



## Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family

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### Key words

arthropod vectoring  
beta-tubulin  
internal transcribed spacer region (ITS)

**Abstract** Current literature accepts 17 species in *Penicillium* section *Sclerotiora*. Several produce colonies in bright yellow to orange colours and have monoverticillate conidiophores, apart from *P. herquei*, *P. malachiteum* and *P. nodositatum*, which are biverticillate. The focus of this paper is to refine the concepts of the species currently accepted in the section and introduce five new species, named after the Dutch Royal family as *P. vanoranjei*, *P. maximae*, *P. amaliae*, *P. alexiae* and *P. arianae*. *Penicillium vanoranjei* produces orange (Dutch = oranje) colonies in culture, and is named after Willem-Alexander Claus George Ferdinand, 'Zijne Koninklijke Hoogheid de Prins van Oranje' (translated from Dutch as: 'His Royal Highness the Prince of Orange') and his family, to coincide with his coronation. We review the current taxonomic positions of *P. lilacinoechinulatum* and *P. nodositatum*, both currently considered to be synonyms of *P. bilaiae*. Sequence data generated in this study show that both species are phylogenetically distinct. *Penicillium lilacinoechinulatum* is closely related to *P. amaliae* sp. nov., whereas *P. nodositatum* does not belong to *Penicillium* sensu stricto. All species were compared morphologically and phylogenetically, based on  $\beta$ -tubulin and calmodulin DNA data. A table summarising the morphological characters of all species is included, together with photomicrographs and recommended DNA markers for identification.

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### INTRODUCTION

In anticipation of recent changes in the International Code of Nomenclature for algae, fungi and plants (ICN, previously known as the International Code of Botanical Nomenclature, ICBN), which ended dual nomenclature in fungi (McNeill et al. 2012), the family *Trichocomaceae* was revised using a four-gene phylogeny by Houbraken & Samson (2011). In that paper, *Penicillium* subgenus *Biverticillium* was separated from *Penicillium* sensu stricto (s.str.) and its species were combined into *Talaromyces* in a subsequent paper (Samson et al. 2011). Species of *Penicillium* s.str. were divided among two subgenera, *Aspergilloides* and *Penicillium*, and 25 sections (Houbraken & Samson 2011). The section *Sclerotiora* represents one of these. Seventeen species were included in section *Sclerotiora* by Houbraken & Samson (2011), most with monoverticillate conidiophores. Some exceptions are *P. herquei*, *P. malachiteum* and *P. nodositatum* (= *P. bilaiae* fide Houbraken & Samson 2011), which produce biverticillate conidiophores. In general, colonies have bright yellow or orange pigments, which may occur in mycelia, sclerotia, ascocarps, soluble pigments or colony reverse pigmentation (Pitt 1979, Savard et al. 1994, Houbraken & Samson 2011, Rivera & Seifert 2011). Also, species often produce loosely funiculate colony textures and conidiophores with short stipes, as seen in *P. bilaiae*, *P. hirayamae* and *P. viticola* (Nonaka et al. 2011).

Section *Sclerotiora* is phylogenetically well defined, with several recent studies introducing new species (Peterson 2000, Peterson et al. 2003, 2004, Nonaka et al. 2011, Rivera & Seifert 2011, Rivera et al. 2012). Rivera & Seifert (2011) addressed the problematic *P. sclerotiorum* complex using a five-gene phylogeny combined with morphological studies, introducing three new species and a phylogenetically accurate description for *P. sclerotiorum*. In a subsequent paper, Rivera et al. (2012) introduced two additional species to the complex.

In this paper, we describe five new species isolated from soil. Although this group is phylogenetically well studied, several species descriptions have not been updated using modern, standardized techniques. Therefore, we summarise the most important morphological characters in table format and provide photomicrographs for the remaining species of section *Sclerotiora* not treated by Rivera & Seifert (2011) or Rivera et al. (2012).

Several names associated with section *Sclerotiora*, including putative synonyms of some species, are also considered in this paper. Based on sequence data, *P. nodositatum* was tentatively placed in synonymy with *P. bilaiae* by Houbraken & Samson (2011). The uncertainty reflected the fact that the latter species is strictly monoverticillate, whereas *P. nodositatum* was described as biverticillate (Valla et al. 1989). The synonymy of *P. nodositatum* with *P. bilaiae* would thus be surprising, and is reconsidered below. *Penicillium multicolor* was previously mentioned as a problematic taxon (Pitt 1979, Houbraken & Samson 2011, Rivera & Seifert 2011). The descriptions by Grigorieva-Manoilova & Poradielova (1915) and Raper & Thom (1949) are at odds, and Pitt (1979) considered *P. multicolor* a dubious name. Two different strains have been considered to represent ex-type cultures, namely (CBS 501.73), closely related to *P. fellutanum* (Houbraken & Samson 2011, Rivera & Seifert 2011), and Raper & Thom's strain (NRRL 2060 = CBS

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134565), considered the ex-type strain by Peterson & Horn (2009). The latter has unique sequences in section *Sclerotiora* (Peterson & Horn 2009, Houbraken & Samson 2011) and we formally describe it as *P. maximae* below. Finally, reconsideration of *P. lilacinoechinulatum*, previously considered a synonym of *P. bilaiae* (Pitt 1979, Pitt et al. 2000), shows it to be a distinct species in section *Sclerotiora*.

## MATERIALS AND METHODS

### Strains

Reference and ex-type strains (summarised in Table 1) were obtained from the public collection of the CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands (CBS), with additional strains retrieved from the working culture collection of the Applied and Industrial Mycology department (DTO) at the same institution. Some strains were originally received and deposited into the collection from J.I. Pitt (Australia), C. Silva Pereira (Portugal), C.M. Visagie and K. Jacobs (South Africa). Many of the South African strains are also deposited in the Canadian Collection of Fungal Cultures associated with the herbarium DAOM, Agriculture & Agri-Food Canada, Ottawa. Raper & Thom's *P. multicolor* strain (NRRL 2060) was provided by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Peoria, United States.

### Morphology

Great care was taken to use standardised conditions for culture incubation (Okuda et al. 2000). Media were dispensed in 90 mm Petri dishes with a volume of 20 mL. Strains were plated onto media from spore suspensions in a three-point pattern using a micropipette and inocula of 0.5–1 µL. Cultures were incubated for 7 d in the dark (Okuda et al. 2000) as described below. Each species was characterised using colony characters on Czapek yeast autolysate agar (CYA), malt extract agar (Oxoid) (MEA), yeast extract sucrose agar (YES), dichloran 18 % glycerol agar (DG18), CYA supplemented with 5 % NaCl (CYAS) and creatine sucrose agar (CREA) grown at 25 °C, with additional CYA plates incubated at 30 and 37 °C. After incubation, strains were described using the models of Pitt (1979) and Frisvad & Samson (2004). All colour names and codes refer to the Methuen Handbook of Colour (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, conidiophores were washed with 70 % ethanol, then mounted in lactic acid to release air bubbles and wash away excess spores. Microscopic examinations were made using a stereo- (Olympus SZX12) and light-microscope (Olympus BX50 and Zeiss Axioskop 2 Plus). Pictures were taken using an Evolution MP digital microscope camera and ImagePro v. 6.0 software. Conidiophore structures were measured with ImagePro v. 6.0 and Nikon NIS-elements D v. 4.0. In species descriptions, average measurements and standard deviations are provided between square brackets, generally based on 30–50 measurements. Plates of photomicrographs were assembled using Adobe® Photoshop® Creative Suite v. 6. The healing brush tool was used to clean up some images for aesthetic reasons, without altering parts of the images of scientific relevance. Colony textures were captured using multiple focal planes and assembled with Helicon Focus v. 4.2 Z-stacking software.

### Low-temperature scanning electron microscopy (SEM)

Strains of *P. vanoranjei* were grown for 2–3 wk on MEA. By this time, micro-colonies with typical sclerotia and conidiophores had developed next to the three major colonies. Colonies were

selected using a dissecting microscope (10–50× magnification, Nikon SMZ 1500), and agar blocks (5 × 5 mm) were cut out with a surgical blade and carefully transferred into a copper cup (diam 10 mm, height 8 mm). To prevent dislodging during freezing, agar blocks were glued to the copper cup with frozen tissue medium (KP-Cryoblock, Klinipath, Duiven, the Netherlands) mixed with one part colloidal graphite (Agar Scientific, Stansted, UK). The copper cup was placed onto wet agar to maintain humid conditions and prevent drying of the sample. The sample was snap-frozen in nitrogen slush and immediately transferred to a JEOL 5600LV scanning electron microscope (JEOL, Tokyo, Japan) equipped with an Oxford CT1500 Cryostation for cryo-electron microscopy (cryoSEM). The sample was sputter-coated by means of a gold target for three times 90 s holding the sample at different angles for an optimal coating. Electron micrographs were acquired with the F4 scan at 4 kV and contrast levels digitally enhanced in Adobe® Photoshop® Creative Suite v. 6.

### DNA extraction, sequencing and phylogenetic analysis

Representative strains for each species were grown on MEA and DNA extracted using the Ultraclean™ Microbial DNA isolation Kit (MoBio, Solana Beach, USA). DNA preps were stored at -20 °C until used for PCR.

Amplification of target genes employed Kapa ReadyMix (Kapa Biosystems, Woburn, USA). Reactions had a final volume of 25 µL, consisting of 12.5 µL ReadyMix, 10.5 µL MilliQ H<sub>2</sub>O, 1 µL DNA and 0.25 µM of both forward and reverse primers. Primer pairs used for amplification and sequencing included ITS1–ITS4 (White et al. 1990) for internal transcribed spacer regions of the nrDNA operon (ITS), Bt2a–Bt2b (Glass & Donaldson 1995) for partial β-tubulin and CMD5–CMD6 (Hong et al. 2006) for partial calmodulin. The thermocycling conditions for amplifications had an initial denaturing step of 94 °C for 5 min, 36 cycles of 94 °C for 45 s, 56 °C for 45 s, 72 °C for 60 s, followed by a final elongation step at 72 °C for 10 min. For some strains, annealing temperatures were dropped to 52 °C to enable better amplification. Sequence reactions were set up using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, CA). The thermocycling 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, with sequences determined on an ABI PRISM 3730xl genetic analyser.

Sequence contigs were assembled and edited using Codon-Code Aligner v. 4.0.1 (CodonCode Corporation, Centerville, USA). A sequence database was established using newly generated sequences and those previously published in GenBank. Strains used for sequence comparisons and their GenBank accession numbers are summarised in Table 1. Alignments were done using MAFFT v. 6.850b (Katoh et al. 2009) using the G-INS-I option. NEXUS files were analysed in PAUP v. 4.0b10 (Swofford 2002) using the BioNJ option (Gascuel 1997) and MEGA v. 5.1 using Maximum Likelihood with the Tamurai-Nei model and Nearest-Neighbour-Interchange option selected. Confidence levels in nodes were determined using bootstrap analyses of 1 000 replicates. Alignment matrices and tree files were deposited in TreeBASE ([www.treebase.org](http://www.treebase.org)) with submission ID 13796. Newly generated sequences were submitted to GenBank, and their accession numbers are included in Table 1.

## RESULTS

### Molecular markers, phylogeny and morphology

Phylogenetic and morphological results show that section *Sclerotiora* contains 22 species, including the five that are newly described here. ITS barcodes were generated for these

Table 1 Strains used for phylogenetic analyses of *Penicillium* section *Sclerotiora*.

Species	Strains	Substrate and locality	GenBank accession no.		
			ITS	Btub	GMD
<i>P. adameitzi</i>	CBS 209.28 <sup>T</sup> = ATCC 10407 = IMI 039751 = NRRL 737	Soil under conifers, Poznan, Poland	JN714929	JN625957	KC773796
	DTO 190A8	Soil, Spanderswoud, Netherlands	KC773822	KC773772	KC773797
<i>P. adameitzioides</i>	CBS 313.59 <sup>T</sup> = ATCC 18306 = IMI 068227 = NRRL 3405	Soil, Japan	JN686433	JN799642	JN686387 <sup>x</sup>
	DTO 78A7 = IBT 23667	Unknown	KC773825	KC773775	KC773800
	DTO 78A9 = IBT 27906	Unknown	KC773826	KC773776	KC773801
	DTO 78F2 = IBT 10870	Unknown	KC773827	KC773777	KC773802
<i>P. alexiae</i>	CBS 134558 <sup>T</sup> = DTO 118H8	<i>Quercus suber</i> forest soil, Tunisia	KC790400	KC773778	KC773803
	CBS 134209 <sup>T</sup> = DTO 183F3 = DAOM 241034 = CV1875	<i>Protea repens</i> infructescence, Stuisbaai, South Africa	JX091443	JX091563	JX141557
<i>P. analiae</i>	CBS 134211 = DTO 181C6 = CV 204	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091444	JX091560	JX141554
	CBS 134212 = DTO 181F7 = DAOM 241031 = CV 401	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091440	JX091558	JX141556
	CBS 134555 = DTO 181A9 = DAOM 241032 = CV 112	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091441	JX091559	JX141553
	CBS 134556 = DTO 181C9 = DAOM 241033 = CV 227	<i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091445	JX091561	JX141555
	CBS 134557 = DTO 57A8	Insect larva, New South Wales, Australia	KC790401	KC773788	KC779542
	CBS 130293 <sup>T</sup> = IBT 27051 = NRRL 28157	Polypore on dead conifer stump, New Mexico, USA	AY313613	KC773779	KC773804
	DTO 190B8	Soil, Spanderswoud, Netherlands	KC773829	KC773780	KC773805
DTO 41A2	Soil, Poland	KC773830	KC773781	KC773806	
DTO 41E6	Soil, Poland	KC773831	KC773782	KC773807	
DTO 42A9	Soil, Poland	KC773832	KC773783	KC773808	
NRRL 35630	Cork bark, Alentejo, Portugal	EF200087	EF198554	EF198582	
NRRL 35633	Cork bark, Alentejo, Portugal	EF200088	EF198555	EF198583	
<i>P. arianeae</i>	CBS 134559 <sup>T</sup> = DTO 20B8	Soil, Spanderswoud, Netherlands	KC773833	KC773784	KC773811
	CBS 221.66 <sup>T</sup> = ATCC 22348 = IMI 113677 = NRRL 3391	Soil, Kiev, Ukraine	JN714937	JN625966	JN626009
<i>P. bilatae</i>	CBS 330.90	Unknown	KC773834	KC773785	KC773812
	DTO 181D8 = CV 255	Mite inside <i>Protea repens</i> infructescence, Stellenbosch, South Africa	JX091437	JX091565	JX141560
<i>P. brocae</i>	CBS 116113 <sup>T</sup> = IBT 26293 = NRRL 31472	Coffee berry borer faeces, Tapachula, Chiapas, Mexico	KC773835	KC773787	KC773814
	DAOM 239914 <sup>T</sup>	Nuts of <i>Juglans nigra</i> , Niagara, Canada	JN686435	JN686366	JN686389 <sup>x</sup>
<i>P. cainii</i>	DAOM 239915	Nuts of <i>Carya ovata</i> , Niagara, Canada	JN686436	JN686367	JN686390
	DAOM 239912 <sup>T</sup>	Gut of the caterpillar <i>Eutelia</i> sp. reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626098	JN625967	JN626010
<i>P. guanacastense</i>	DAOM 239913	Gut of the caterpillar <i>Eutelia</i> sp. reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626099	JN625968	JN626011
	CBS 336.48 <sup>T</sup> = ATCC 10118 = IMI 28809 = NRRL 1040	Leaf of <i>Agauria pirifolia</i> , France	JN626101	JN625970	JN626013
<i>P. herquei</i>	CBS 136.22 = NRRL 2113	Unknown, France	JN626100	JN625969	JN626012
	CBS 347.51 = ATCC 18237 = IMI 107651 = NRRL 3450	Ex-type of <i>P. luteooceruleum</i> , Wakamoto corn and rice cake, Nehira, Japan	JN617703	JN625971	JN626014
<i>P. herquei</i>	CBS 229.60 <sup>T</sup> = ATCC 18312 = IMI 078255 = NRRL 143	Milled rice, Thailand	JN626095	JN625955	JN626003
	CBS 134207 = DTO 182B9 = DAOM 241115 = CV 887	Fynbos soil, Riverlands Nature Reserve, Malmesbury, South Africa	JX091453	JX091572	JX141568
<i>P. hirayamae</i>	CBS 134208 = DTO 182D2 = DAOM 241116 = CV 916	Fynbos soil, Riverlands Nature Reserve, Malmesbury, South Africa	JX091454	JX091573	JX141569
	CBS 238.65	Corn meal, South Africa	JN626096	JN625956	JN626004
<i>P. jacksonii</i>	DAOM 239937 <sup>T</sup>	Forest soil, Queensland, Australia	JN686437	JN686368	JN686391
	DAOM 239938	Forest soil, Queensland, Australia	JN686438	JN686369	JN686392
<i>P. johnkrugii</i>	DAOM 239943 <sup>T</sup>	Forest soil, Langkawi, Kedah, Malaysia	JN686447	JN686378	JN686401
	DAOM 239939	Rainforest soil, Langkawi, Kedah, Malaysia	JN686443	JN686374	JN686397
	DAOM 239940	Forest soil, Langkawi, Kedah, Malaysia	JN686444	JN686375	JN686398
	DAOM 239941	Forest soil, Langkawi, Kedah, Malaysia	JN686445	JN686376	JN686399
	DAOM 239942	Forest soil, Langkawi, Kedah, Malaysia	JN686446	JN686377	JN686400
	DAOM 239944	Forest soil, Langkawi, Kedah, Malaysia	JN686448	JN686379	JN686402
	DAOM 239945	Forest soil, Langkawi, Kedah, Malaysia	JN686449	JN686380 <sup>x</sup>	JN686403
<i>P. jugoslavicum</i>	DAOM 239946	Forest soil, Langkawi, Kedah, Malaysia	JN686450	JN686381 <sup>x</sup>	JN686404
	CBS 192.87 <sup>T</sup> = IMI 314508	Seed of <i>Helianthus annuus</i> , Yugoslavia	KC773836	KC773789	KC773815
<i>P. levitum</i>	CBS 345.48 <sup>T</sup> = ATCC 10464 = IMI 039735 = NRRL 705	Modelling clay, USA	GU981607	JN714938	JN714939
	CBS 454.93 <sup>T</sup> = ATCC 18309 = IMI 068211	Soil, Japan	KC773837	KC773790	KC773816
<i>P. lilacinoehinulatum</i>	CBS 134563 = DTO 17E2	Soil, Spanderswoud, Netherlands	KC773791	KC773791	KC773817
	CBS 134564 = DTO 17I3	Soil, Spanderswoud, Netherlands	KC773792	KC773792	KC773818
	CBS 134560 = DTO 42A2	Soil, Poland	KC773793	KC773793	KC773819



<i>P. malachiteum</i>	CBS 647.95 = IBT 17515	Soil, Japan	KC773838	KC773794	KC773820
<i>P. mallochii</i>	DAOM 239917 <sup>†</sup>	Caterpillar on <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626104	JN625973	JN626016
	DAOM 239919	Midgut of the caterpillar <i>Ciftheronia lobesis</i> reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626106	JN625975	JN626018
	DAOM 239922	Hindgut of the caterpillar <i>Rothschildia lebeau</i> reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626109	JN625978	JN626021
	DAOM 239925	Guts of the caterpillar <i>Ciftheronia lobesis</i> reared on leaves of <i>Cochlospermum vitifolium</i> , Santa Rosa, Costa Rica	JN626112	JN625980	JN626023
	DAOM 239926	Frag of the caterpillar <i>Rothschildia lebeau</i> reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626111	JN625981	JN626024
	DAOM 239927	Gut of the caterpillar <i>Rothschildia lebeau</i> reared on leaves of <i>Spondias mombin</i> , Santa Rosa, Costa Rica	JN626113	JN625982	JN626025
<i>P. maximae</i>	CBS 134565 <sup>†</sup> = NRRL 2060	Weathering treated cellophane, Florida, USA	EU427298	KC773795	KC773821
<i>P. multicolor</i>	CBS 501.73 <sup>†</sup> = ATCC 24723 = IMI 174716	Soil, Russia	KC790402	JN799645	JN799646
<i>P. sclerotiorum</i>	CBS 287.36 <sup>†</sup> = ATCC 10494 = IMI 040569 = NRRL 2074	Air, Java, Indonesia	JN626132	JN626001	JN626044
	CBS 118889	Soil, Korea	JN686454	JN686385 <sup>x</sup>	JN686408
	CBS 128.65	Forest litter, Leopoldville, Zaire	JN686452	JN686383 <sup>x</sup>	JN686406
	CBS 258.55	Culture contaminant, Istanbul, Turkey	JN686453	JN686384 <sup>x</sup>	JN686407
	DAOM 239930	Forest soil, Hua Hin, Thailand	JN626129	JN625998	JN626041
	DAOM 239931	Forest soil, Barron Falls, Queensland, Australia	JN626130	JN625999	JN626042
	DAOM 239932	Forest soil, Barron Falls, Queensland, Australia	JN626131	JN626000	JN626043
	NRRL 32583	Coffee seeding crown, Kauai, Hawaii, USA	JN626133	JN626002	JN626045
<i>P. vanoranjei</i>	CBS 134406 <sup>†</sup> = DTO99H6	<i>Quercus suber</i> forest soil, Tunisia	KC695696	KC695686	KC695691
	CBS 134404 = DTO99F3	<i>Quercus suber</i> forest soil, Tunisia	KC695694	KC695684	KC695689
	CBS 134405 = DTO99G1	<i>Quercus suber</i> forest soil, Tunisia	KC695695	KC695685	KC695690
	CBS 134407 = DTO119G8	<i>Quercus suber</i> forest soil, Tunisia	KC695692	KC695682	KC695687
	CBS 134408 = DTO120C8	<i>Quercus suber</i> forest soil, Tunisia	KC695693	KC695683	KC695688
	DAOM 239933	Forest soil, Barron Falls, Queensland, Australia	JN686439	JN686370	JN686393
	DAOM 239934	Forest soil, Atherton, Queensland, Australia	JN686440	JN686371	JN686394
	DAOM 239935	Rainforest soil, Atherton, Queensland, Australia	JN686441	JN686372	JN686395
	DAOM 239936	Rainforest soil, Atherton, Queensland, Australia	JN686442	JN686373	JN686396

<sup>x</sup> In Rivera et al. (2011) some GenBank accession numbers were listed incorrectly and are corrected here. Those published as JN686779–686788 are correctly JN686379–686388

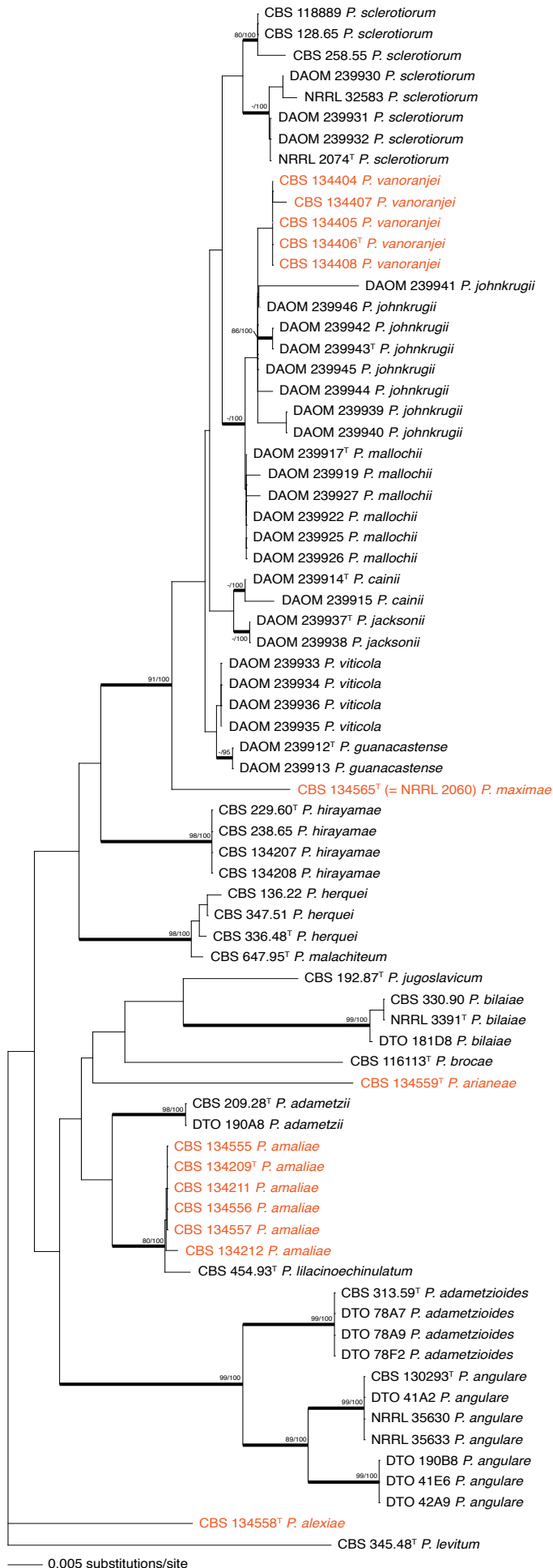
new species and are listed in Table 1. Analysis of the ITS,  $\beta$ -tubulin and calmodulin gene regions resulted in coherent, monophyletic clades (Fig. 1 & 2). The aligned datasets for ITS,  $\beta$ -tubulin and calmodulin were respectively 513, 355 and 478 bp long, including alignment gaps. Tree topologies were identical for both Maximum Likelihood and Neighbour-Joining analysis. ITS barcodes were suitable identification markers, with all species having unique sequences, although some have only two or three base pair differences, i.e. *P. malachiteum* and *P. herquei*, as well as *P. amaliae* and *P. lilacinoechinulatum* (Fig. 1). Therefore, as is normal in *Penicillium*, a secondary identification marker is recommended for accurate and robust identification of sibling species. ITS barcodes and proposed markers for all species accepted in the section are summarised in the taxonomy section below and in Table 1. Intraspecific sequence variations seem to be the norm for section *Sclerotiora*, as also noted by Rivera & Seifert (2011). This is especially true among strains of *P. hirayamae*, *P. johnkrugii*, *P. mallochii*, *P. angulare*, *P. amaliae* and *P. sclerotiorum*. However, morphological examination revealed no consistent morphological or phylogenetic characters that could separate putative species within these clades.

The phylogenies consistently resolved strains into three main clades (Fig. 2). These include the *P. sclerotiorum* complex (clade 1), species closely related to *P. adametzii* (clade 2) and the *P. herquei* complex (clade 3). Assignment of specific morphological characters to individual clades is not straightforward. Characters are summarised in Table 2 and in Fig. 3–5. The *P. herquei* complex contains two species that produce biverticillate conidiophores, in contrast with species assigned to clades 1 and 2, which are monoverticillate. Clades 1 and 2 are more difficult to define. Generally, species in clade 1 have colonies in orange colours and lack the strongly coloured, soluble pigments such as those generally seen in species of clade 2. However, *P. johnkrugii* (clade 1) lacks orange colours in colonies, because the sclerotia remain white or grey on media other than MEA. Micromorphology also cannot be used to characterise clades 1 and 2. Species of both clades are monoverticillate, typically have vesiculated stipes and produce conidia in a variety of shapes and ornamentations.

Our phylogenetic analysis confirmed the status of NRRL 2060 (CBS 134565) as a unique taxon, as suggested by Peterson & Horn (2009) and Houbraeken & Samson (2011), described as *P. maximae* below. The ex-type strain of *P. lilacinoechinulatum* (CBS 454.93) resolved separately from *P. bilaiae*, along with several strains previously deposited in the DTO collection, and as a sister taxon to *P. amaliae*, newly described below. Morphologically, *P. lilacinoechinulatum* has weaker sporulation on most media, and slower growth on CYAS (Fig. 3). More noticeable, however, is the absence of brown or yellow exudates consistently produced in *P. bilaiae* on CYA and its slower growth at 37 °C. Compared to *P. amaliae*, *P. lilacinoechinulatum* has stronger acid production on CREA and more restricted growth at 37 °C. Newly generated sequences of the ITS (KC790403) and  $\beta$ -tubulin (KC790399) gene regions of *P. nodositatum* (CBS 333.90, ex-type) show that it is closely related to *P. kabunicum* and does not belong in *Penicillium* s.str. (Houbraeken & Samson 2011). This corresponds with morphological features of its conidiophores, which are symmetrically biverticillate. The phialides are not penicillium-like, but are broad and taper into very fine and long necks, rather similar to those of some *Talaromyces* species, e.g. *T. verruculosus*.

The phylogenies revealed that several strains with unique morphological characters are grouped in distinct clades. These are considered new species and described in the taxonomy section as *P. vanoranjei*, *P. maximae*, *P. amaliae*, *P. alexiae* and *P. arianae*. Following these descriptions, morphological

ITS



characters of the remaining section *Sclerotiora* species not treated by Rivera & Seifert (2011) are summarised in Table 2 and illustrated in Fig. 3–5.

### Scanning electron microscopy of *P. vanoranjei*

Characters observed from samples prepared from a well developed colony and a young microcolony were similar (Fig. 7). Sclerotia had a highly complex and well organised structure. Cell walls were distinctly and consistently roughened and cells appeared to be linked by some kind of extracellular matrix (Fig. 7e). On the majority of the sclerotia, characteristic sheet-like structures were observed. Conidial roughening and size develop with age (Fig. 7h–j).

### TAXONOMY

*Penicillium vanoranjei* Visagie, Houbraken, Samson, *sp. nov.*  
— MycoBank MB803782; Fig. 3, 5–7

*ITS barcode.* KC695696.

*Alternative markers.* KC695686 ( $\beta$ -tubulin), KC695691 (calmodulin).

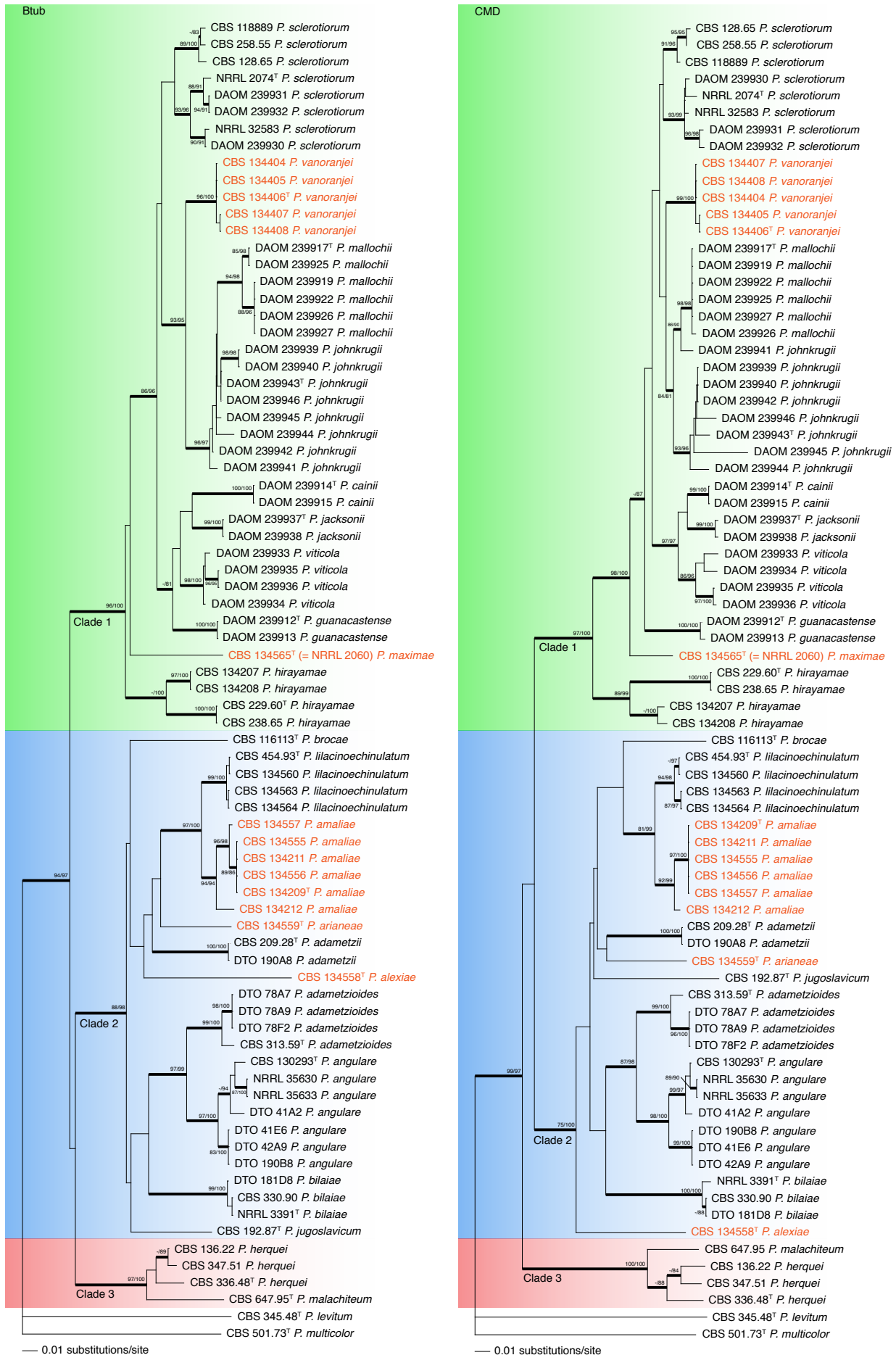
*Etymology.* Latin, *vanoranjei*: named, in reference to the orange (Dutch = oranje) coloured colonies produced by this species, after Willem-Alexander Claus George Ferdinand, 'Zijne Koninklijke Hoogheid de Prins van Oranje' (translated from Dutch as: 'His Royal Highness the Prince of Orange') and the new King of the Netherlands upon the retirement of Queen Beatrix on 30 April 2013.

*Diagnosis* — Bright orange sclerotia dominate colony appearance on most media. Conidiophores monoverticillate; stipes smooth walled, vesiculate; phialides 8.5–12.5  $\times$  3–3.5  $\mu$ m; conidia smooth, spheroidal to subspheroidal, 2.5–3.5  $\times$  2.5–3  $\mu$ m.

*Colony morphology* — Colony diam, 7 d, in mm: CYA 21–28; CYA 30  $^{\circ}$ C 25–30; CYA 37  $^{\circ}$ C 12–18; MEA 22–30; YES 30–35; DG18 15–25; CYAS 17–23; CREA 10–20.

CYA, 25  $^{\circ}$ C, 7 d: Colonies slightly raised at centre, radially sulcate, abundant bright orange sclerotia produced; margins low, narrow, entire; mycelia white near margin, orange elsewhere; texture floccose; sporulation absent to sparse, conidial colour *en masse* cannot be determined; exudate bright orange, soluble pigment inconspicuously orange, reverse pigmentation brownish orange to Burnt Sienna (6C7–7D8). CYA, 30  $^{\circ}$ C, 7 d: Colonies showing no differences from those grown on CYA at 25  $^{\circ}$ C. MEA, 25  $^{\circ}$ C, 7 d: Colonies slightly raised at centre, radially sulcate, abundant bright orange sclerotia produced; margins low, narrow, entire; mycelia white near margin, orange elsewhere; texture floccose; sporulation sparse and only at colony centre, conidial colour *en masse* difficult to determine precisely, greyish green; exudate bright orange, soluble pigment absent, reverse pigmentation brownish orange (6A8–6C8). YES, 25  $^{\circ}$ C, 7 d: Colonies moderately deep, raised at centre, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white near margin, orange elsewhere; texture floccose; sporulation absent; exudate absent, soluble pigment absent, reverse pigmentation brownish orange to Burnt Sienna (6C7–7D8). CREA, 25  $^{\circ}$ C, 7 d: Moderate to strong acid production. *Conidiophores* strictly monoverticillate; *stipes* smooth walled, 65–220  $\times$  2.5–3  $\mu$ m, vesicle 4.5–6  $\mu$ m (5.3  $\pm$  0.6); *phialides* ampulliform, 10–20 per stipe, 8.5–12.5  $\times$  3–3.5  $\mu$ m (10.6  $\pm$  0.9  $\times$  3.3  $\pm$  0.2); *conidia* smooth to slightly rough walled, connectives

**Fig. 1** Neighbour-Joining tree based on ITS nucleotide sequences, showing the relationship of species in the section *Sclerotiora*. *Penicillium levitum* (CBS 345.48<sup>T</sup>) was chosen as outgroup. Bootstrap values above 80 % for Maximum Likelihood and Neighbour-Joining are presented at nodes (ML-bs/NJ-bs), with a hyphen (-) indicating no support. (<sup>T</sup> = ex-type). Coloured names indicate strains that belong to the new species.



**Fig. 2** Neighbour-Joining trees based on β-tubulin and calmodulin nucleotide sequences, showing the relationship of species in the section *Sclerotiora*. *Penicillium levitum* (CBS 345.48<sup>T</sup>) and *P. multicolor* (CBS 501.73<sup>T</sup>) were chosen as outgroups for the β-tubulin and calmodulin phylogenies. Bootstrap values above 80 % Maximum Likelihood and Neighbour-Joining are presented at nodes (ML-bs/NJ-bs), with a hyphen (-) indicating no support. (T = ex-type). Coloured names indicate strains that belong to the new species.

Table 2 Summary of morphological features for the identification of species of *Penicillium* section *Sclerotiora*.

Species	Growth rates (in mm)						Colony characters				Conidiophores				Conidia	
	CYA	CYA 30 °C	CYA 37 °C	MEA	YES	DG18	CYAS	Acid on CREA	Sclerotia colour	Sclerotia	Soluble pigments	Reverse colour on CYA	Branching	Stipe roughening	Roughening	Shape
<i>P. canthii</i> <sup>1</sup>	23–29	n.a.	n.a.	28–35	31–34	n.a.	n.a.	strong	absent	absent	absent	golden to brownish yellow	monoverticillate/single metula rare	rough	finely rough	spheroid
<i>P. guanacastense</i> <sup>1</sup>	25–33	n.a.	n.a.	29–33	n.a.	n.a.	n.a.	strong	absent	absent/yellow	orange	monoverticillate/single metula rare	finely rough	finely rough	spheroid	
<i>P. hirayamae</i> <sup>1</sup>	25–33	30–33	25–33	25–30	29–35	8–15	29–33	weak	present	absent	orange	monoverticillate/single metula rare	smooth	smooth	spheroid to subspheroid	
<i>P. jacksonii</i> <sup>1</sup>	30–33	n.a.	n.a.	31–37	30–32	n.a.	n.a.	strong	absent	absent	yellow	monoverticillate/single metula rare	smooth to rough	finely rough	spheroid	
<i>P. johnkrugii</i> <sup>1</sup>	30–38	n.a.	n.a.	26–36	28–38	n.a.	n.a.	strong	present	white/grey	yellow/orange	monoverticillate	smooth to finely roughened	finely rough	spheroid to subspheroid	
<i>P. maillochii</i> <sup>1</sup>	29–39	n.a.	n.a.	19–35	n.a.	n.a.	n.a.	strong	absent	absent/orange	yellow/orange	monoverticillate/single metula rare	smooth to finely roughened	finely rough	spheroid to subspheroid	
<i>P. maxinae</i> <sup>1</sup>	34–37	32–37	no growth	34–37	40–43	15–22	27–30	absent	absent	absent	reddish brown	monoverticillate/single metula rare	smooth	smooth	ellipsoid	
<i>P. sclerotiorum</i> <sup>1</sup>	18–40	n.a.	n.a.	15–32	20–44	n.a.	n.a.	strong	present	orange	orange/reddish yellow/brown	monoverticillate	smooth to finely roughened	finely rough	subspheroid to ellipsoid	
<i>P. vanoranjii</i> <sup>1</sup>	21–28	25–30	12–18	22–30	30–35	15–25	17–23	moderate to strong	present	bright orange	brownish orange/reddish brown	monoverticillate	smooth	smooth to finely rough	spheroid to subspheroid	
<i>P. viticola</i> <sup>1</sup>	26–36	n.a.	n.a.	25–40	29–33	n.a.	n.a.	strong	absent	absent	yellow / orange	monoverticillate/single metula rare	rough	smooth	spheroid	
<i>P. adametzii</i> <sup>2</sup>	27–35	27–37	10–16	28–35	27–36	9–16	18–21	absent	absent	absent	pale to yellow brown	monoverticillate	smooth	smooth to finely rough	spheroid to subspheroid	
<i>P. adamezoides</i> <sup>2</sup>	20–26	15–20	no growth	20–26	20–30	17–21	15–24	weak	absent	reddish brown	deep brown	monoverticillate	smooth	smooth to finely rough	subspheroid	
<i>P. alexiae</i> <sup>2</sup>	18–21	8–15	no growth	18–21	21–26	15–17	15–18	absent	present	white	brown	monoverticillate	smooth	smooth	subspheroid	
<i>P. amaliae</i> <sup>2</sup>	20–30	22–30	3–11	27–33	30–36	14–24	24–27	weak	absent	n.a.	light to dull yellow	monoverticillate	smooth	rough	spheroid to subspheroid	
<i>P. angulare</i> <sup>2</sup>	13–25	2–5	no growth	10–20	16–28	12–20	10–20	weak to moderate	absent	absent	pale yellow	monoverticillate	smooth	smooth	subspheroid to ellipsoid	
<i>P. arianaeae</i> <sup>2</sup>	9–12	no growth	no growth	10–14	14–16	11–15	9–10	absent	absent	n.a.	pale orange/greyish yellow	monoverticillate	smooth	thick rough walled	spheroid	
<i>P. bilaiae</i> <sup>2</sup>	25–33	25–30	13–15	25–28	29–31	20–22	25–29	strong	absent	n.a.	deep brown	monoverticillate/single metula rare	smooth	rough	spheroid to subspheroid	
<i>P. brocae</i> <sup>2</sup>	17–23	15–18	no growth	14–22	25–32	14–16	16–20	moderate	absent	absent	yellow	monoverticillate	smooth	finely rough	spheroid	
<i>P. jugoslavicum</i> <sup>2</sup>	20–22	no growth	no growth	21–25	27–30	15–17	24–25	moderate	absent	absent	pale yellow	monoverticillate	smooth	finely rough to rough	ellipsoid	
<i>P. ilicinochinulatum</i> <sup>2</sup>	25–30	30–33	2–4	27–32	32–35	17–20	22–25	moderate	absent	absent	light to dull yellow	monoverticillate	smooth	rough	spheroid to subspheroid	
<i>P. herquelii</i> <sup>3</sup>	25–35	25–34	no growth	30–40	30–40	15–25	5–15	absent	absent / sometimes present	yellow	olive brown/brown	biverticillate	smooth	smooth to rough	ellipsoid	
<i>P. malachitum</i> <sup>3</sup>	20–25	5–10	no growth	28–32	28–32	17–21	10–17	absent	present	yellow	olive brown/brown	biverticillate	smooth	smooth to rough	ellipsoid	

<sup>1</sup> Data from Rivera & Seifert (2011) and Rivera et al. (2012).<sup>2,3</sup> Indicative of clade (from Fig. 2) to which species belong.

n.a. Data not available.



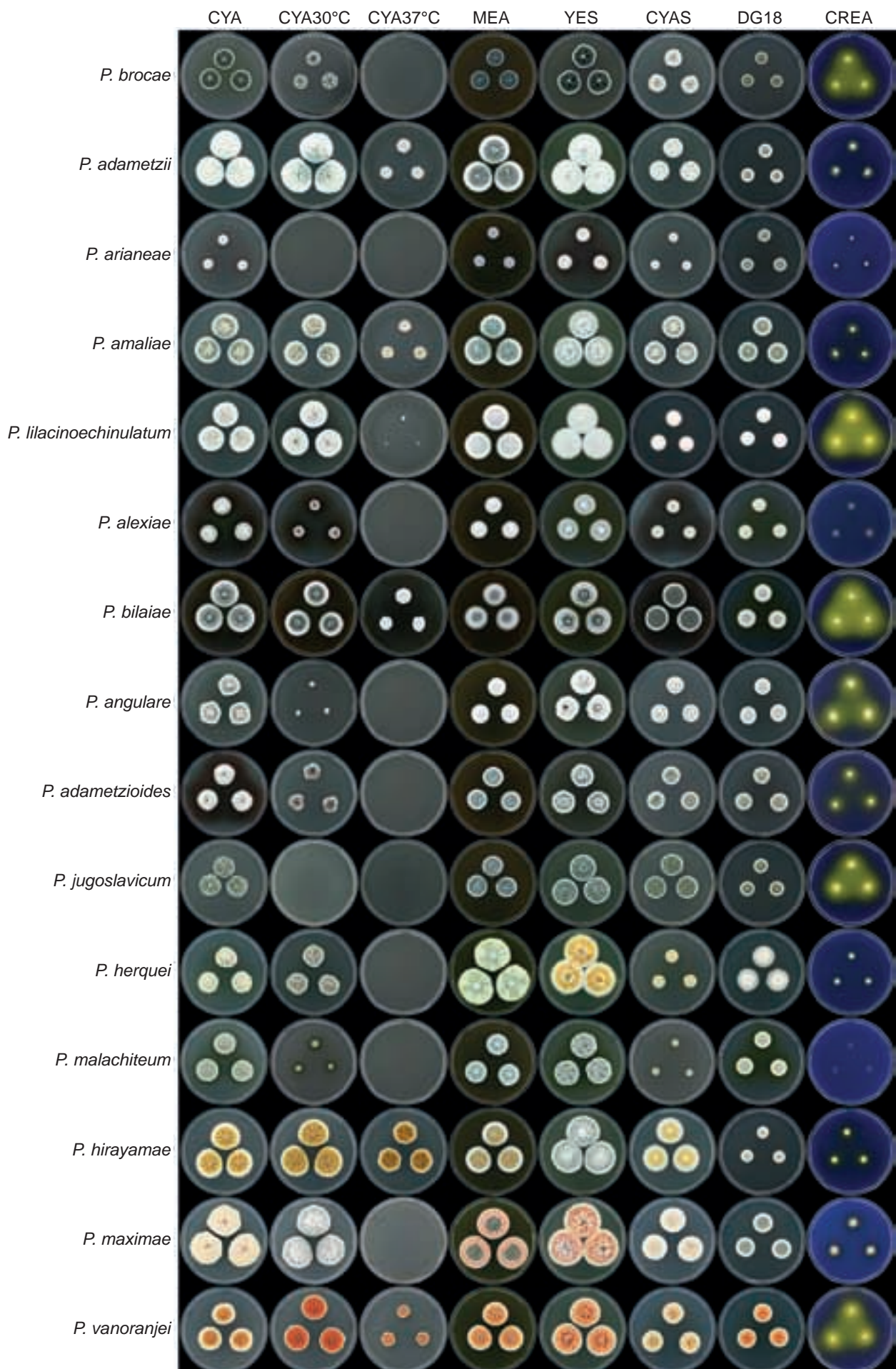
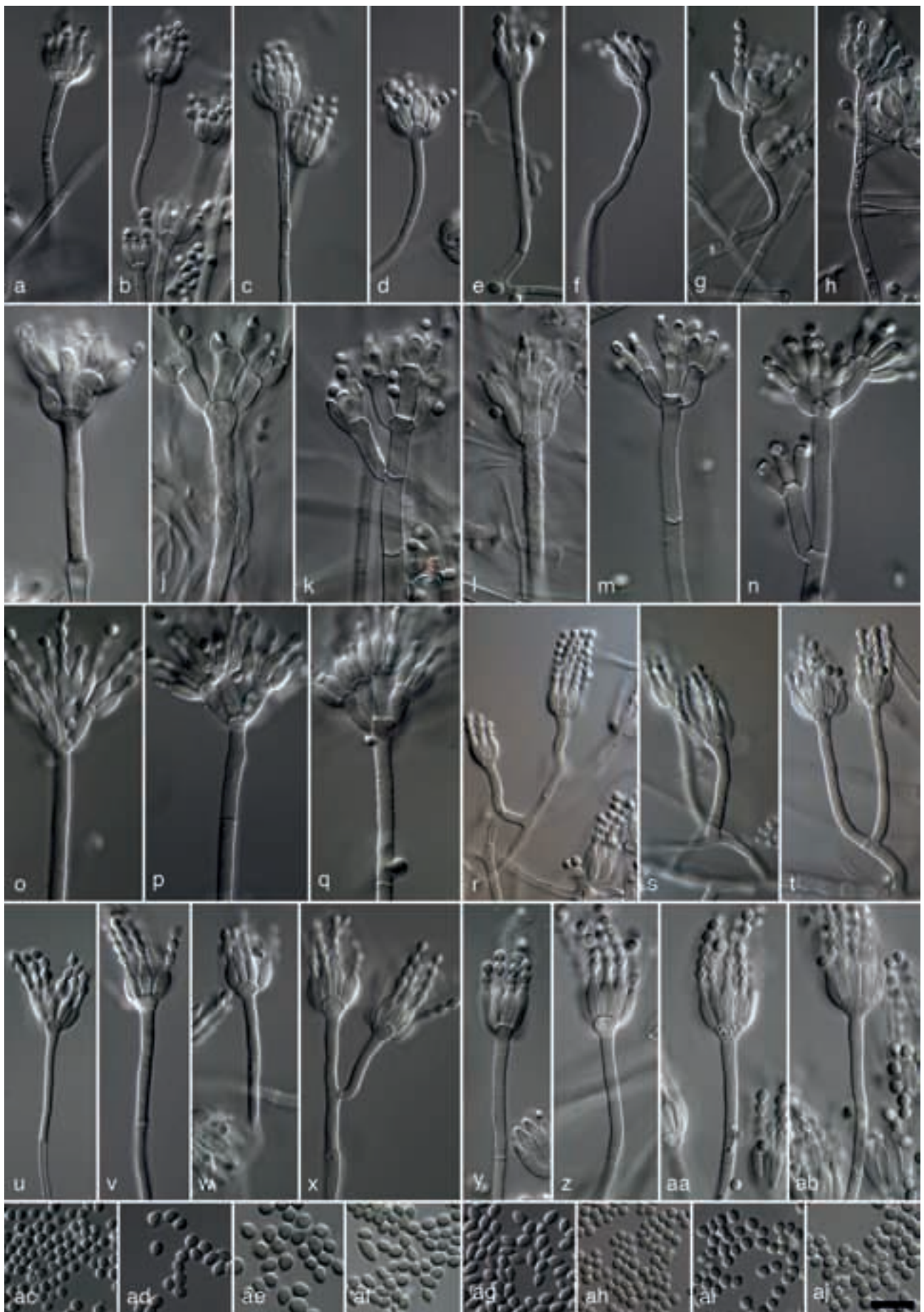


Fig. 3 Overview of colony characters in *Penicillium* section *Sclerotiora* species treated in this paper.





**Fig. 4** Overview of conidiophores (a–af) and conidia (ag–an) in *Penicillium* section *Sclerotiora* species treated in this paper: a–d, ag, *P. brocae*; e–h, ah, *P. adametzii*; i–l, ai, *P. arianae*; m–p, aj, *P. amaliae*; q–t, ak, *P. lilacinoechinulatum*; u–x, al, *P. alexiae*; y–ab, am, *P. bilaiae*; ac–af, an, *P. angulare*. — Scale bar in an = 10 µm, applies to a–an.



**Fig. 5** Overview of conidiophores (a–ab) and conidia (ac–aj) in *Penicillium* section *Sclerotiora* species treated in this paper: a–d, ac. *P. adametzioides*; e–h, ad. *P. jugoslavicum*; i–n, ae, af. *P. herquei*; o–q, ag. *P. malachiteum*; r–t, ah. *P. hirayamae*; u–x, ai. *P. maximae*; y–ab, aj. *P. vanoranjei*. — Scale bar in an = 10  $\mu$ m, applies to a–aj.



visible, spheroidal to subspheroidal,  $2.5\text{--}3.5 \times 2.5\text{--}3 \mu\text{m}$  ( $3.0 \pm 0.2 \times 2.9 \pm 0.2$ ), average width/length =  $0.95 \pm 0.04$ ,  $n = 39$ ; sclerotia bright orange,  $85\text{--}190 \times 70\text{--}150 \mu\text{m}$ .

*Specimen examined.* TUNISIA, Tabarka, *Quercus suber* forest soil, 2 Feb. 2009, collected by C. Silva Pereira, CBS H-21145, holotype, culture ex-type CBS 134406 (= DTO 99H6 = AHS3SF\_13).

*Additional strains examined.* TUNISIA, Tabarka, *Quercus suber* forest soil, 2 Feb. 2009, collected by C. Silva Pereira, cultures CBS 134404 (= DTO 99F3 = AHS3SF\_2); CBS 134405 (= DTO 99G1 = AHS3SF\_10); CBS 134407 (= DTO 119G8); CBS 134408 (= DTO 120C8).

**Notes** — *Penicillium vanoranjei* is characterised by colonies dominated by bright orange sclerotia. Orange sclerotia also occur in *P. sclerotiorum* and *P. johnkrugii* in the *P. sclerotiorum* species-complex (clade 1), as recently revised by Rivera & Seifert (2011). *Penicillium sclerotiorum* was distinguished from *P. johnkrugii* based on more abundant conidiogenesis on CYA, orange sclerotia on most media and subspheroidal to ellipsoidal conidia in the former species, in contrast to orange sclerotia produced only on MEA and spheroidal to subspheroidal conidia in the latter. The new species is easily distinguished from *P. sclerotiorum* by the absence of conidiogenesis on CYA and spheroidal to subspheroidal conidia. The orange sclerotia of *P. vanoranjei*, produced on most media, distinguish it from *P. johnkrugii*. Also, the new species has more restricted growth on CYA. It must be noted that roughness on conidia of *P. vanoranjei*, although easily visible on SEM, was inconspicuous with light microscopy.

***Penicillium maximae*** Visagie, Houbraken, Samson, *sp. nov.*  
— MycoBank MB803783; Fig. 3, 5, 8

*ITS barcode.* EU427298.

*Alternative markers.* KC773795 ( $\beta$ -tubulin), KC773821 (calmodulin).

*Etymology.* Latin, *maximae*: named after 'Hare Koninklijke Hoogheid Prinses Máxima der Nederlanden' (translated from Dutch as: 'Her Royal Highness Princess Máxima of the Netherlands'), wife of Prince Willem-Alexander of the Netherlands.

**Diagnosis** — A ring of orange mycelia masks sporulation on MEA. Conidiophores mostly monoverticillate, a minor proportion biverticillate; stipes smooth walled; phialides  $6.5\text{--}10 \times 2.5\text{--}3 \mu\text{m}$ ; conidia smooth and ellipsoidal,  $3\text{--}3.5 \times 2.5\text{--}3 \mu\text{m}$ . Sclerotia are not produced.

**Colony morphology** — Colony diam, 7 d, in mm: CYA 34–37; CYA 30 °C 32–37; CYA 37 °C no growth; MEA 34–37; YES 40–43; DG18 15–22; CYAS 27–30; CREA 12–15.

CYA, 25 °C, 7 d: Colonies angular in outline, radially sulcate, moderately raised, with an orange pink colour; margins low, narrow, entire; mycelia white and pinkish orange; texture floccose; sporulation sparse, conidial colour *en masse* cannot be determined; exudate orange, soluble pigment orange, reverse pigmentation reddish brown (8E8) at centre fading to orange (6B7). CYA, 30 °C, 7 d: Colonies similar to CYA at 25 °C, except for denser sporulation, conidia *en masse* greyish green (25D5). MEA, 25 °C, 7 d: Colonies moderately deep, lightly radially sulcate, fluffy pinkish orange mycelia dominate margins and masks conidiogenesis; margins low, narrow, entire; mycelia white and pinkish orange elsewhere; texture floccose to somewhat velutinous in some areas; sporulation dense at centre, conidial colour *en masse* dark green (26F5); exudate clear, soluble pigment absent, reverse pigmentation brown to reddish brown (7E8–8E8). YES, 25 °C, 7 d: Colonies moderately deep, randomly sulcate; margins low, narrow (2 mm), entire; mycelia white and pinkish orange; texture floccose; sporulation sparse, conidial colour *en masse* cannot be determined; exudate absent, soluble pigment absent, reverse pigmentation

reddish brown (8E8) at centre fading to orange (6B7). CREA, 25 °C, 7 d: Acid not produced.

**Conidiophores** monoverticillate, with a low proportion biverticillate; stipes smooth walled,  $45\text{--}150 \times 2\text{--}3 \mu\text{m}$ , vesicle  $3\text{--}6 \mu\text{m}$  ( $4.5 \pm 0.7$ ); branches when present only two,  $11\text{--}38 \mu\text{m}$ ; phialides ampulliform,  $8\text{--}16$  per stipe,  $6.5\text{--}10 \times 2.5\text{--}3 \mu\text{m}$  ( $8.4 \pm 0.2 \times 2.8 \pm 0.8$ ); conidia smooth walled, ellipsoidal,  $3\text{--}3.5 \times 2.5\text{--}3 \mu\text{m}$  ( $3.0 \pm 0.2 \times 2.5 \pm 0.04$ ), average width/length =  $0.8 \pm 0.04$ ,  $n = 38$ ; sclerotia not produced.

*Specimen examined.* USA, Florida, weathering treated cellophane, 1945, collected by L. White, CBS H-21144, holotype, culture ex-type CBS 134565 (= NRRL 2060 = DTO 244C7).

**Notes** — *Penicillium maximae* typically produces fast growing colonies with pinkish orange mycelia that mask sporulation underneath. This colour is especially striking on MEA. The species is basal to the *P. sclerotiorum* species complex, as is *P. hirayamae*. Morphologically it most closely resembles *P. sclerotiorum*. However, the absence of sclerotia, lack of acid production on CREA and lack of sporulation on CYA distinguish *P. maximae* from *P. sclerotiorum*.

***Penicillium amaliae*** Visagie, Houbraken & K. Jacobs, *sp. nov.*  
— MycoBank MB803784; Fig. 3, 4, 9

*ITS barcode.* JX091443.

*Alternative markers.* JX091563 ( $\beta$ -tubulin), JX141557 (calmodulin).

*Etymology.* Latin, *amaliae*: Named after Catharina-Amalia Beatrix Carmen Victoria 'Prinses der Nederlanden, Prinses van Oranje-Nassau' (translated from Dutch as: 'Princess of the Netherlands, Princess of Orange-Nassau'), first daughter of Princess Máxima of the Netherlands and Prince Willem-Alexander of the Netherlands.

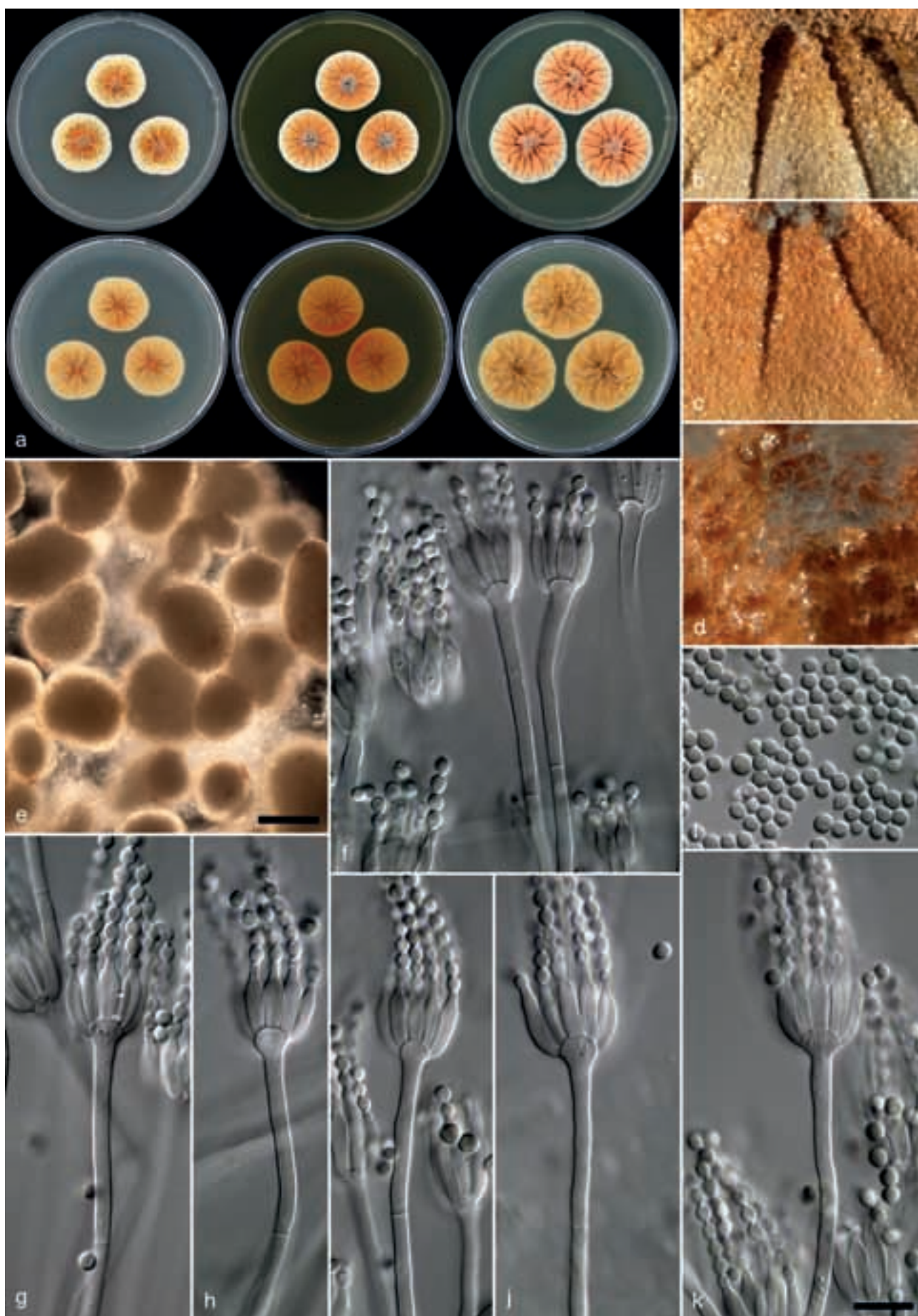
**Diagnosis** — Conidial colour on all media pale green. Colonies on CYA at 37 °C  $3\text{--}11$  mm, no acid produced on CREA. Conidiophores strictly monoverticillate; stipes smooth walled and ending in a swollen vesicle; phialides  $6.5\text{--}9 \times 2.5\text{--}3.5 \mu\text{m}$ ; conidia rough walled, spheroidal to subspheroidal,  $2\text{--}3 \times 2\text{--}2.5 \mu\text{m}$ . Sclerotia are not produced.

**Colony morphology** — Colony diam, 7 d, in mm: CYA 20–30; CYA 30 °C 22–30; CYA 37 °C 3–11; MEA 27–33; YES 30–36; DG18 14–24; CYAS 24–27; CREA 10–18.

CYA, 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidial colour *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 pale yellow (2A3–2A4), in some isolates a darker dull yellow (3B4). CYA, 30 °C, 7 d: Colonies showing no differences from those grown on CYA at 25 °C. MEA, 25 °C, 7 d: Colonies low, planar; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidial colour *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, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, narrow (2 mm), entire; mycelia white; texture velutinous; sporulation moderately dense, conidial colour *en masse* green to greyish green (27D4–27D5), when sporulation denser, 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. CREA, 25 °C, 7 d: Weak acid production.

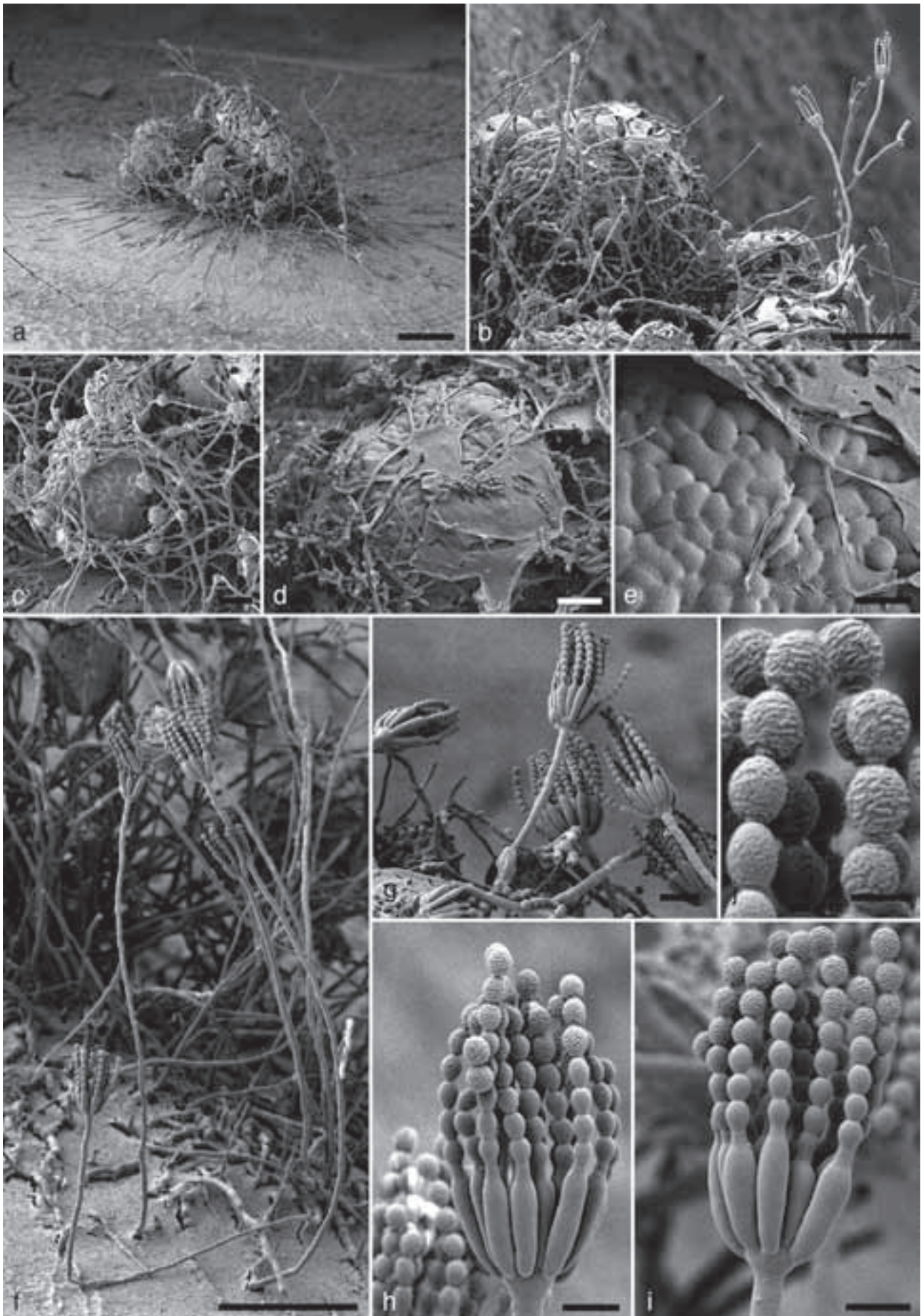
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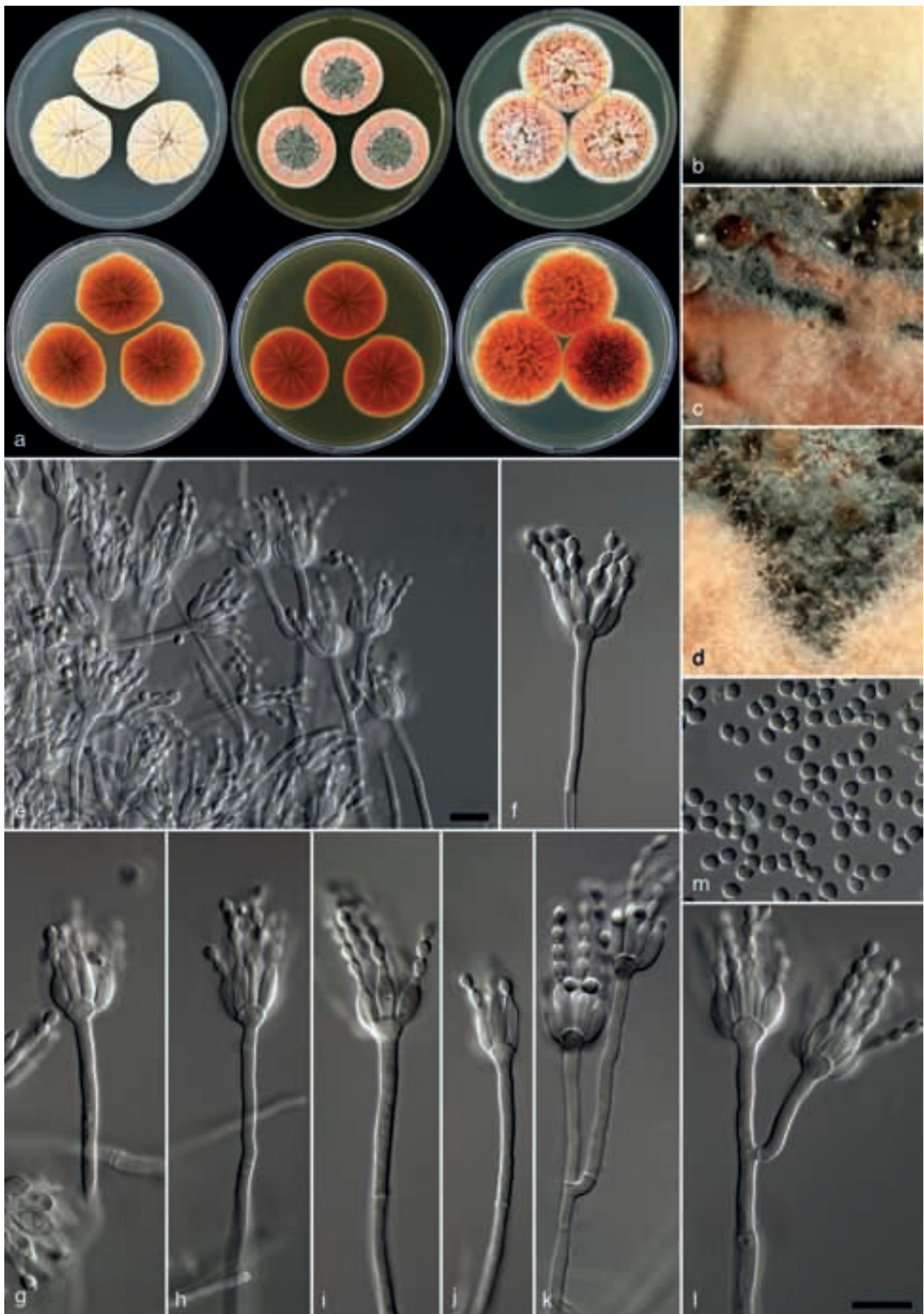
**Fig. 6** *Penicillium vanoranjei* (CBS 134406<sup>T</sup>). a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse); b. texture on CYA; c, d. texture on MEA; e. sclerotia; f–k. conidiophores; l. conidia. — Scale bar in e = 100  $\mu$ m; in k = 10  $\mu$ m, applies to f–l.





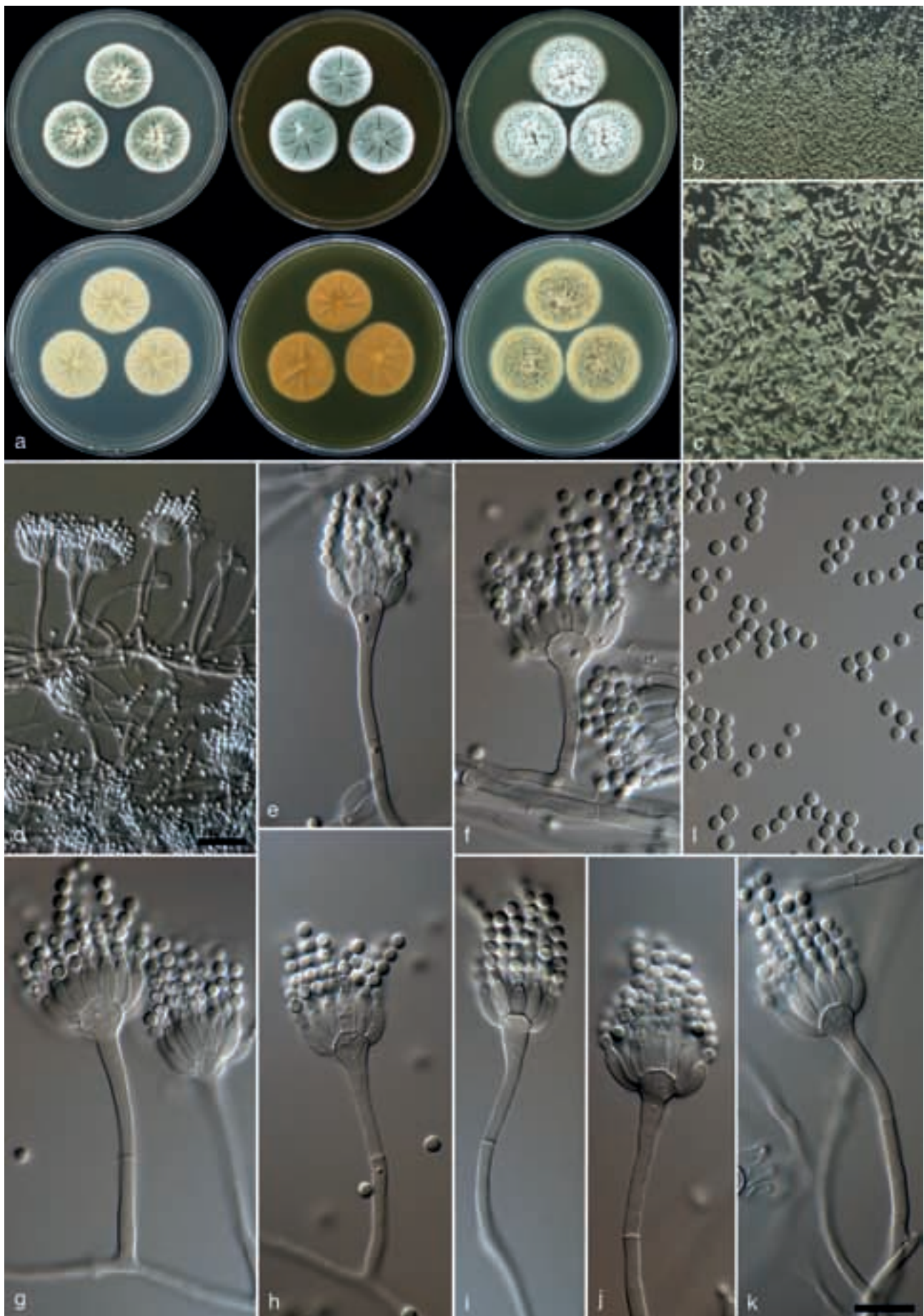
**Fig. 7** Scanning electron microscope pictures showing characteristic features of *P. vanoranjei* (CBS 134406<sup>T</sup>). a, b. Young colony showing development of sclerotia together with conidiophores; c–e. sclerotia produced on MEA, showing the sheets of dried-out exudates covering sclerotia; f–j. conidiophores and conidia; SEM pictures clearly show that conidial roughness develops as conidia become older. Also, connectives between conidia visible in (j) makes it possible for this species to support very long chains of conidia. — Scale bars: a = 100  $\mu$ m; b, f = 50  $\mu$ m; c, d = 20  $\mu$ m; e, g = 10  $\mu$ m; h, i = 5  $\mu$ m; j = 2  $\mu$ m.





**Fig. 8** *Penicillium maximae* (CBS 134565<sup>T</sup>) 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 bars = 10  $\mu$ m; l applies to f–m.



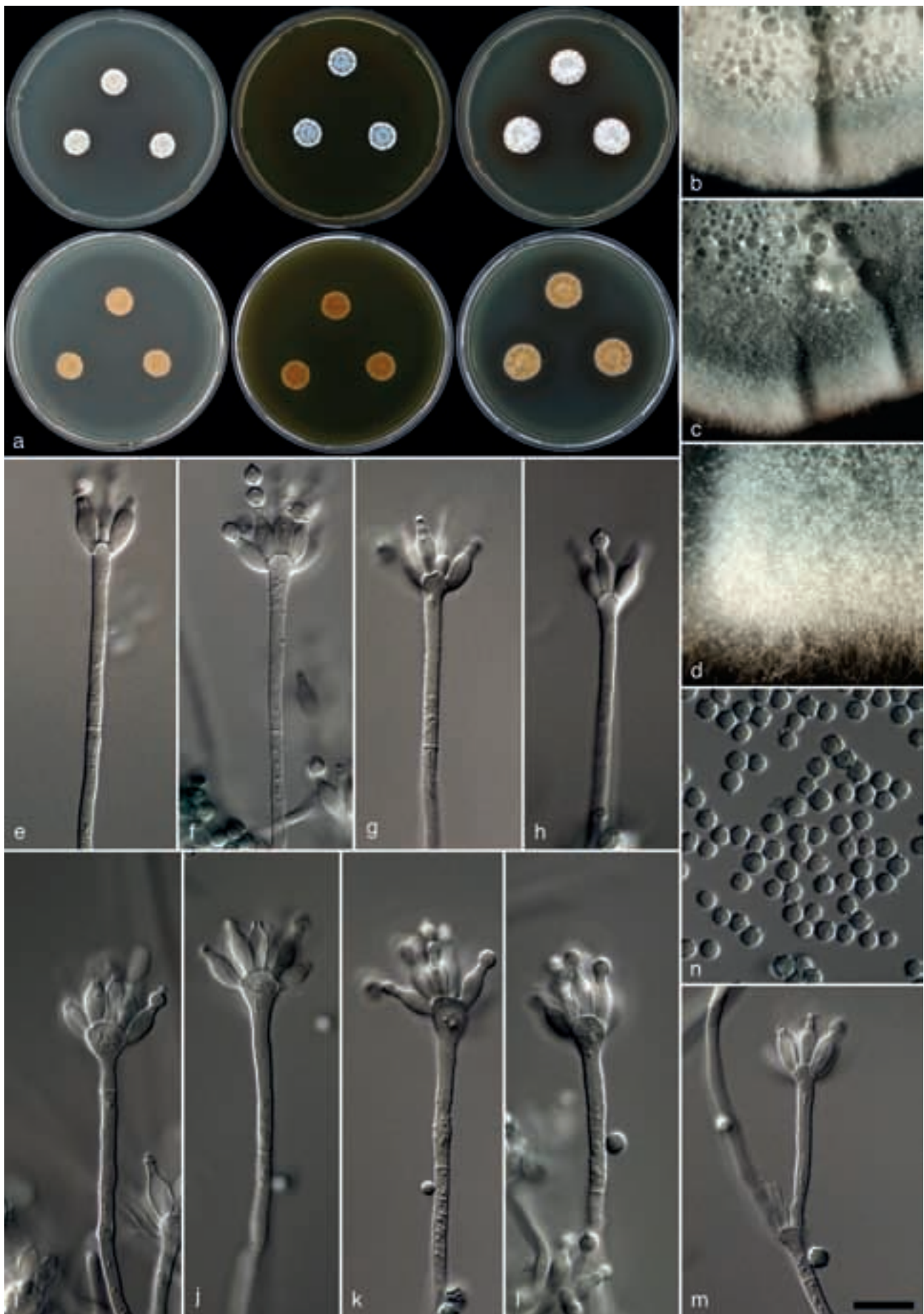


**Fig. 9** *Penicillium amaliae* (CBS 134209<sup>T</sup>). a. Colonies on CYA, MEA and YES from left to right (top = obverse, bottom = reverse); b. texture on CYA; c. texture on MEA; d–k. conidiophores; l. conidia. — Scale bar in e = 100 µm; k = 10 µm, applies to e–l.



**Fig. 10** *Penicillium alexiae* (CBS 134558<sup>T</sup>). 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 e = 100  $\mu$ m; k = 10  $\mu$ m, applies to f–l.





**Fig. 11** *Penicillium arianae* (CBS 134559<sup>T</sup>). 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 n = 10  $\mu$ m, applies to e–n.



*Conidiophores* strictly monoverticillate; *stipes* smooth walled, 20–95 × 2–3 µm, vesicle 4.5–7(–9) µm (6.3 ± 0.8); *phialides* ampulliform, 12–24 per stipe, 6.5–9 × 2.5–3.5 µm (7.7 ± 0.5 × 2.9 ± 0.2); *conidia* rough walled, connectives visible, spheroidal to subspheroidal, 2–3 × 2–2.5 µm (2.5 ± 0.1 × 2.4 ± 0.1), average width/length = 0.93 ± 0.04, n = 127; sclerotia not produced.

*Specimen examined.* SOUTH AFRICA, Western Cape Province, Struisbaai, *Protea repens* infructescence, 1 Aug. 2009, collected by C.M. Visagie, CBS H-21141, holotype, culture ex-type CBS 134209 (= CV 1875 = DTO 183F3 = DAOM 241034).

*Additional strains examined.* AUSTRALIA, New South Wales, Katandra Nature Reserve, insect larva, 2003, collected by A.L. Markovina, received via J.I. Pitt, CBS 134557 (= DTO 57A8). – SOUTH AFRICA, Western Cape Province, Stellenbosch, *Protea repens* infructescence, collected by C.M. Visagie, cultures CBS 134555 (= CV 112 = DTO 181A5 = DAOM 241032), CBS 134556 (= CV 227 = DTO 181C9 = DAOM 241033), CBS 134212 (= CV 401 = DTO 181F7 = DAOM 241031); Struisbaai, *P. repens* infructescence, collected by C.M. Visagie, CBS 134210 (= CV 1722 = DTO 183D1); CBS 134211 (= CV 204 = DTO 181C6).

**Notes** — *Penicillium amaliae* is characterised by monoverticillate conidiophores that have a vesiculate stipe and produce spheroidal rough walled conidia. It grows relatively well on all media. Conidial colour *en masse* is pale green, a character also observed in *P. lilacinoechinulatum*. Phylogenetically, it resolves as a distinct clade closely related to *P. lilacinoechinulatum*, *P. adametzii* and *P. brocae* (Fig. 2). The numerous phialides of *P. amaliae* distinguish it from *P. adametzii* and *P. brocae*. Also, the growth of *P. brocae* is more restricted than the new species, whereas *P. adametzii* grows faster at 30 and 37 °C. *Penicillium lilacinoechinulatum* is its closest relative, distinguished by its faster growth at 30 °C, slower growth at 37 °C, and stronger acid produced on CREA.

***Penicillium alexiae*** Visagie, Houbraken, Samson, *sp. nov.* — MycoBank MB803785; Fig. 3, 4, 10

*ITS barcode.* KC790400.

*Alternative markers.* KC773778 (β-tubulin), KC773803 (calmodulin).

*Etymology.* Latin, *alexiae*: Named after Alexia Juliana Marcela Laurentien 'Prinses der Nederlanden, Prinses van Oranje-Nassau' (translated from Dutch as: 'Princess of the Netherlands, Princess of Orange-Nassau') and second daughter of Princess Máxima of the Netherlands and Prince Willem-Alexander of the Netherlands.

**Diagnosis** — Colonies on CYA 18–21 mm, CYA 30 °C 8–15 and CYA 37 °C no growth; brown soluble pigment commonly produced on most media. Acid not produced on CREA. White sclerotia produced on CYA and MEA. Conidiophores monoverticillate; *stipes* smooth walled; *phialides* 8.5–10.5 × 2.5–3.5 µm; *conidia* smooth walled, subspheroidal, 2.5–3 × 2–3 µm.

**Colony morphology** — Colony diam, 7 d, in mm: CYA 18–21; CYA 30 °C 8–15; CYA 37 °C no growth; MEA 18–21; YES 21–26; DG18 15–17; CYAS 15–18; CREA 10–14.

CYA, 25 °C, 7 d: Colonies raised high at centre, radially sulcate, white sclerotia present; margins low, narrow, irregular; mycelia white; texture velutinous; sporulation moderately dense, conidial colour *en masse* greyish green (25B4); exudate absent, soluble pigment reddish brown, reverse pigmentation brown (7E8) at centre, paler brown (6E8) elsewhere. CYA, 30 °C, 7 d: Colonies crateriform, having a greyish colour, with no sporulation. MEA, 25 °C, 7 d: Colonies low, radially sulcate, white sclerotia present; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense, conidial colour *en masse* greyish green (25B4); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (6C7). YES, 25 °C, 7 d: Colonies low, raised at centre, radially sulcate; margins low, narrow, entire; mycelia white; texture velutinous;

sporulation moderately dense, conidial colour *en masse* greyish green (25B4); exudate absent, soluble pigment pale brown, reverse pigmentation brownish orange (6C7) at centre, yellowish grey elsewhere (2B2); CREA, 25 °C, 7 d: Acid not produced.

*Conidiophores* strictly monoverticillate; *stipes* smooth walled, 50–185 × 2.5–3 µm, vesicle 3.5–4.5 µm (4.0 ± 0.3); *phialides* ampulliform, 5–12 per stipe, 8.5–10.5 × 2.5–3.5 µm (9.6 ± 0.6 × 3.0 ± 0.3); *conidia* smooth walled, subspheroidal, 2.5–3 × 2–3 µm (2.6 ± 0.1 × 2.4 ± 0.2), average width/length = 0.91 ± 0.04, n = 45; *sclerotia* white, 70–165 × 70–135 µm.

*Specimen examined.* TUNISIA, Tabarka, *Quercus suber* forest soil, 2 Feb. 2009, collected by C. Silva Pereira, CBS H-21142, holotype, culture ex-type CBS 134558 (= DTO 118H8 = FERS1SF\_05).

**Notes** — *Penicillium alexiae* typically produces slow growing colonies with a brown soluble pigment on most media. This is a character also observed for *P. bilaiae* and *P. adametzii*. The faster growth of *P. bilaiae* at 30 and 37 °C on CYA and strong acid production makes it distinct. Acid is not produced by *P. alexiae*, in comparison with *P. adametzii*, which exhibits weak acid production. One of the most striking features of *P. alexiae* is the production of the white sclerotia. Considering these species micromorphology, *P. alexiae* is easily distinguished from both *P. bilaiae* and *P. adametzii*, because its non-swollen vesicles result in a compact conidiophore.

***Penicillium arianae*** Visagie, Houbraken, Samson, *sp. nov.* — MycoBank MB803786; Fig. 3, 4, 11

*ITS barcode.* KC773833.

*Alternative markers.* KC773784 (β-tubulin), KC773811 (calmodulin).

*Etymology.* Latin, *arianae*: named after Ariane Wilhelmina Máxima Ines 'Prinses der Nederlanden, Prinses van Oranje-Nassau' (translated from Dutch as: 'Princess of the Netherlands, Princess of Orange-Nassau') and third daughter of Princess Máxima of the Netherlands and Prince Willem-Alexander of the Netherlands.

**Diagnosis** — Colonies restricted on most media, on CYA 9–12 mm, with no growth above 30 °C, MEA 10–14 mm. Conidiophores monoverticillate; *stipes* smooth walled, with slightly vesiculate ending; *phialides* 7.5–10 × 3–4 µm; *conidia* spheroidal and have thick rough walls, 3–3.5 × 3–3.5 µm. Sclerotia are not produced.

**Colony morphology** — Colony diam, 7 d, in mm: CYA 9–12; CYA 30 °C no growth; CYA 37 °C no growth; MEA 10–14; YES 14–16; DG18 11–15; CYAS 9–10; CREA 3–4.

CYA, 25 °C, 7 d: Colonies crateriform, radially sulcate at margins; margins low, narrow, irregular; mycelia white at the margin; texture floccose; sporulation absent to sparse, conidial colour *en masse* greenish grey to pale green (25A2–25A3); exudate clear, soluble pigment inconspicuously brownish, reverse pigmentation pale orange (5A3) and greyish yellow (4B3). CYA, 30 °C, 7 d: No growth observed. MEA, 25 °C, 7 d: Colonies crateriform, radially sulcate; margins low, narrow, somewhat irregular; mycelia white; texture floccose; sporulation sparse to sometimes moderately dense, conidial colour *en masse* greyish green (25D4); exudate absent, soluble pigment absent, reverse pigmentation brownish orange (5C5). YES, 25 °C, 7 d: Colonies crateriform, radially and concentrically sulcate; margins low, narrow, irregular; mycelia white; texture floccose; sporulation absent to sparse, conidial colour *en masse* greenish white; exudate absent, soluble pigment reddish brown, reverse pigmentation pale yellow (4A4–4A5); CREA, 25 °C, 7 d: No acid produced.

*Conidiophores* strictly monoverticillate; *stipes* smooth walled, 30–180 × 2.5–3 µm, vesicle 4–7 µm (5.5 ± 1.0); *phialides* ampulliform, 6–12 per stipe, 7.5–10 × 3–4 µm (8.5 ± 0.7

× 3.5 ± 0.2); *conidia* thick, rough walled, spheroidal, 3–3.5 × 3–3.5 µm (3.2 ± 0.2 × 3.2 ± 0.2), average width/length = 0.98 ± 0.03, n = 30; *sclerotia* not produced.

*Specimen examined.* THE NETHERLANDS, Bussum, Spanderswoud, soil, Apr. 2006, collected by J. Houbraken, L. Janson & R. Samson, CBS H-21143, holotype, culture ex-type CBS 134559 (= DTO 20B8).

Notes — *Penicillium arianeae* has restricted growth on all media. This character easily distinguishes it from its closest relatives, *P. amaliae*, *P. lilacinoechinulatum* and *P. adametzii*. Its thick, rough walled conidia are also unique in this section.

## ACCEPTED SPECIES IN SECTION SCLEROTIORA HOUBRAKEN & SAMSON

In: *Penicillium* subgenus *Aspergilloides*.  
Type species: *Penicillium sclerotiorum*.

We accept the following names in the section *Sclerotiora*. As discussed at more length below, *P. nodositatum*, previously classified in this section (Houbraken & Samson 2011) is removed and does not belong in *Penicillium* s.str. In this list, GenBank accession numbers to ITS barcodes and alternative identification markers are provided (BT = β-tubulin; CMD = calmodulin).

***Penicillium adametzii*** K.M. Zalesky, Bull. Int. Acad. Polon. Sci., Cl. Sci. Math., Ser. B, Sci. Nat. 1927: 507. 1927. (MB119777). — Herb.: IMI 39751. Ex-type: CBS 209.28 = ATCC 10407 = IMI 039751 = MUCL 29106 = NRRL 737. ITS barcode: JN714929. (Alternative markers: BT = JN625957; RPB2 = JN121455; CMD = KC773796).

***Penicillium adametzioides*** S. Abe ex G. Sm., Trans. Brit. Mycol. Soc. 46: 335. 1963 ≡ *Penicillium adametzioides* S. Abe, J. Gen. Appl. Microbiol., Tokyo 2: 68. 1956 (nom. inval., Art. 36). (MB302372). — Herb.: CBS 313.59. Ex-type: CBS 313.59 = ATCC 18306 = FAT1302 = IFO 6055 = IMI 068227 = NRRL 3405 = QM 7312. ITS barcode: JN686433. (Alternative markers: BT = JN799642; RPB2 = JN406578; CMD = JN686388).

***Penicillium alexiae*** Visagie, Houbraken & Samson (this study). (MB 803785). — Herb.: CBS-H 21142. Ex-type: CBS 134558 = DTO 118H8 = FERS1SF\_05. ITS barcode: KC790400. (Alternative markers: BT = KC773778; CMD = KC773803).

***Penicillium amaliae*** Visagie, Houbraken & Jacobs (this study). (MB803784). — Herb.: CBS-H 21141. Ex-type: CBS 134209 = CV 1875 = DTO 183F3 = DAOM 241034. ITS barcode: JX091443. (Alternative markers: BT = JX091563; CMD = JX141557).

***Penicillium angulare*** S.W. Peterson, E.M. Bayer & Wicklow, Mycologia 96: 1289. 2004. (MB487891). — Herb.: BPI 842268. Ex-type: CBS 130293 = IBT 27051 = NRRL 28157. ITS barcode: (representative strain NRRL 28140) AY313613. (Alternative markers: BT = KC773779; RPB2 = JN406554; CMD = KC773804).

***Penicillium arianeae*** Visagie, Houbraken & Samson (this study). (MB 803786). — Herb.: CBS-H 21143. Ex-type: CBS 134559 = DTO 20B8. ITS barcode: KC773833. (Alternative markers: BT = KC773784; CMD = KC773811).

***Penicillium bilaiae*** Chalab., Bot. Mater. Otd. Sporov. Rast. 6: 165. 1950. (MB302379). — Herb.: IMI 113677. Ex-type: CBS 221.66 = ATCC 22348 = ATCC 48731 = CCRC 31675 = FRR 3391 = IJFM 5025 = IMI 113677 = MUCL 31187 = VKMF-854. ITS barcode: JN714937. (Alternative markers: BT = JN625966; RPB2 = JN406610; CMD = JN626009).

***Penicillium brocae*** S.W. Peterson, Jeann. Pérez, F.E. Vega & Infante, Mycologia 95: 143. 2003. (MB373658). — Herb.: BPI 841763. Ex-type: CBS 116113 = IBT 26293 = NRRL 31472. ITS barcode: AF484398. (Alternative markers: BT = KC773787; RPB2 = JN406639; CMD = KC773814).

***Penicillium cainii*** K.G. Rivera & Seifert, Stud. Mycol. 70: 147. 2011. (MB563159). — Herb.: DAOM 239914. Ex-type: CCFC 239914. ITS barcode: JN686435. (Alternative markers: BT = JN686366; CMD = JN686389).

***Penicillium guanacastense*** K.G. Rivera, Urb & Seifert, Mycotaxon 119: 324. 2012. (MB563044). — Herb.: DAOM 239912. Ex-type: CCFC 239912. ITS barcode: JN626098. (Alternative markers: BT = JN625967; CMD = JN626010).

***Penicillium herquei*** Bainier & Sartory, Bull. Soc. Mycol. France 28: 121. 1912. (MB536431). — Herb.: IMI 28809. Ex-type: CBS 336.48 = NRRL 1040 = ATCC 10118 = BIOURGE 452 = FRR 1040 = IFO 31747 = IMI 28809 = MUCL 29213 = NCTC 1721 = QM 1926 = Thom 4640.447. ITS barcode: JN626101. (Alternative markers: BT = JN625970; RPB2 = JN121494; CMD = JN626013).

***Penicillium hirayamae*** Udagawa, J. Agric. Sci. (Tokyo) 5: 6. 1959. (Teleomorphic synonym: *Eupenicillium hirayamae* D.B. Scott & Stolk). (MB302402). — Herb.: IMI 78255. Ex-type: CBS 229.60 = ATCC 18312 = IFO 6435 = IMI 078255 = IMI 078255ii = NHL 6046 = NRRL 143 = QM 7885. ITS barcode: JN626095. (Alternative markers: BT = JN625955; RPB2 = JN121459; CMD = JN626003).

***Penicillium jacksonii*** K.G. Rivera & Seifert, Stud. Mycol. 70: 151. 2011. (MB563160). — Herb.: DAOM 239937. Ex-type: CCFC 239937. ITS barcode: JN686437. (Alternative markers: BT = JN686368; CMD = JN686391).

***Penicillium johnkrugii*** K.G. Rivera & Seifert, Stud. Mycol. 70: 151. 2011. (MB563161). — Herb.: DAOM 239943. Ex-type: CCFC 239943. ITS barcode: JN686447. (Alternative markers: BT = JN686378; CMD = JN686401).

***Penicillium jugoslavicum*** C. Ramírez & Munt.-Cvetk., Mycopathologia 88: 65. 1984. (MB124173). — Herb.: CBS 192.87. Ex-type: CBS 192.87 = IJFM 7785 = IMI 314508. ITS barcode: KC411688. (Alternative markers: BT = KC773789; RPB2 = JN406618; CMD = KC773815).

***Penicillium lilacinoechinulatum*** S. Abe ex G. Sm., Trans. Brit. Mycol. Soc. 46: 335. 1963 ≡ *Penicillium lilacinoechinulatum* S. Abe, J. Gen. Appl. Microbiol., Tokyo 2: 54, 1956 (nom. inval., Art. 36). (MB120793). — Herb.: CBS 454.93. Ex-type: CBS 454.93 = ATCC 18309 = FAT 84 = FRR 3451 = IFO 6231 = IMI 068211 = QM 7289. ITS barcode: AY157489. (Alternative markers: BT = KC773790; CMD = KC773816).

***Penicillium malachiteum*** (Yaguchi & Udagawa) Houbraken & Samson, Stud. Mycol. 70: 47. 2011 ≡ *Chromocleista malachitea* Yaguchi & Udagawa, Trans. Mycol. Soc. Japan 34: 102. 1993 (Houbraken & Samson 2011). (MB561971). — Herb.: CBS 647.95. Ex-type: CBS 647.95 = IBT 17515. ITS barcode: KC773838. (Alternative markers: BT = KC773794; CMD = KC773820).

***Penicillium mallochii*** K.G. Rivera, Urb & Seifert, Mycotaxon 119: 322. 2012. (MB563043). — Herb.: DAOM 239917. Ex-type: CCFC 239917. ITS barcode: JN626104. (Alternative markers: BT = JN625973; CMD = JN626016).

***Penicillium maximae*** Visagie, Houbraken & Samson (this study). (MB803783). — Herb.: CBS-H21144. Ex-type: CBS 134565 = NRRL2060 = DTO 244C7. ITS barcode: EU427298. (Alternative markers: BT = KC773795; CMD = KC773821).



***Penicillium sclerotiorum*** J.F.H. Beyma, Zentralbl. Bakteriol., 2. Abt., 96: 418. 1937. (MB277708). — Herb.: IMI 40569. Ex-type: CBS 287.36 = ATCC 10494 = IFO 6105 = IMI 040569 = NRRL 2074 = QM 1938 = VKMF-353. ITS barcode: JN626132. (Alternative markers: BT = JN626001; RPB2 = JN406585; CMD = JN626044).

***Penicillium vanoranjei*** Visagie, Houbraken & Samson (this study). (MB803782). — Herb.: CBS-H 21145. Ex-type: CBS 134406 = DTO 99H6 = AHS3SF\_13. ITS barcode: KC695696. (Alternative markers: BT = KC695686; CMD = KC695691).

***Penicillium viticola*** Nonaka & Masuma, Mycoscience 52: 339. 2011. (MB516048). — Herb.: TNS-F38702. Ex-type: JCM 17636 = FKI-4410. ITS barcode: AB606414. (Alternative markers: BT = AB540174; CMD = JN686393 (representative strain DAOM 239933)).

## DISCUSSION

Houbraken & Samson (2011) introduced *Penicillium* section *Sclerotiora* for their 'clade 2', based on a four-gene phylogeny (RPB1, RPB2, *Tsr1*, *Cct8*) and accepted 17 species. The section corresponds to clade 3 of Peterson (2000). Rivera & Seifert (2011) and Rivera et al. (2012) recently revised the type species for the section, *P. sclerotiorum*, introduced five new species, redefined the concept of *P. sclerotiorum*, and provided a dichotomous key to the species of this clade. As such, we did not further consider known species from this clade here, although *P. vanoranjei* and *P. maximae* belong there.

Morphological characters that distinguish species of *Penicillium* section *Sclerotiora* are summarised in Table 2, mostly modified from data provided by Rivera & Seifert (2011), and illustrated in Fig. 3–5. This data is meant to facilitate species identification in conjunction with sequence data. GenBank accession numbers for ITS barcodes and supplementary identification markers to all known species of the section are given in the taxonomy section above. Although phenotypic characters that define the three clades of the section could not be identified, species identification using only morphology is possible. Colony morphology of strains grown under standardised conditions was especially informative. Conidiophore morphology among the different species is very similar. There are exceptions, however, with *P. herquei* and *P. malachiteum* both producing biverticillate conidiophores. Houbraken & Samson (2011) distinguished these two species by production of cleistothecia in *P. malachiteum*. We found growth at 30 °C a more useful character, with *P. malachiteum* showing restricted growth compared to *P. herquei*. It should be noted, however, that preliminary data show that this clade represents a species complex, with several species undescribed. As such, for this study we considered only type strains and a couple of representative strains with identical sequences. For the monoverticillate species in the *P. adametzii* complex (clade 2), growth rates at 30 and 37 °C are taxonomically informative. For example, *P. arianae* and *P. jugoslavicum* do not grow at 30 °C, whereas only *P. adametzii*, *P. amaliae*, *P. bilaiae*, *P. hirayamae* and *P. lilacinoechinulatum* were able to grow at 37 °C. Also, acid production on CREA is a reliable taxonomic character. In general, most species in section *Sclerotiora* produce conidiophores with smooth walled stipes and spheroidal to subspheroidal conidia.

SEM observations of *P. vanoranjei* revealed a sheet-like extracellular matrix that might act as a kind of glue or protection against adverse conditions including drying. *Penicillium vanoranjei* produces large amounts of exudates in some colonies. We have often observed the drying of exudate droplets in older cultures, leaving a thin, sheet-like coloured matrix behind.

This mechanism could be responsible for the covering of the sclerotia shown in the SEM micrographs (Fig. 7b–e). Conidial wall texture is informative in section *Sclerotiora* but difficult to interpret with light microscopy. SEM photographs show the development of *P. vanoranjei* conidia in basipetal chains, and the development of conidial roughening as conidia expand (Fig. 7f–i).

Grigorieva-Manoilova & Poradielova (1915) originally isolated *P. multicolor* from soil in Russia and described it as having short stipes ending in vesicles. They reported that colony colours range from yellow to orange to red. Raper & Thom (1949) used several strains isolated from America as representative for *P. multicolor*, but noted that their identification of these strains (i.e. NRRL 2060) might be doubtful. Pitt (1979) considered *P. multicolor* a confused name and did not accept it in his treatment of the genus. Peterson & Horn (2009) treated NRRL 2060 as the ex-type of *P. multicolor*. However, this strain has features inconsistent with the original description by Grigorieva-Manoilova & Poradielova (1915). Based on sequence data, it was shown that the ex-type for *P. multicolor* (CBS 501.73 = ATCC 24723 = IMI 174716) is closely related to *P. fellutanum*, in section *Charlesia* (Houbraken & Samson 2011). Rivera & Seifert (2011) considered it as a tentative synonym of *P. fellutanum*. To resolve this confusion, we introduce and describe *P. maximae* above for NRRL 2060, depositing the herbarium material as CBS-H21144 (= CBS 134565 ex-type). *Penicillium multicolor* in the sense of CBS 501.73 will be included in a future study of section *Charlesia*. However, we recommend KC790402 as the species ITS DNA barcode, with the alternative  $\beta$ -tubulin marker JN799645, for identification of the species.

Valla et al. (1989) originally described *P. nodositatum* and deposited strains in CBS. Houbraken & Samson (2011) included the ex-type strain (CBS 330.90) in their phylogenies, reporting it had identical sequences to the ex-type strain (CBS 221.66) of *P. bilaiae*. However, they noted problems with the synonymy of *P. nodositatum* with *P. bilaiae* because it produces biverticillate conidiophores (Valla et al. 1989), in contrast to the monoverticillate conidiophores of *P. bilaiae* (Chalabuda 1950, Pitt 1979, Savard et al. 1994). We traced the problem to incorrect accession numbers in the CBS database. Although the herbarium specimen was submitted with accession number CBS 330.90, the accepted species list endorsed by the International Commission of *Penicillium* and *Aspergillus* (ICPA, <http://www.aspergilluspenicillium.org/>) included the living ex-type strain of the herbarium specimen as CBS 333.90. The source of this error in the database cannot be traced. However, the living culture CBS 330.90 represents *P. bilaiae* with monoverticillate conidiophores, whereas *P. nodositatum* (CBS 333.90<sup>T</sup>) is closely related to *P. kabunicum* and does not belong in *Penicillium* s.str. (Houbraken & Samson 2011). Herbarium material for *P. nodositatum* has subsequently been lost. In future, the strain will be lectotypified and epitypified in a study focused on the classification of species rejected from *Penicillium* s.str. by Houbraken & Samson (2011).

The isolation of *P. amaliae* from both South Africa and Australia is intriguing. The South African isolates were obtained from *Protea repens* infructescences as part of an ecological study focused on mite dispersal of *Penicillium* in this niche. The occurrence of this species in Australia from insect larvae thus suggests a pattern. Similarly, a closely related species, *P. brocae*, was described from coffee-berry borers and their faeces and guts in Mexico (Peterson et al. 2003). The idea of *Penicillium* species being transported or vectored by mites and insects is not new (Peterson et al. 2003, Hubert et al. 2004, Seifert et al. 2004, Visagie et al. 2009). However, it has never been demonstrated as a mutualistic relationship. In a similar ecological study from Fynbos, a mutualistic association was

shown between *Ophiostoma* species and mites (Roets et al. 2007). Our preliminary data does suggest that at least some *Penicillium* species have a similar mutualistic association (Visagie 2012). Visagie (2012) showed that *Penicillium* populations in the diverse Fynbos biome in South Africa are distinct at the different sites sampled. Postulating a mutualistic relationship and positive correlation for the dispersal of *Penicillium* populations in this niche may help explain the distribution patterns of these species and make us re-evaluate the idea of water and wind as the only primary dispersal mechanisms in *Penicillium*.

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