat is, however, not well explored. *Penicillium* taxonomists did well to record the sources from where their strains were isolated. However, as collection strategies are usually unsystematic, this only serves to identify possible "preferred" habitats.

The Fynbos presents a unique study area. It is one of the most botanically diverse regions on earth, especially when β- and γ-diversity is considered (Myers *et al*. 2000, Goldblatt & Manning 2002). Factors that are believed to promote this diversity include fire regimes, low nutrient soils, variable geography and landscape features, and the extreme temperature fluctuations between summer and winter months (Goldblatt & Manning 2002). Although plant diversity is well studied in this area, very little is known about the fungal diversity (Crous *et al*. 2006, Crous *et al*. 2011).

Crous *et al*. (2006) predicted 171 500 fungi to occur in South Africa, based on a 1:7 plant to fungi ratio. Should one extrapolate this to the fynbos, using the 9 030 plant species that occur here, more or less 63 210 fungi are estimated to occur in this region of which 42 000 are expected to be novel (Crous *et al*. 2006, Roets *et al*. 2009). However, to date only 780 fungi have been described from South Africa (Crous *et al*. 2006). With regards to *Penicillium* s.l. in the Fynbos, Visagie (2008) reported this genus as one of the dominant groups in soils. Unfortunately, reports on species that occur in Fynbos are limited (Allsopp *et al*. 199 Marais & Wingfield 1994) with only four new species recently described from this habitat (Visagie *et al*. 2009, Visagie & Jacobs 2012).

Plant species often present unique micro-niches for the study of insects (Zwolfer 1979). For example, serotinous *Protea* species such as *Protea repens* house their seeds in specialized structures (infructescences) that form after pollination of their inflorescences (Coetzee & Giliomee 1987, Rebelo 1995, 2001). Infructescences serve as aboveground storage structures for seeds that are released after extreme drought or veld fires. Before release, these structures become occupied by a diverse array of organisms including insects (Wright & Samways 1999, 2000, Fleming & Nicolson 2003), mites (Roets *et al*. 2007, Roets *et al*. 2008, Roets *et al*. 2011, Theron 2011) and fungi (Wingfield *et al*. 1988, Marais & Wingfield 1992, Wingfield & Van Wyk 1993, Marais & Wingfield 1994 1997, Marais *et al*. 1998, Marais & Wingfield 2001, Lee & Crous 2003, Lee *et al*. 2003, Lee *et al*. 2005, Marincowitch *et al*. 2008, Roets *et al*. 2008).

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Previous studies have reported numerous fungi occurring on and inside *Protea* species, however, only two studies mentioned the presence of *Penicillium* s.l. inside the infructescences (Marais & Wingfield 1994, Roets 2006). Even though they rarely get mentioned in these fungal surveys, a preliminary study found it to be one of the dominant molds in *P. repens* infructescences (Visagie personal observation), together with ophiostomatoid fungi (Roets *et al*. 2005). *Protea repens* inflorescences and infructescences present a favorable environment for the growth of *Penicillium* species as there is an abundance of carbohydrates in the form of nectar that accumulates inside the bracts. The closed nature of these structures also ensures a fairly humid growth environment for fungi residing here (Roets *et al*. 2012). Roets *et al*. (2012) monitored temperature and humidity of three *Protea* species infructescences during February, which in general are the peak of summer in the western Cape. Data showed that infructescences on average had higher temperatures than ambient air and often reached temperatures close to 40 °C. The influence of chemical compositions and temperature inside the infructescences were reported to be important characters that influence ophiostomatoid fungal populations residing in these infructescences. How these factors influence the *Penicillium* s.l. populations, however, is still unclear.

The closed nature of infructescences presents a challenge for dispersal of fungi within. It is commonly accepted that *Penicillium* s.l., with its hydrophobic conidia, use wind currents as primary dispersal strategy (Thom 1910, Raper & Thom 1949, Pitt 1979, Frisvad & Samson 2004). Furthermore, water can also easily aid in dispersal of these fungi, especially when it produces ascospores (Pitt 1979). However, air and water dispersal is probably not a major contributor to fungal dispersal from infructescences, and other means of dispersal must be utilized. Vectored dispersal via arthropods may represent a feasible alternative in this environment. It has been shown that ophiostomatoid fungi from *Protea* infructescences are dispersed via arthropods (Roets *et al*. 2009, 2011). For these fungi there is a three-way association between fungi, mites and insects where mites transport the ophiostomatoid fungal spores between infructescences with the aid of beetle vectors. The association between the mites and the ophiostomatoid fungi is thought to be mutualistic (Roets *et al*. 2007).

Similar to ophiostomatoid fungi, transport of *Penicillium* s.l. spores from *Protea* infructescences may be dependent on arthropods. Previous studies have shown that mites can vector *Penicillium* spores in pack-houses (Hubert *et al*. 2004). A mutualistic association have, however, not been shown between *Penicillium* and mites. As previous surveys have indicated the existence of high numbers of *Penicillium* strains inside *Protea repens* infructescences (Marais & Wingfield 1994, Visagie personal observation) the possibility of dispersal via mites and their influence on the ecology of these fungi would be interesting to evaluate.

Surveys have found *Penicillium* s.l. as one of the dominant fungal groups in Fynbos soils and *Protea repens* infructescences (Allsopp *et al*. 1987, Marais & Wingfield 1994, Visagie 2008, Visagie *et al*. 2009, Visagie & Jacobs 2012, Visagie 2012 chapter 2 & 3). This high diversity and abundance of *Penicillium* s.l. in soil is not unexpected when previous soil surveys from other parts of the world are considered (Christensen *et al*. 2000). The high abundance and diversity of these fungi inside *Protea* infructescences and the origin of inoculum for these is, however, unknown. Various hypotheses could account for the origin of inoculum. *Penicillium* s.l. populations may be specific to infructescences and are dispersed from one flower to the next via mites and pollinators, such as in the case of ophiostomatoid fungi from this niche. Alternatively, these fungi originate from soil and reach infructescences via air currents or soil-associated mites.

The *Protea*-infructescence habitat and surrounding environment was identified as a good base from which to start exploring *Penicillium* and *Talaromyces* species diversity in the Fynbos as a whole and forms the basis of various other studies (CHAPTERS 2 & 3). In the present study we evaluate the diversity of *Penicillium* and *Talaromyces* species associated with the infructescences of *Protea repens*. We aim to determine whether plant locality and/or infructescence age may play a role in the diversity and assemblages present and whether the species present are specific to this niche or may have been transported from soil. In addition, we evaluate whether mites and/or air currents may be involved in the dispersal of *Penicillium* between *P. repens* infructescences.

Materials and Methods

Sampling and isolation protocols

Three mature *P. repens* individuals were selected at random from three sites with Fynbos vegetation; Stellenbosch mountain (33°56’48.21”S, 18°52’49.45”E), Riverlands Nature Reserve (33°29’47.33”S, 18°35’27.42”E) and Struisbaai (34°45’1.96”S, 19°58’52.72”E). These sites represent Boland granite, Atlantis sand and Agulhas sand Fynbos, respectively (Muchina & Rutherford 2010). From each individual we collected three mature infructescences, each one representing a different age (1 to 3 years old). Soil samples were collected from the base of each plant. Soil samples were homogenized, which resulted in one soil sample that was associated with each *Protea repens*

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individual. In addition, fungal spores from 50L air sampled at *ca*. 1.5 m height were filtered onto potato dextrose agar (PDA) supplemented with 100 ppm streptomycin, 50 ppm chloramphenicol and 2 ppm dichloran at each of these *P. repens* individuals using a MAS-Eco® air sampler (MBV, Switzerland).

PDA was used for all subsequent isolations from samples. *Penicillium* and *Talaromyces* strains were isolated from soil and infructescence samples, as well as from the mite populations collected from inside the infructescences. Mites were removed from infructescences by shaking them onto 1% water agar plates. Mites were sedated by dipping a small piece of cotton wool (*ca*. 1 cm diam.) in chloroform and placing it in the Petri dish containing the mites. Individual mites were transferred to PDA plates (*ca*. 10 per plate) using a fine needle and incubated on the lab bench (±21°C) for 5–7 days. To prevent movement of mites between plates, plates were sealed with parafilm and placed on water traps. These traps consisted of a flat plastic container (60 by 100 cm) that was filled with soapy water to break surface tension. Test tube racks served as platforms for plates and were placed inside these containers. For isolation of fungi from infructescences, the inner bracts and florets were washed with 100 mL ddH_2O and the solution diluted to 10^{-6}. One milliliter of each dilution was plated onto PDA in triplicate. For isolation of *Penicillium* and *Talaromyces* from soil samples, a soil solution (5g/100mL) was diluted to 10^{-2} in triplicate. One milliliter of each dilution was plated onto PDA in triplicate. All plates were incubated on the lab bench for 7 days after which colonies representing *Penicillium* and *Talaromyces* were transferred onto Czapek yeast autolysate agar (CYA) (Pitt 1979) and stored as water plugs at 5°C.

Species identification

Single spore strains were identified to species level using morphological and genetic data (CHAPTER 2 & 3). Strains were incubated at 25°C on CYA (+ 5, 30 & 37 °C), MEA, YES, G25N and CREA media (Pitt 1979, Frisvad & Samson 2004), under standardized conditions (Visagie & Jacobs 2012), and characterized after 7d of growth. Strains were placed into their respective macro-morphological groups, and further characterized using micromorphology. Strains representing each morphological group were selected for further phylogenetic comparisons. DNA extractions were made from 8d old MEA colonies with the ZR Fungal/Bacterial DNA Kit™ (ZymoResearch Corporation, USA). DNA markers selected for phylogenetic comparisons included ITS, β-tubulin, Calmodulin, Elongation Factor 1-α and RPB2. Sequence contigs were assembled with Codon Code Aligner (CodonCode Corporation, USA). Data sets, containing numerous ex-type cultures of described species and our representative samples, were aligned in MAFFT (Katoh *et al*. 2009) and analyzed in PAUP*4.0b10 (Swofford 2000) using the BioNeighbour-Joining option. These multigene phylogenies combined with the morphological data were used for species identifications (Chapter 2 & 3).

Data analyses

A species presence-absence data matrix was created for each sample collected APPENDIX 1. Species accumulation curves were estimated from Cole rarefaction (Coleman 1981, Coleman *et al*. 1982, Colwell *et al*. 2004, Colwell 2006, Jumpponen 2011) calculated in EstimateS V8.2 (Robert K. Colwell, USA). Graphs include curves for combined data from all sampling sites for soil, air and *Protea repens* infructescences (including those from mites therein), as well as separate curves for *Protea repens* infructescence samples collected (combined infructescence and mite data) from each site.

Species richness and composition of *Penicillium* and *Talaromyces* communities were compared between the different sites and habitats (i.e. soil, air and *Protea repens* infructescences). First, data from all sites were combined in order to compare species richness and composition of samples collected from soil, air and *P. repens* communities (including those from infructescence and mites), as well for communities from *P. repens* infructescences only and mites only. Second, species richness and composition between the three sites were compared. This was done for a combined data set that included all samples collected from each of the three sites, as well as sub-datasets that only included species isolated from either soil, air and *Protea repens* (including infructescence and mites). Again we compared communities from each site only with those isolated from either only *Protea repens* infructescences or only from mite populations. Thirdly, comparisons of *Penicillium* and *Talaromyces* species richness and composition from different aged *Protea repens* infructescences were made. This was done for both the combined datasets (including all sites) as well as for each individual site separately.

The effects of various factors on *Penicillium* and *Talaromyces* communities were compared using Multi-dimensional scaling (MDS) and statistically evaluated using Permutational multivariate analysis of variance analyses (permANOVA) (Smith *et al*. 2011, Maciá-Vicente *et al*. 2012). MDS analyses were conducted to evaluate groupings of *Penicillium* and *Talaromyces* communities from the different samples collected (infructescences (including mites), soil and air) from the three sites. For these analyses, the Sørenson's index was calculated from presence-absence data in Primer6 (Primer-E Ltd, United Kingdom) (Sørenson 1948, Gochenaur 1978, Martin *et al*. 1993, Byrd *et al*. 2000, da Silva *et al*. 2005, Dexter *et al*. 2012). PermANOVA's (Anderson

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2001, Anderson *et al.* 2008) were used to calculate the t- and p-values for the similarity of the fungal assemblages between sampled habitats (infructescences, soil, air) and collection sites using 999 unrestricted permutations of raw data.

The influence of infructescence age and the effect of plant individual (from which collection was made) on fungal communities were also tested. This was done with species composition data (presence/absence) for *Protea repens* individual infructescences collected from each separate plant (3 infructescences, age 1, 2 and 3-years old from three individual plants per site). For these analyses the effect of sampling site was controlled by analyzing data separately for each respective site. PermANOVA analyses were conducted on these data (again using Sørenson's index and 999 unrestricted permutations of raw data) to determine the significance of effect of plant individual and infructescence age on the similarity of fungal communities found within infructescences.

We tested whether *Penicillium* and *Talaromyces* species from infructescences originated from environmental samples other than infructescences by comparing species composition of fungi within *Protea repens* infructescences (separating data from mites and infructescences, respectively) to those collected from air and soil samples (air and soil data combined). Data collected from each plant individual was analyzed separately. Data were presented as Area-Proportional Venn Diagrams (www.bioinforx.com) with calculated Sørenson's similarity index values for the communities collected from the various habitats also indicated.

Results

Isolations and species identifications

Penicillium was found to be one of the dominant fungal genera in soil and *Protea repens* samples collected. In general, *Cladosporium* was dominant in air samples collected. In total *ca.* 2 500 fungal strains were obtained from *Protea repens* infructescences, mites, soil and air samples. Other genera identified include *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Talaromyces* and *Trichoderma*. Among these, 1 688 *Penicillium* and 199 *Talaromyces* strains were isolated. Based on morphology, isolates were placed into their respective *Penicillium* and *Talaromyces* groups. Phylogenetic analyses of multiple gene regions confirmed most of these groupings and their species identities. We characterized and identified 61 *Penicillium* and 15 *Talaromyces* species. Of these, 30 are considered novel and will be described in future studies.

Adequacy of sampling

Species accumulation curves were calculated for combined data of soil, air and *Protea repens* samples collected from all sites (FIGURE 1), as well as data for *Protea repens* infructescences collected from each site separately (FIGURE 2). From all the curves it is clear that not all species were isolated with curves not approaching saturation. This was also observed in the curves for the *Protea repens* infructescences from each site.

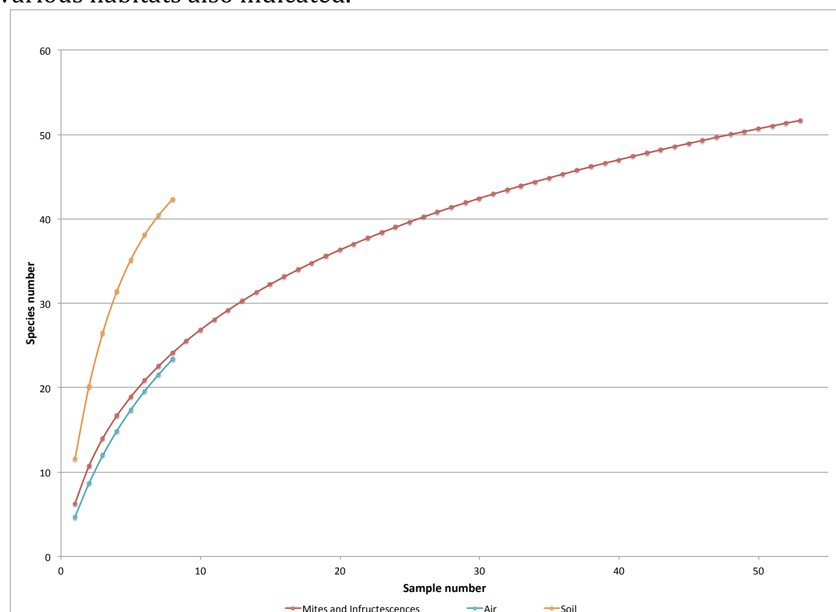

FIGURE 1: Species accumulation curves using Cole rarefaction for *Penicillium* and *Talaromyces* communities collected from *Protea repens* infructescence (mite data included), air and soil samples from three sites in the Fynbos Biome.

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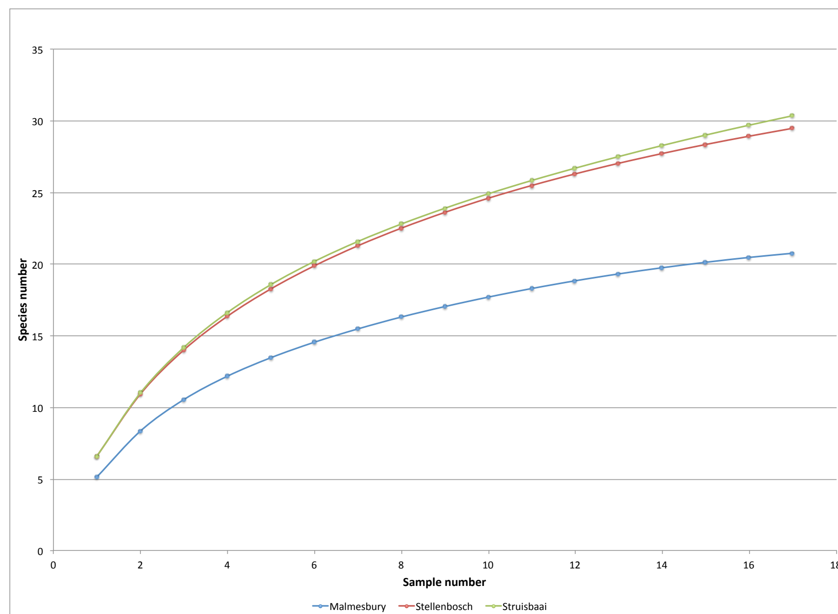

FIGURE 2: Species accumulation curves using Cole rarefaction for *Penicilium* and *Talaromyces* communities collected from *Protea repens* infructescences (including mites) from three sampling sites, Malmesbury, Stellenbosch and Struisbaai.

Penicillium and *Talaromyces* communities associated with the infructescences of *Protea repens*, compared to air and soil

Species composition of communities collected from different sampling sites and habitats (soil, air, *Protea repens* infructescences) were compared using species richness data (TABLE 1). The largest number of species collected from the different habitats sampled was from infructescences (including the mites) followed by those from soil. The communities from infructescences also had the greatest number of unique species (TABLE 1). The air samples contained by far the lowest number of species. With regards to species richness between different sampling sites, Struisbaai soil seems to be less diverse than soil from Malmesbury and Stellenbosch, with only 9 species isolated compared to 25 and 26, respectively. For total infructescence communities, sites had fairly similar species numbers, although large numbers of these were restricted to particular sites. Comparisons of communities from different sampled habitats indicated that although many species are shared, almost half of species collected were habitat specific. This pattern was also observed when communities from different sites were compared.

Species diversity and composition from *Protea repens* infructescences of different age groups are presented in TABLE 1.2. In general, infructescence age seems to have no effect on the richness of its *Penicilium* and *Tararomyces* communities. The largest proportion of species was shared between the one, two and three year old infructescences.

Table 1.1: Total species richness of *Penicillium* and *Talaromyces* collected from air, soil, *Protea repens* infructescences and mites from infructescences at three sites in the Fynbos Biome of South Africa. Values between brackets represent number of species that were only found at a particular site

	Total	Malmesbury	Stellenbosch	Struisbaai
All samples	76	40 (18)	44 (18)	34 (12)
Soil	44 (18)	25 (14)	26 (15)	9 (1)
Air	25 (4)	16 (10)	11 (7)	7 (1)
P. repens infructescences and mites	52 (24)	21 (7)	30 (12)	31 (15)
P. repens infructescence only	44 (12)	17 (4)	24 (9)	27 (15)
P. repens mites only	40 (8)	17 (5)	24 (11)	21 (9)

Table 1.2: Total species richness of *Penicillium* and *Talaromyces* collected from 1, 2 and 3 year old *Protea repens* infructescences. Values between brackets represent number of species that were only found at a particular aged infructescence

	1 year old	2 years old	3 years old
All sites			
Total (mites and infructescences)	37 (11)	34 (7)	31 (3)
Mites only	25 (5)	25 (6)	27 (5)
Infructescence only	25 (5)	25 (6)	27 (5)
Malmesbury			
Total (mites and infructescences)	18 (4)	12 (1)	14 (1)
Mites only	12 (3)	8 (1)	12 (4)
Infructescence only	15 (3)	11 (1)	11 (0)
Stellenbosch			
Total (mites and infructescences)	22 (7)	18 (4)	16 (2)
Mites only	15 (4)	14 (4)	15 (2)
Infructescence only	16 (6)	15 (6)	10 (1)
Struisbaai			
Total (mites and infructescences)	21 (5)	19 (4)	20 (4)
Mites only	12 (2)	11 (2)	16 (5)
Infructescence only	19 (4)	17 (4)	16 (4)

Effect of plant locality and/or infructescence age on the assemblage composition of *Penicillium* and *Talaromyces* spp. associated with the infructescences of *Protea repens*

MDS diagrams (FIGURE 3) indicate that communities from the sampled habitats strongly group together according to sampling sites. Results of permANOVA analyses confirmed that sampling sites had a significant influence on community similarity in infructescences (df = 2, F = 7.5648, P = 0.001), air (df = 2, F = 2.7334, P = 0.01) and soil (df = 2, F = 15.495, P = 0.004). On the other hand, infructescence age from combined sites had no significant influence on community similarity (df = 2, F = 1.387, P = 0.16). Due to small sample sizes, we could only compare community similarity

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between sites for the infructescence habitat *post hoc*. Statistically, community composition differed between sites (Malmesbury: Stellenbosch, t = 2.879, P = 0.001; Malmesbury: Struisbaai, t =2.699, P = 0.001; Stellenbosch: Struisbaai, t = 2.6892, p = 0.001).

Since collection site had such a large effect on community composition of *Penicillium* and *Talaromyces*, a second set of MDS's and permANOVAS were conducted to test the effect of infructescence age and plant individual on fungal communities at each separate sampling site (FIGURE 4). No groupings were obvious for samples collected from Malmesbury and Struisbaai. However, some level of grouping was observed at the Stellenbosch site. PermANOVA results confirmed that infructescence age and plant individual had no influence on community composition at the Malmesbury and Struisbaai sites (TABLE 2.1). At Stellenbosch, however, a significant result was obtained for the effect of infructescence age and plant individual on *Penicillium* and *Talaromyces* community composition. *Post hoc* analyses showed that communities from one year old and two year old infructescences were significantly different, as well those from two - and three year old infructescences. Communities from one year old and three year old infructescences were statistically similar (TABLE 2.2). *Post hoc* analyses of plant individual data showed that only one comparison was close to significant. However, due to the small sample size, this may be incidental and remains to be tested on a larger scale.

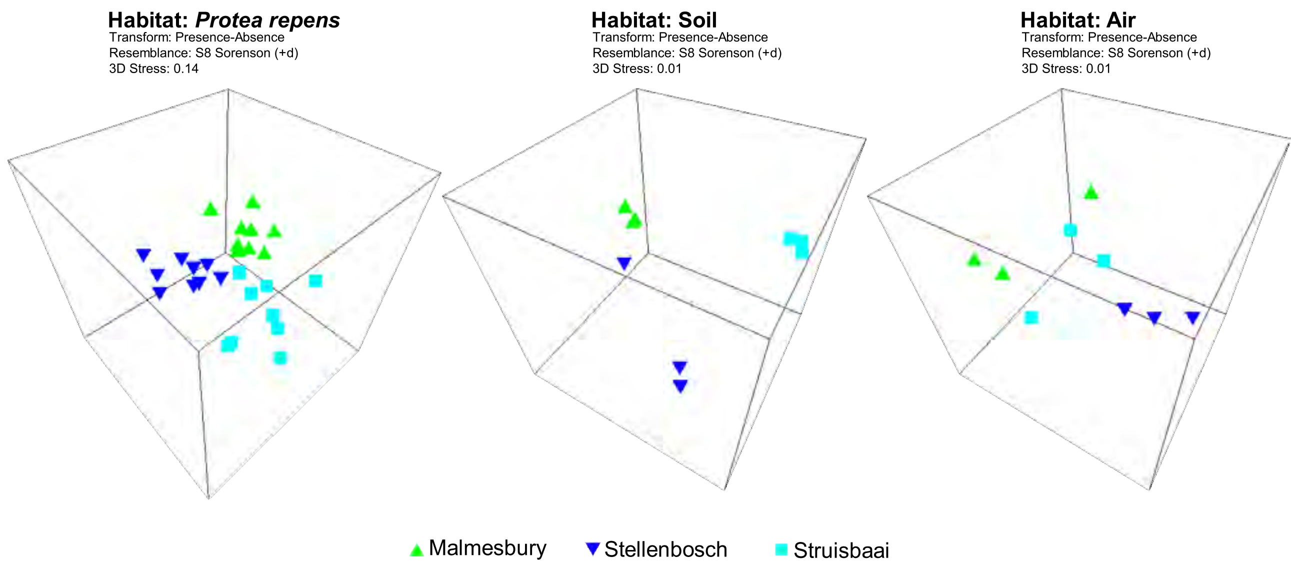

FIGURE 3: Multi-dimensional scaling diagrams of *Penicillium* and *Talaromyces* community similarities between different sampling sites from different habitats (*Protea repens* infructescences, soil and air).

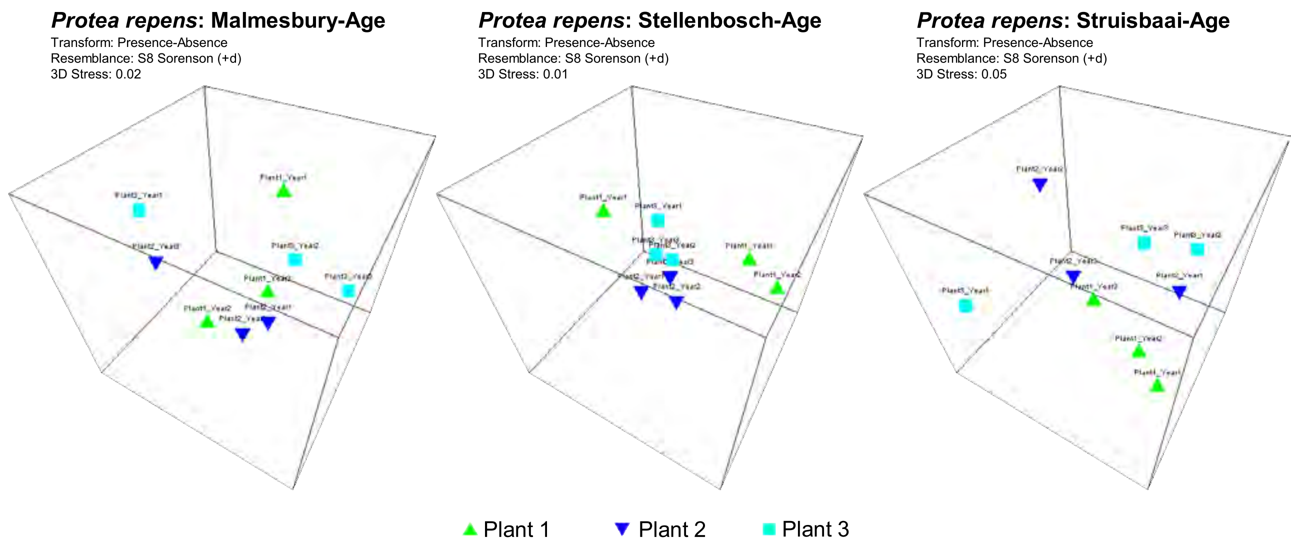

FIGURE 4: Multi-dimensional scaling diagrams of *Penicillium* and *Talaromyces* community similarities between different aged *Protea repens* infructescences and plant individuals at each sampling site.

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Table 2.1: Permanova results for testing significance of various factors that might influence community compositions

Factor	Degrees of Freedom (dF)	Test statistic (F)	P-value
Protea repens: Malmesbury-Age			
individual	2	0.741	0.741
age	2	0.941	0.941
Protea repens: Struisbaai-Age			
individual	2	1.2804	0.246
age	2	1.0466	0.423
Protea repens: Stellenbosch-Age			
individual	2	2.8732	0.034
age	2	3.3519	0.017

Table 2.2: Pair-wise comparisons of factors that might influence community compositions in *Protea repens* from Stellenbosch

Factor	Degrees of Freedom (dF)	Test statistic (t)	P-value
Pair-wise comparisons between different individuals from Stellenbosch			
Plant1 x Plant2	2	1.906	0.113
Plant1 x Plant3	2	1.5144	0.218
Plant2 x Plant3	2	1.6907	0.053
Pair-wise comparisons between different aged infructescences from			
Year1 x Year2	2	1.8681	0.021
Year1 x Year3	2	1.5691	0.066
Year2 x Year3	2	2.4456	0.013

Possible dispersal methods of *Penicillium* and *Talaromyces*

To test whether vectored transport might be possible via mites, area proportional Venn diagrams and Sørenson's similarity indexes were calculated for comparisons of communities collected from the different habitats per individual plant. Thus, for each plant individual we compared fungal communities from mites, infructescences and surrounding habitats (soil and air community data combined) (FIGURE 5). The diagrams clearly illustrate that at the Malmesbury and Stellenbosch sites, soil and air communities are different from fungal communities from mites and infructescences. It was also observed that the mite and infructescence communities from individual plants were more similar than either of these are to soil and air communities. This was, however, not observed for plant individuals sampled at Struisbaai. In general more species were shared between infructescences and mites and the surrounding soil and air communities from Struisbaai, compared to the other two sites. Observations from Venn diagrams were confirmed by the Sørenson's similarity indexes, which in general showed higher values between infructescence and mite communities. However, this difference in indexes was less for the Struisbaai comparisons.

Discussion

Surveys from Fynbos have shown that *Penicillium* and *Talaromyces* are both in numbers and species diversity, one of the dominant genera in the fynbos biome (Allsopp *et al*. 1989, Visagie 2008, Visagie *et al*. 2009, Visagie 2012). Its occurrence in the enclosed woody infructescences of *Protea repens* have also been reported before (Marais & Wingfield 1994, Roets 2006). The current study aims to describe various aspects of the ecology of *Penicillium* and *Talaromyces* and provide a baseline data set for future ecological studies. *Ca*. 2500 fungal isolates were collected from *Protea repens* infructescences, soil and air. Subsequent characterization resulted in the identification of 76 species (61 *Penicillium*; 15 *Talaromyces*). Thirty of the species were found to be novel based on morphological characterization and multi-gene phylogenetic analyses. The taxonomy of these was treated in CHAPTERS 2 & 3. These results indicate that the Fynbos harbor immensely diverse and virtually unexplored *Penicillium* and *Talaromyces* communities.

Species richness of *Penicillium* and *Talaromyces* associated with the infructescences of *Protea repens*, air and soil, and their specificity

Comparisons of species richness suggested that site locality has a significant effect on fungal distribution. Forty-eight of the total 76 species isolated were only found at one particular site. As the three sites sampled in this study represent three unique Fynbos types, it is expected that many more *Penicillium* and *Talaromyces* species will be found from the other Fynbos vegetation types. Species accumulation curves indicated that not all species were isolated from the few sampling events in this study. Thus, additional species can be expected from the three sites sampled in this study. Interestingly, species richness and numbers of unique species encountered from *Protea repens* infructescences were particularly high. However, how these communities compare to other serotinous *Protea* species is not clear, data which will be important for clarification of host specificity of these taxa.

During isolations, species such as *P. toxicarium* and *P. atrolazulinum* were so abundant that the infructescence solutions had to be diluted down to 10^{-5} and 10^{-6} in order to observe single colonies. As such, species that occur in low abundance could have been missed using our particular isolation method. Direct isolation of *Penicillium* conidiophores from the *Protea* bracts is, however, also not considered a viable method as numerous species are missed. This technique will also turn out to be too labor intensive. The use of alternative isolation media or incubation conditions is a more plausible approach in finding unsampled *Penicillium* and *Talaromyces* taxa. It is well documented from taxonomic studies that various growth conditions have an effect on the growth characters of a particular species. These growth factors include for instance pH, carbon source, incubation temperature and water-potential. Heat treatment of samples has also been shown to result in additional species isolated that were previously missed (Pitt 1979).

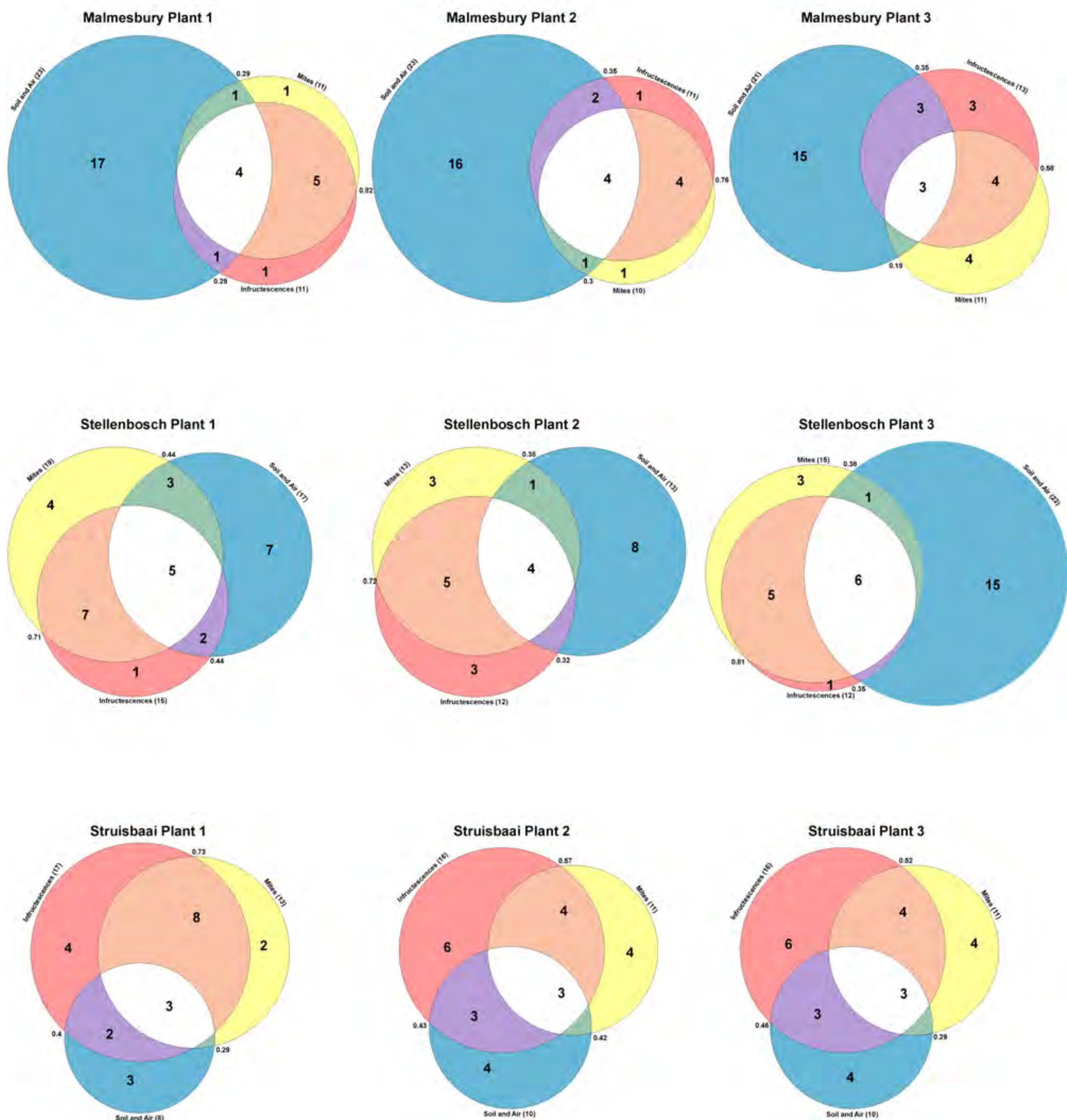

FIGURE 5: Community composition comparisons for each individual plant sampled at Malmesbury, Stellenbosch and Struisbaai. Communities include combined data for non-infructescence samples (soil + air), as well as those from mites and from *Protea repens* infructescences. Numbers on outside of intersections represent the respective Sørenson's similarity index values between those particular communities. Numbers inside diagrams, represent number of species from that particular niche. Colors correspond to habitat; blue - soil and air, red - infructescences, yellow - mites, purple - infructescences + soil and air, green - soil and air + mites, orange - infructescences + mites.

Therefore, exposing communities in a sample to these different conditions may promote/restrict the growth of particular species. As a result, these selective conditions may result in the isolation of species that are often overgrown by more abundant and better growing species. Full inventories are important for a better understanding of the taxonomy, dynamics and evolution of species within genera. Therefore, the above-mentioned alternative isolation techniques are suggested for future studies aiming to do inventories of *Penicillium* and *Talaromyces* in the Fynbos.

However, based on the accumulation curves, the isolation technique used in the study was considered adequate for observing general trends in community composition. More inclusive sampling efforts from these and other sites would, however, be valuable in future studies on the ecology of particular species.

In a study of fungal diversity from soil, Christensen *et al*. (2000) reported that on average *Penicillium* s.l. accounted for 21% of the 90 species reported in 90 soil microfungal surveys. This is slightly lower from the 35% of the 50–75 species reported by an earlier study (Christensen 1989).

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Christensen *et al*. (2000) also reported that a large proportion of species isolated from soil surveys represents novel species. This trend was also observed in the current Fynbos study. In total, 44 species were isolated from soil samples and of these 21 species are considered novel. Although Struisbaai displayed low diversity, 25 and 26 species were respectively isolated from Stellenbosch and Malmesbury. Also, only eleven species occurred at both sites. Although data was not collected of what percentage *Penicillium* make up of the total fungal species, it was observed that *Penicillium* s.l. was by far the dominant genus when isolations were made from soil dilutions. Allsopp *et al*. (1987) studied fungal communities from proteiod rhizospheres, at the same Malmesbury site sampled in this study, and isolated 14 *Penicillium* s.l. spp., 21% of the total number. However, Allsopp *et al*. (1987) only reported on species that was obtained from more than 10 samples and thus probably missed a large number of species that occur in low abundance. It is clear that we have only started to uncover the true diversity of *Penicillium* s.l. species that occur in Fynbos soils. The same is true for communities that occur in air samples. Data collected for an indoor air project conducted in the Stellenbosch region (Borchardt *et al*. 2010) showed that *Penicillium* s.l. and *Cladosporium* are dominant in air samples, similar to what was found in this study. In addition, some of the species isolated from the Fynbos environment, i.e. soil, air or *Protea repens*, were common in indoor environments. More or less 50 *Penicillium* and *Talaromyces* taxa were isolated during the indoor project, with many of them that still need to be identified. Indoor environments form an important part of *Penicillium* and *Talaromyces* biodiversity in the Western Cape and needs to be explored further. The population dynamics between the communities from the natural Fynbos and various indoor environments also needs investigation, since this data will be valuable for a better understanding of the roles these species play in our everyday lives.

Assemblage composition of *Penicillium* and *Talaromyces* communities from the infructescences of *Protea repens*, soil and air

Site locality or Fynbos type was shown to have a significant effect on *Penicillium* and *Talaromyces* communities. Site location was also shown to have a significant effect on the community compositions of the soil and air samples. The communities from air were, however, more variable than results observed for *Protea repens* and soil. The effect of plant individual from which infructescences were collected on species composition was not significant, except for plants from Stellenbosch. Also, in general, infructescence age had no significant effect on fungal communities, except for those from Stellenbosch where age differences between 1 & 2 year old and 2 & 3 year old communities were found to be significant, although this may not be meaningful and needs to be tested with a more exhaustive sampling approach. However, it was observed that infructescences collected at the Stellenbosch site were in a better condition than those collected from the other two sites, with less bore holes and thus fewer disturbances from insects that could affect communities. In order to minimize the possible effect of these disturbances, future sampling events should try and avoid infructescences with these bore holes. However, the idea that these insects might influence the communities only adds to the idea that mites and insects could disperse *Penicillium* and *Talaromyces* species. Also, if the insects do not alter the communities inside the infructescences, we are left with the question why specific species were found dominant from the one to two year old infructescences and how they got into the flowers. If via air currents, there must be an inoculum source of these species, possibly from another plant host since soil were shown to have different community compositions.

As collection site seems to be the most important factor in determining *Penicillium* and *Talaromyces* community composition from various Fynbos habitats it will be valuable to collect samples from as many Fynbos types possible, when conduction biodiversity assays. Also, since site locality had a significant effect on *Penicillium* and *Talaromyces* communities, future sampling efforts should be focused on a single Fynbos vegetation type when addressing ecological questions. For focused ecological studies, a larger sampling effort than the one followed in this study may be required.

Possible dispersal methods of *Penicillium* and *Talaromyces* species associated with the infructescences of *Protea repens*

Results indicate that *Penicillium* and *Talaromyces* communities were largely unique between soil, air and *Protea repens* infructescences. A large portion of the species found in infructescences likely did not originate from soil or air samples. These differences were observed at all sites. The reasons for such specificity are unclear, but may include various niche requirements such as chemistry, temperature and humididity (eg. Roets *et al*. 2012). On the other hand, it might not be specific at all and only by chance that specific species are found inside infructescences. Although specificity can be questioned for communities, the common occurrence and dominance of species such as *P. toxicarium* inside these infructescences collected from all three sites, suggest that there is some form of selectivity. Future studies should thus compare the growth requirements and competitive abilities of species associated with infructescences and soil.

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Infructescences present fungi with micro-niches (Zwolfer 1979) that are well protected from dispersal elements such as rain and wind. This was corroborated by our results that indicated very little overlap between *Penicillium* and *Talaromyces* species from infructescences and air. Since communities from infructescences at particular sites were very similar, it is hypothesized that *Penicillium* and *Talaromyces* species are mainly dispersed between these structures via mites. In turn, mites are most probably dispersed by insects similar to the situation of ophiostomatoid fungi in this niche (Roets *et al*. 2009, 2011). Mite dispersal of *Penicillium* and *Talaromyces* from this habitat is supported by our results that showed that fungal communities from mites are remarkably similar to those from the infructescences themselves. These mites thus have a proven ability to acquire and transport spores over short distances, similar to findings by Hubert *et al*. (2004). Abbot (2000) proposed that extended structures such as the ascocarps of ophiostomatoid fungi or synnema produced by some *Penicillium* and *Talaromyces* species aids in the dispersal via arthropod vectors. However, the present study identified only a few synnema producing taxa, with very few synnemata observed on bracts of the infructescences. Species associated with mites may thus not need synnema to acquire spores such as is commonly the case for insect dispersed species. During direct isolations from *Protea* bracts, a high density of conidiophores that resemble those of *Penicillium* was observed in nectar rich regions. The shear density of these conidiophores will result in insects acquiring spores, whether by chance or choice.

The precise nature of the symbiosis between mites and the various *Penicillium* and *Talaromyces* species from infructescences is unclear, but may be mutualistic for some taxa, as was suggested for some ophiostomatoid fungi (Roets *et al*. 2007). It was, however, noted during isolations that some mites were phoretic and laid eggs in the fluffy yellow colonies of *P. toxicarium*, one of the most commonly isolated species in the study (data not shown). Future studies should test for the dispersal of these fungi via mites using emergence chambers (Ferro *et al*. 2011) followed by fungal isolations. Hereafter, it should be investigated if these fungal spores can be "inoculated" into uncolonised tissues under natural and controlled conditions. Feeding studies of mites using different fungal taxa should help to clarify whether the association between the fungi and mites are mutualistic (Roets *et al*. 2007). The idea of some *Penicillium* or *Talaromyces* having mutualistic associations with insects is not new. Peterson *et al*. (2003) described *P. brocae* from coffee berry borers. A mutualistic association was also proposed since the fungus produce sterols that are crucial for the insects' development. Seifert *et al*. (2004) reported two *Penicillium* and four *Talaromyces* species from insect galls that did not show evidence of bore holes. They also hinted that extrolites might play a role in attracting insects. Extrolite data for species isolated and described in this study might thus prove to be valuable information for further exploring the idea of attraction via extrolites.

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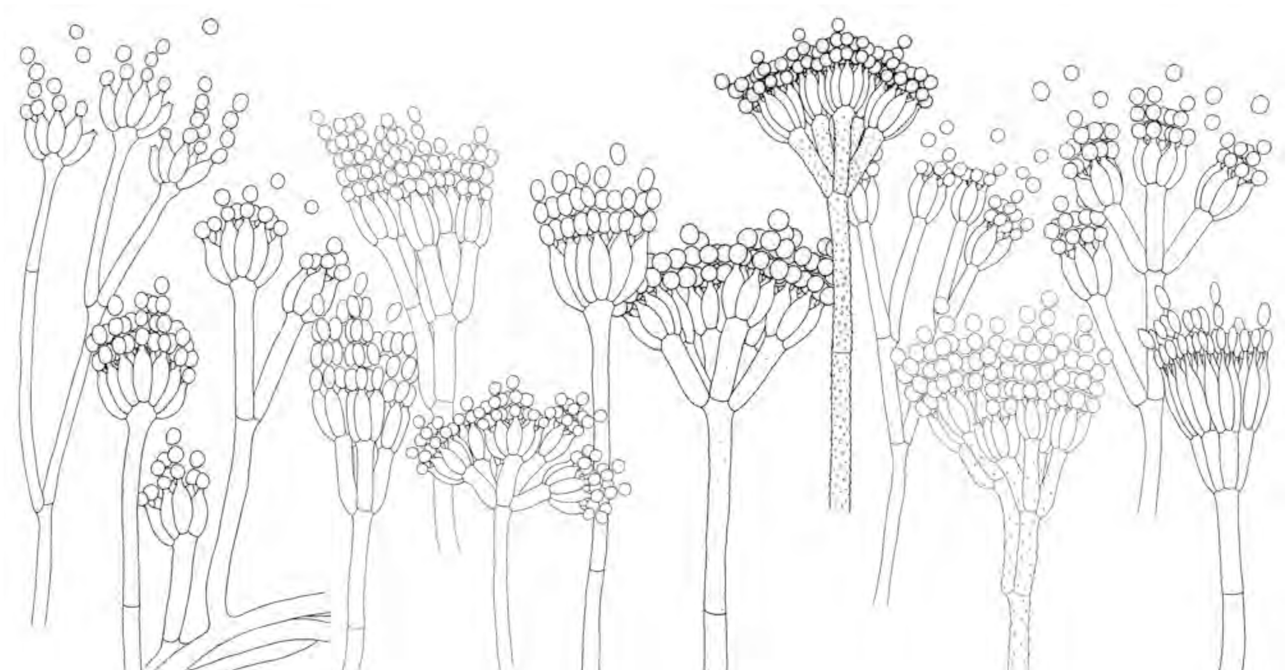
CHAPTER 5
General discussion and conclusions



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General discussion and conclusions

Penicillium is considered to be one of the most ubiquitous and cosmopolitan fungi worldwide (Raper & Thom 1949, Pitt 1979). Despite its economic importance (Frisvad & Samson 2004), not much is known about this genus from South Africa (Schutte 1992). In a review of *Penicillium* occurring in southern Africa, Schutte (1992) highlighted that a large number of South African strains are misidentified. This was attributed to the poor baseline knowledge of the genus in South Africa and compounded by a great degree of morphological variation often seen in strains. Schutte (1992) thus highlighted the need for classifying South African *Penicillium* species based on modern taxonomic techniques. Almost 20 years have since passed, but the current study aimed to do exactly this and, therefore, set the following objectives:

1. To isolate *Penicillium* and *Talaromyces* strains associated with *Protea repens* infructescences, mites living inside the infructescences, as well as surrounding soil and air, from three distinct Fynbos types.
2. To characterize Fynbos strains based on morphology and multi-gene phylogenies.
3. To provide descriptions of *Penicillium* and *Talaromyces* species that occurs in Fynbos, including full color photoplates and line drawings where considered informative.
4. To provide identification keys to species isolated and described during this study.
5. To investigate *Penicillium* community distribution patterns and ecology from three Fynbos types.

The Fynbos, situated at the southwestern tip of Africa, is considered to be one of the world's 25 biodiversity hotspots (Myers *et al*. 2000). The area contains *ca*. 9030 plant species, of which 70% are endemic (Myers *et al*. 2000, Goldblatt & Manning 2002). The Fynbos display a high beta diversity, which mean that species turnover is high across an environmental or habitat gradient. This high species turnover is also observed in identical habitats along geographical gradients, referred to as gamma diversity (Cowling *et al*. 1992, Goldblatt and Manning 2002). An association for diversity between plants and fungi are often made for fungal species predictions (Hawksworth 1991, 2001, Crous *et al*. 2006). Based on the well-studied plant and fungal associations of the British Islands, Hawskworth (1991, 2001) estimated that for every plant species there is six associated fungal species. A 1.5 million species estimate was thus proposed. Crous *et al*. (2006) used a similar concept and predicted that the plant:fungi ratio is closer to 1:7 in South Africa, which resulted in an 171 500 fungal species estimate, 63 200 for the Fynbos. However, the 1:7 ratio is expected to be much higher for the Fynbos, but the little knowledge on fungal host relations in this area makes estimates difficult (Crous *et al*. 2006). Based on results from this study, we know that locality has a significant impact on *Penicillium* and *Talaromyces* communities (CHAPTER 4), with 52 species isolated from *Protea repens* at three different collection sites. We also found that the individual plant had no significant effect on the communities present. Although this is based on a smaller sampling regime than what is ideal, it does indicate that this ratio should be much higher to compensate for the high species turnover in the Fynbos. Therefore, the 63 200 fungal species estimate for Fynbos is considered a gross underestimate.

Based on previous surveys from Fynbos soil (Visagie 2008, Visagie *et al*. 2009, Visagie & Jacobs 2012), it is known that *Penicillium* and *Talaromyces* are some of the dominant fungal genera in this environment when diversity and abundance are considered. This project isolated 61 *Penicillium* and 15 *Talaromyces* species from *Protea*, soil and air, and found these fungi most abundant with the isolation techniques used in the study. Previous surveys from *Protea* infructescences indicated that ophiostomatoid fungi, *Acremonium*, *Cladosporium* and *Penicillium* s.l. were dominant members (Marais & Wingfield 1994, Lee *et al*. 2005, Roets 2006, Marincowitz *et al*. 2008). However, the species richness of *Pencillium* (52 species) found inside these infructescences were unexpected. Amongst the 72 species isolated, a total of 25 *Penicillium* (CHAPTER 2) and 5 *Talaromyces* (CHAPTER 3) species were novel. These genera were thus found to be diverse, abundant and rather unique to the specific areas. Data also indicate that we have only started to uncover the true diversity of *Penicillium* and *Talaromyces* in the Fynbos (CHAPTER 4). For future studies, it will not only be important to know how communities compare at additional collection sites, but also what communities look like in other *Protea* species. It seems highly unlikely that each *Protea* species will have unique communities, especially if these fungi's inoculum source is not *Protea* but rather are dispersed from other sources. Therefore, additional habitats and collection sites will be an important aspect for uncovering the ecology of these important fungi.

Houbraken & Samson (2011) redefined the family *Trichocomaceae*, providing a new sectional classification of the *ca*. 250 *Penicillium* spp. known to science. This was mainly based on a four-gene phylogeny, which included the RPB1, RPB2, Tsr1 and Cct8 gene regions. The family was divided into three new families, with *Penicillium* subgenus *Biverticillium* species transferred to the

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monophyletic genus *Talaromyces* (treated in chapter 4). Houbraken & Samson (2011) divided *Penicillium* into 25 sections based on this phylogeny and additional data from previous studies, which focused on specific closely related groups such as the section *Lanata-Divaricata* and *Sclerotiora* (Mercado-Sierra *et al*. 1998, Peterson *et al*. 2000, Peterson 2000, Pitt *et al*. 2000, Peterson & Sigler 2002, Frisvad & Samson 2004, Peterson *et al*. 2005 Peterson & Horn 2009, Wang & Zhuang 2009, Barreto *et al*. 2011, Houbraken *et al*. 2010, Houbraken *et al*. 2011a,b). This follows the idea of the Peterson (2000) study, which used an ITS phylogeny to show that the use of conidiophore branching patterns for subgeneric classification of *Penicillium* is superficial and did not reflect true evolutionary events in the genus. He also proposed that clades observed in the ITS phylogeny, could represent a subgeneric classification in future. The ITS region was recently instated as the official DNA barcode for fungi (Schoch *et al*. 2012). As such, we used ITS to classify Fynbos strains into the sections proposed by Houbraken & Samson (2011). To a large extent, the same phylogenetic clades/sections were observed for the ITS phylogeny in this study (CHAPTER 2, FIGURE 1). However, not all taxa used in the Houbraken & Samson (2011) study were included in this phylogeny, which might have an influence on observed clades. Although ITS generally do not distinguish between closely related species (Skouboe *et al*. 1999, Peterson 2000, Seifert *et al*. 2007, Houbraken *et al*. 2011), it was found to be useful for the classification of species on a sectional level. For classification on a species level, the β-tubulin, Calmodulin, Elongation Factor 1-α and RPB2 genes were used. However, β-tubulin, Calmodulin and Elongation Factor 1-α contain variable introns that make alignments across a diverse genus such as *Penicillium* problematic (Seifert *et al*. 2007, Houbraken & Samson 2011). As such, these genes were used in phylogenies that focused on each section. Representative Fynbos strains were also included in RPB2 phylogenies done for each section. Even though the Elongation Factor 1-α dataset is very restricted, representative sequences for Fynbos species are provided for a number of clades for use in future studies. Since ITS do not distinguish between closely related *Penicillium* species, a secondary barcode is necessary (Seifert *et al*. 2007, Schoch *et al*. 2012). Currently there is no gene selected as this secondary barcode. Data obtained from this study, show that the combinations of the above mentioned genes provided reliable and consistent results for most species recognized in this study. Since RPB2 is very easy to align across the family *Trichocomaceae* (Houbraken & Samson 2011), it has been suggested as a candidate for a secondary barcode (Houbraken, Seifert personal communication). The gene also contains enough phylogenetic information to distinguish between closely related species. However, we found RPB2 to be a challenge to reliably amplify, much more so than for β-tubulin and Calmodulin. The difficulty to amplify this region thus defeats the purpose of a DNA barcode (Seifert *et al*. 2007, Schoch *et al*. 2012). Since alignments should not present an issue for comparisons between closely related species, we propose the use of β-tubulin as a secondary barcode, because it is easy to amplify, contains enough phylogenetic information and importantly has a big database available. However, for taxonomic studies the RPB2 genes have obvious advantages above both β-tubulin, mainly alignments, and should thus be included in all future taxonomic studies.

In a rather broad study like this, DNA phylogenies were considered as the only logical method for comparisons to previously described *Penicillium* species. However, morphological comparisons between phylogenetically closely related species were done where possible. Full DNA sequence databases were thus crucial for this study, in order to identify close relatives. Attempts were made to have at least one representative sequence for each recognized *Penicillium* and *Talaromyces* species in the dataset, preferably its ex-type culture. However, a minor proportion of ex-type culture sequences were missing from this study. Most notable was species from the section *Torulomyces* (= genus *Torulomyces*), which we could not obtain. In a number of other sections, dataset were not always complete for all the genes studied. However, between the different genes most accepted species were represented. More important was the problem we found when comparing species with only one representative strain. Intraspecies variation exists. This became apparent in this study, with *P. sumatrense* as a good example (Houbraken *et al*. 2011). This species showed variation in its morphology and DNA sequences. Clades observed for *P. sumatrense* strains from Fynbos correlated with these morphological observations. However, when all the strains identified as *P. sumatrense* by Houbraken *et al*. (2011) are added to the comparisons, these differences became less pronounced and no concordance between data was observed. For the species accepted and described here, in cases where variation was observed and delineation was not clear a broad species concept was applied (eg. *P. rubefaciens* and *P. restrictum*). In these cases, the ideal situation would thus be to find additional representative strains from different habitats and study the relationship between these groups of strains and possibly species.

The circumscription of a large number of species in *Penicillium* is still unresolved. This complicated the classification of Fynbos species. Broad species

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concepts were thus also applied in these cases. One clade of Fynbos strains (CV6, CV7, CV15 and CV728) resolved as distinct but very close to *P. glabrum* (CHAPTER 2, FIGURE 10). Morphological differences include a red colony reverse for the Fynbos strains. However, without a thorough review of species delineation in *P. glabrum*, this clade cannot be described as novel. Strains identified as *P. thomii* (FIGURE 10) were also problematic. Morphologically, Fynbos strains represented two distinct morphogroups. However, phylogenetically they represented three species. Representative strains of previously assigned synonym species of *P. thomii* could not be obtained for comparisons. However, strain CV1189 and the CV851 clade seems to be distinct from *P. thomii*. Strain CV461 is most probably *P. crocicola* based on RPB2 data. It is interesting to note that tree topology in this clade shifts depending on the gene analyzed. With the uncertainty about the circumscription of *P. thomii*, the Fynbos strains were identified as two morphogroups in *P. thomii.*

Similar taxonomic issues were also observed in section *Exilicaulis*, most notably *P. rubefaciens*. In this particular clade, the phylogenetic position of strain CV1015 (FIGURES 2, 3, 5) shifted depending on the gene analyzed. Morphologically, this group of strains shows minor differences that seem to be clade specific. Without a broader range of morphological characters and possibly secondary metabolite data, as well as additional strains added into comparisons, these four phylogenetic clades have to be considered as part of the *P. rubefaciens* complex. Additional examples of broad species concepts accepted in this study are *P. restrictum* (FIGURES 2, 4, 5), *P. fynbosense* (FIGURES 15, 16) and *P. novae-zeelandiae* (FIGURES 15, 16). Although broad species concepts were often applied for a number of problematic taxa, this study provides a good basis to start to address these problematic taxa. The large number of strains isolated for instance that were identified as *P. restrictum* will prove to be valuable for delineation of the large number of species in this complex. Additional characters on additional media or secondary metabolite analysis are suggested in order to resolve these.

Houbraken & Samson (2011) highlighted the need for finding morphological or physiological characters that define the particular sections of *Penicillium*. Although many of the species in each section share very similar morphologies, there always seem to be exceptions. In the biverticillate clade of section *Exilicaulis*, all species examined produced rough walled stipes. However, *Penicillium corylophilum* was one exception that is characterized by smooth walled stipes. Colony morphology on CYA, MEA and YES does, however, clearly illustrate the close relationship between these species. Previously described section *Aspergilloides* species typically grow fast on media. However, from Fynbos a group of four new species were introduced into the section that has restricted growth. At first, the aim of the identification key was to have morphological characters guide the user to a particular section, from where section specific keys could be used. This was attempted, but after no morphological feature could be found that reliably linked all species to their specific sections, it was abandoned. The use of statistical analysis such as multivariate analysis might prove useful for determining if and which combination of morphological characters is taxonomically informative for each section. This type of analysis will prove invaluable for creating a key to all species in the genus.

Based on a polyphasic species concept, the Fynbos study identified and described 61 *Penicillium* species (25 novel species) and 15 *Talaromyces* species (5 novel species). These Fynbos species represented a diverse range of sections and morphological characters. The biggest proportion of isolates belonged to sections *Exilicaulis* and *Aspergilloides*. This was mainly due to *P. glabrum*, *P. toxicarium* and *P. atrolazulinum* that were present in almost all samples analyzed. *Penicillium bilaiae* and the new species *P. compactum* from section *Sclerotiora*, were also well represented in most samples. The omnipresence of *P. glabrum* is probably not unexpected since it is commonly isolated worldwide from a wide range of habitats (Raper & Thom 1949, Pitt 1979, Barreto *et al*. 2011). *Penicillium bilaiae* is reported to have soil as its primary habitat. However, we found *P. bilaiae* to also be common in *Protea repens* infructenscences. *Penicillium toxicarium* was reported to be common on rice from Japan (Uraguchi 1971, Pitt 1979) and Serra *et al*. (2008) reported that *P. toxicarium* is common on cork planks from Portugal. We also found *P. toxicarium* in all samples collected, except for the air. From the dilution technique used in the study, *P. toxicarium* was also observed to be the most abundant species in infructescences. Also, together with *P. atrolazulinum*, it was always present on plates incubated with mites from the infructescences. On these plates, it was noted that mites always preferred to digest and lay eggs in the bright yellow floccose colonies of *P. toxicarium*. Whether this is a true association or by pure chance will be investigated in a future study. This study forms part of a big future project that will focus on distribution and dispersal patterns of *Penicillium* and *Talaromyces* species in South Africa. One aspect of this is to investigate preferred/primary habitats of the different species. In other words, where do the diversity we see in Fynbos come from? This idea was explored in CHAPTER 4. However, it is clear from our data that species might have a much wider host range than originally thought. *Penicillium* is often considered to have evolved from soil (Pitt

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1979). *Protea* infructescences, one of the main focus areas for this study, present fungi with a unique microhabitat (Zwolfer 1979). Pirozynski & Hawksworth (1988) predicted that 50% of fungi that occur in microhabitats have evolved with their host. The idea that some *Penicillium* and *Talaromyces* species have evolved with *Protea* species is definitely interesting. Exploring this idea will unlock a large amount of new knowledge on the ecological behavior of these fungi. Where do they live, from where did they evolve, how are they dispersed from and into infructescences, are all questions that will be explored in future.

Schutte (1992) mentioned the need for the classification of South African *Penicillium* species using modern taxonomic concepts, in order to reduce the large number of misidentifications common in South African literature. This study is the first survey of its kind for South Africa and creates the baseline knowledge needed for similar future studies. Descriptions were based on fresh isolates and thus represent the species as close to the wild type possible. Species were also characterized by multigene sequences. The sequences, combined with morphological data, generated in this study will thus serve as a reliable means for future identification of these species. The identification key included in this study will aid morphological identifications, with full color photoplates presented an important part of the identification process. Great care was taken to present the plates and descriptions in the most logical and user friendly way. As a result, this work is considered invaluable for not only species identifications, but also will form the template for future studies on *Penicillium* from South Africa.

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