Short title: Visagie & Yilmaz: Six new *Penicillium* species

Along the footpath of *Penicillium* discovery: Six new species from the Woodville Big Tree Forest Trail

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ABSTRACT

In this study, we studied the diversity of *Penicillium* occurring in soil collected along the Woodville Big Tree Forest Trail situated close to the coastal town of Wilderness in South Africa. Strains were accessioned into a collection and then identified to species based on ß-tubulin DNA sequences, which is the recommended DNA barcode for the genus. The 74 strains were found to represent 18 species, including six we consider undescribed. Here we introduce them as *Penicillium claroviride, P. kalander, P. mattheeae, P. outeniquaense, P. subfuscum,* and *P. umkhoba*. Phylogenetic comparisons were made and genealogical concordance was demonstrated for these new species using DNA sequences from nuc rDNA ITS1-5.8S-ITS2 (ITS barcode), ß-tubulin, calmodulin and RNA polymerase II second largest subunit. Notes on morphological characters distinguishing the new species from their close relatives are provided.

KEY WORDS: *Eurotiales*; fungal diversity; fynbos; multigene phylogenies, 6 new taxa

INTRODUCTION

Soil typically contains diverse fungal communities which play important ecological roles like the decomposing of organic materials, recycling of nutrients and many more. Worldwide, surveys exploring fungal diversity in soil often report *Penicillium* as one of the dominant genera (Christensen and others 2000), while many species were described from soil historically (Biourge 1923; Pitt 1980; Ramírez 1982; Raper and Thom 1949; Thom 1930). However, not all fungi isolated from this complex matrix are considered soil-borne with a high proportion of species most probably transients (Barron 1968; Domsch and others 1980). A survey conducted in the fynbos region of South Africa that included soil, found the region to be a hotspot for *Penicillium* diversity and subsequently introduced 29 new species among the 61 identified (Visagie and others 2021). It also hinted that South Africa with its species-rich and diverse vegetation types (Mucina and Rutherford 2006) still has many new *Penicillium* species awaiting discovery.

Penicillium is a speciose genus with 513 species currently accepted (Houbraken and others 2020; updated by 2022/09/05). Identifications based on morphology have, however, been greatly problematic and the literature is strewn with misidentifications (Frisvad 1989). Sequence-based identifications in theory should have prevented incorrect identifications. In practice, however, it still occurs, which led to a large collaborative effort in the last decade to standardize *Penicillium* taxonomy (Houbraken and others 2020; Houbraken and others 2021; Visagie and others 2014). The key component of these efforts was the updated 'accepted species list' with each entry linked to important data, including MycoBank numbers, collection numbers of both type and ex-type cultures, its mode of reproduction, and GenBank accession

numbers of the nuc rDNA ITS1-5.8S-ITS2 (ITS barcode), ß-tubulin (*BenA*), calmodulin (*CaM*) and RNA polymerase II second largest subunit (*RPB2*) sequences (Houbraken and others 2020). Species were classified into a hierarchical infrageneric structure at subgenus, section, and series level based on a multigene phylogeny of ITS, *BenA, CaM* and *RPB2*, that where appropriate was supported by morphological characters or ecological traits. This classification has proven helpful when working with such a species rich genus (Houbraken and others 2020; Houbraken and Samson 2011). Efforts were also made to standardize working methods of *Penicillium*, with Visagie and others (2014) recommending growth media and incubation conditions for morphological characterization and subsequent descriptions.

In our latest efforts to expand knowledge on *Penicillium* species diversity, soil samples were collected during a hike along the Woodville Big Tree walking path that forms part of the Wilderness section of the Garden Route National Park. This park is home to the historic and affectionally named 'Big Tree', a 31-meter high, ±850-year-old Outeniqua Yellowwood (*Afrocarpus falcatus*). The park, situated in the greater fynbos region, forms a pocket of Southern Afrotemperate forest with a warm and moist climate. Fungal isolations from collected soil resulted in 18 *Penicillium* species identified, including the discovery of the six new *Penicillium* species described below.

MATERIALS AND METHODS

Sampling, Isolations, and Preservations

Six soil samples were collected in 2018 along the Woodville Big Tree Forest Trail. Plant debris was removed from the surface and soil was collected to a depth of 5 cm using a sterile spatula.

Samples were kept at cool temperatures in resealable plastic storage bags until processing. For isolations, 5 g soil was added to 100 ml sterile dH_2O. This suspension was serially diluted to 1:1000. Each dilution (1 ml) was plated onto potato dextrose agar (PDA) containing streptomycin (100 ppm) and chloramphenicol (50 ppm). Plates were incubated at 25 C for 5–7 d, after which colonies of interest were purified onto Czapek yeast autolysate agar (CYA). After incubating plates at 25 C for 7 d, strains were grouped based on colony appearance and conidiophore characteristics.

Strains were accessioned into the CN working collection housed at FABI (Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa) and preserved as spore suspensions in 10% glycerol at -80 C. Strains belonging to new species were deposited into the CMW (FABI, University of Pretoria, Pretoria, South Africa) and CBS (Westerdijk Institute, Utrecht, the Netherlands) culture collections. Accession numbers are provided in TABLE 1.

Table 1: *Penicillium* strains isolated from the Woodville Big Tree Forest Trail, Wilderness, South Africa

Species	Strains	Section	Series	GenBank accession numbers			
				ITS	*BenA*	*CaM*	*RPB2*
P. adametzii	CN014B7	*Sclerotiorum*	*Adametziorum*	MT949901	MT957401	MT957447	MT957473
P. adametzii	CN014B8	*Sclerotiorum*	*Adametziorum*	MT949902	MT957402	MT957448	MT957474
P. adametzii	CN014F7	*Sclerotiorum*	*Adametziorum*	MT949920	MT957430	MT957467	MT957493
P. adametzii	CN014F8	*Sclerotiorum*	*Adametziorum*	MT949921	MT957431	n.a.	MT957494
P. adametzii	CN014F9	*Sclerotiorum*	*Adametziorum*	n.a.	MT957432	n.a.	n.a.
P. adametzii	CN014G1	*Sclerotiorum*	*Adametziorum*	n.a.	MT957433	n.a.	n.a.
P. cairnsense	CN014H2	*Citrina*	*Westlingiorum*	n.a.	MT957435	n.a.	n.a.
P. claroviride	CMW 56197 = CBS 147458 = CN014D2 (ex-type)	*Canescentia*	*Atroveneta*	MT949909	MT957414	MT957456	MT957482
P. claroviride	CMW 56198 = CBS 147418 = CN014D3	*Canescentia*	*Atroveneta*	MT949910	MT957415	MT957457	MT957483
P. crocicola	CN014G9	*Aspergilloides*	*Thomiorum*	n.a.	MT957434	MT957468	MT957495
P. glabrum	CN014F4	*Aspergilloides*	*Glabra*	n.a.	MT957429	n.a.	n.a.
P. jacksonii	CN014D1	*Sclerotiorum*	*Sclerotiorum*	MT949908	MT957413	MT957455	MT957481
P. jacksonii	CN014E8	*Sclerotiorum*	*Sclerotiorum*	MT949919	MT957426	MT957466	MT957492
P. janczewskii	CMW 56632 = CBS 147413 = CN014B9	*Canescentia*	*Canescentia*	n.a.	MT957403	MT957449	MT957475
P. janczewskii	CN014C1	*Canescentia*	*Canescentia*	n.a.	MT957404	n.a.	n.a.
P. kalander	CMW 56202 = CN014E1 (ex-type)	*Sclerotiorum*	*Sclerotiorum*	MT949914	MT957421	MT957461	MT957487
P. kalander	CMW 56203 = CN014E2	*Sclerotiorum*	*Sclerotiorum*	MT949915	MT957422	MT957462	MT957488
P. kalander	CMW 56204 = CN014E3	*Sclerotiorum*	*Sclerotiorum*	MT949916	MT957423	MT957463	MT957489
P. kalander	CMW 56205 = CBS 147422 = CN014E4	*Sclerotiorum*	*Sclerotiorum*	MT949917	MT957424	MT957464	MT957490
P. kalander	CMW 56390 = CBS 147423 = CN014E5	*Sclerotiorum*	*Sclerotiorum*	MT949918	MT957425	MT957465	MT957491
P. mattheeae	CMW 56388 = CBS 147415 = CN014C5 (ex-type)	*Aspergilloides*	*Saturniformia*	MT949904	MT957408	MT957451	MT957477
P. mattheeae	CMW 56195 = CBS 147416 = CN014C6	*Aspergilloides*	*Saturniformia*	MT949905	MT957409	MT957452	MT957478
P. mattheeae	CMW 56633 = CBS 147417 = CN014C7	*Aspergilloides*	*Saturniformia*	MT949906	MT957410	MT957453	MT957479
P. melinii	CN014I6	*Exilicaulis*	*Lapidosa*	n.a.	MT957440	n.a.	n.a.
P. melinii	CN014I7	*Exilicaulis*	*Lapidosa*	n.a.	MT957441	n.a.	n.a.
P. melinii	CN014I8	*Exilicaulis*	*Lapidosa*	n.a.	MT957442	n.a.	n.a.
P. onobense	CN014H3	*Lanata-Divaricata*	*Simplicissima*	n.a.	MT957436	MT957469	MT957496
P. outeniquaense	CMW 56387 = CBS 147414 = CN014C2 (ex-type)	*Citrina*	*Westlingiorum*	MT949903	MT957405	MT957450	MT957476
P. subfuscum	CN014A6	*Lanata-Divaricata*	*Simplicissima*	MW329997	MW340969	MW340970	MW340971
P. subfuscum	CMW 56196 = CBS 147455 = CN014C9 (ex-type)	*Lanata-Divaricata*	*Simplicissima*	MT949907	MT957412	MT957454	MT957480
P. subspinulosum	CN014D7	*Aspergilloides*	*Spinulosa*	n.a.	MT957419	n.a.	n.a.
P. subspinulosum	CN014D8	*Aspergilloides*	*Spinulosa*	n.a.	MT957420	n.a.	n.a.
P. subspinulosum	CN014E9	*Aspergilloides*	*Spinulosa*	n.a.	MT957427	n.a.	n.a.
P. subspinulosum	CN014F1	*Aspergilloides*	*Spinulosa*	n.a.	MT957428	n.a.	n.a.
P. umkhoba	CMW 56199 = CBS 147456 = CN014D4	*Sclerotiorum*	*Herqueorum*	MT949911	MT957416	MT957458	MT957484
P. umkhoba	CMW 56200 = CBS 147457 = CN014D5 (ex-type)	*Sclerotiorum*	*Herqueorum*	MT949912	MT957417	MT957459	MT957485
P. umkhoba	CMW 56201 = CBS 147419 = CN014D6	*Sclerotiorum*	*Herqueorum*	MT949913	MT957418	MT957460	MT957486
P. vagum	CN014C4	*Aspergilloides*	*Longicatenata*	n.a.	MT957407	n.a.	n.a.
P. vagum	CN014H5	*Aspergilloides*	*Longicatenata*	n.a.	MT957438	n.a.	n.a.
P. vancouverense	CN014C8	*Citrina*	*Westlingiorum*	n.a.	MT957411	n.a.	n.a.
P. vancouverense	CN014H4	*Citrina*	*Westlingiorum*	n.a.	MT957437	n.a.	n.a.
P. vancouverense	CN014H6	*Citrina*	*Westlingiorum*	n.a.	MT957439	n.a.	n.a.
P. westlingii	CN014C3	*Citrina*	*Westlingiorum*	n.a.	MT957406	n.a.	n.a.

DNA extraction, sequencing, identifications and phylogenetic analyses

DNA was extracted from colonies grown on CYA for 7 d using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, California, USA). DNA was accessioned into the CN-DNA collection and stored at -20 C.

All strains were identified based on *BenA* sequences. For new or rare species, the ITS, *CaM* and *RPB2* were also sequenced. Gene regions were amplified using PCR conditions and primer pairs (V9G & LS266 for ITS; Bt2a & Bt2b for *BenA*; cmd5 & cmd6 for *CaM*; and RPB2-RPB2F1 & RPB27CRa for *RPB2*) as suggested by Visagie and others (2014) and Houbraken and others (2020). PCRs were prepared in 25 µl volumes containing 0.15 µl MyTaq™ DNA polymerase (Bioline, Meridian Bioscience, USA), 5 µl 5X MyTaq™ Reaction Buffer (BioLine), 0.5 µl of each primer (10 µM), 0.5 µl template DNA and 18.35 µl MilliQ H_2O.

Bidirectional sequencing reactions were set up using the BigDye Terminator 3.1 ready reaction mix (Perkin-Elmer, Warrington, United Kingdom). Sequencing reactions were analyzed at the University of Pretoria sequencing facility on an ABI PRISM™ 3500xl Auto-sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were assembled into contigs and edited in Geneious Prime 2022 (BioMatter Ltd., Auckland, New Zealand). Obtained sequences were compared to a locally curated reference sequence dataset based on ex-type sequences listed by Houbraken and others (2020) and expanded using sequences of additional reference strains published in various taxonomic revisions. Based on these identifications, a subset of sequences was extracted and used for phylogenetic comparisons (SUPPLEMENTARY TABLE 1). Newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/), with accession numbers listed in TABLE 1. All alignments were uploaded to the University of

Pretoria's research data repository hosted on Figshare (https://doi.org/10.25403/UPresearchdata.21115984).

Datasets were aligned in MAFFT 7.490 (Katoh and Standley 2013) using the G-INS-I option. Alignments were trimmed and adjusted in Geneious Prime. Datasets were partitioned based on gene regions, as well as introns and exons. The most appropriate nucleotide substitution model for each partition was selected based on the Akaike information criterion (Akaike 1974) using PartitionFinder 2.1 (Lanfear and others 2017). Phylogenies were performed using Maximum Likelihood (ML) and Bayesian tree Inference (BI). ML was performed using IQtree 2.1.3 (Nguyen and others 2015) with support in nodes calculated using a bootstrap analysis with 1000 replicates. BI analyses were performed in MrBayes 3.2.7a (Ronquist and others 2012) using three sets of four chains (one cold and three heated) and were stopped using the stoprule option at an average standard deviation for split frequencies of 0.01. Trees were visualized using Treeviewer 2.0.1 (https://treeviewer.org/) and edited in Affinity Publisher 1.10.4 (Serif (Europe) Ltd, Nottingham, UK). ML and BI tree topologies did not differ and thus ML trees were used to present results with both bootstrap values and posterior probabilities shown for supported branches.

Morphology

New species were characterized using the standardized protocols recommended in Visagie and others (2014). Briefly, characters were recorded on CYA, CYA with 5 % NaCl (CYAS), Dichloran-Glycerol agar (DG18: Oxoid CM0729), malt extract agar (MEA; Oxoid CM0059), oatmeal agar (OA), yeast extract sucrose agar (YES) and creatine sucrose agar (CREA), prepared in 90 mm Petri dishes. Inoculations were made in a three-point fashion and incubated in the dark for 7 d

at 25 C, with additional CYA plates incubated at 5, 10, 15, 20, 30 and 37 C. Colour names and codes used in descriptions follow Kornerup and Wanscher (1967). Colonies were captured with a Sony a6400 camera equipped with a Sony FE 90mm f/2.8 Macro G OSS lens. Microscopic observations were made using Zeiss AXIO Imager.A2 compound and AXIO Zoom.V16 microscopes equipped with an AxioCaM 512 color camera driven by Zen Blue 3.2 software (Carl Zeiss CMP GmbH, Göttingen, Germany). Extended Depth of Field analysis and stacking of colony texture micrographs were performed using Helicon Focus 7.5.4 (HeliconSoft, Kharkiv, Ukraine). Plates were prepared in Affinity Photo 1.10.4 (Serif (Europe) Ltd, Nottingham, UK). For aesthetic purposes, some micrographs were edited using the "inpainting brush tool" without altering areas of scientific significance.

RESULTS

Identifications and phylogenetic analyses

Isolations from six soil samples resulted in the recovery of 95 strains. These were identified as *Clonostachys rosea* (n = 1), *Ilyonectria cyclaminicola* (n = 2), a putative new *Metarhizium* species (n = 9), a new *Talaromyces* (n = 9), and a putative new genus (n = 6) closely related to *Monascus*. The remaining 74 strains were identified as *Penicillium,* representing 18 species, namely *P. adametzii* (n = 13), *P. cairnsense* (n = 1), *P. crocicola* (n = 2), *P. glabrum* (n = 1), *P. jacksonii* (n = 2), *P. janczewskii* (n = 2), *P. melinii* (n = 13), *P. onobense* (n = 1), *P. subspinulosum* (n = 9), *P. vagum* (n = 2), *P. vancouverense* (n = 11), *P. westlingii* (n = 1) and the six new species described below. Culture accessions, their identities and GenBank accession numbers are summarized in TABLE 1.

For the new species, ITS performed poorly as an identification marker in contrast to *BenA*, *CaM* and *RPB2* that distinguished our new species from all other known species. Phylogenies were calculated for each new species based on its series classification (FIGS. 1–6), with SUPPLEMENTARY TABLE 2 summarizing dataset characteristics, partitioning schemes and substitution models applied during data processing. Phylogenies resolved the new species in distinct, well supported clades, and demonstrated genealogical concordance. An exception was observed in the *CaM* phylogeny for series *Westlingiorum* (FIG. 4) where CBS: 126422 resolved distinct from other strains of *P. godlewskii*. A similar result was found by Houbraken and others (2011), but based on a multigene phylogeny, morphology and extrolite profiles, CBS: 126422 adhered to their concept of *P. godlewskii*. Here we use a phylogenetic species concept to delineate our new species. We formally describe them below and provide additional notes on their phylogenetic relationships and compare their morphologies with known species.

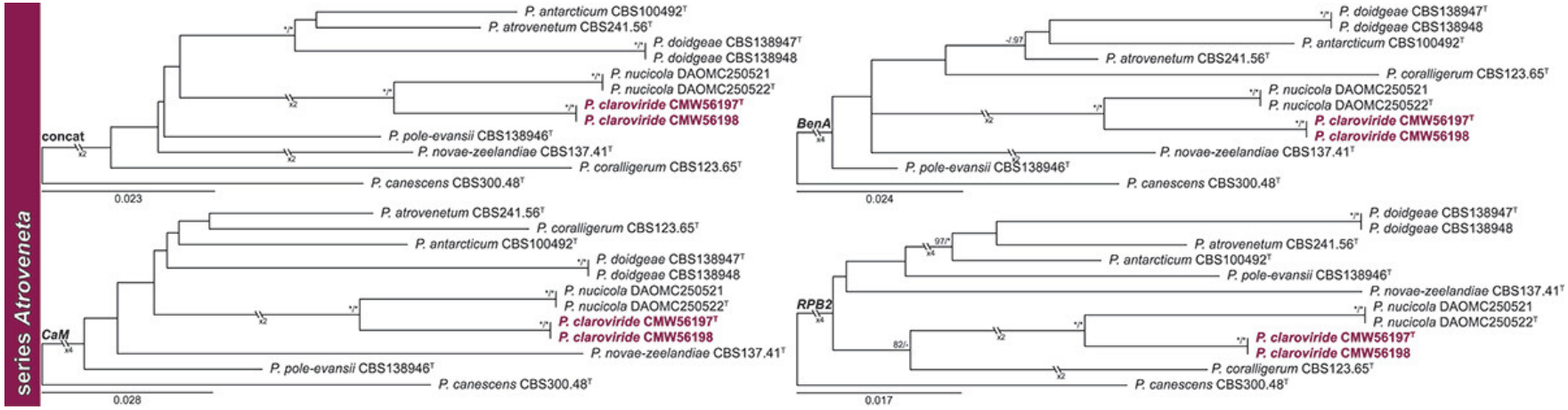
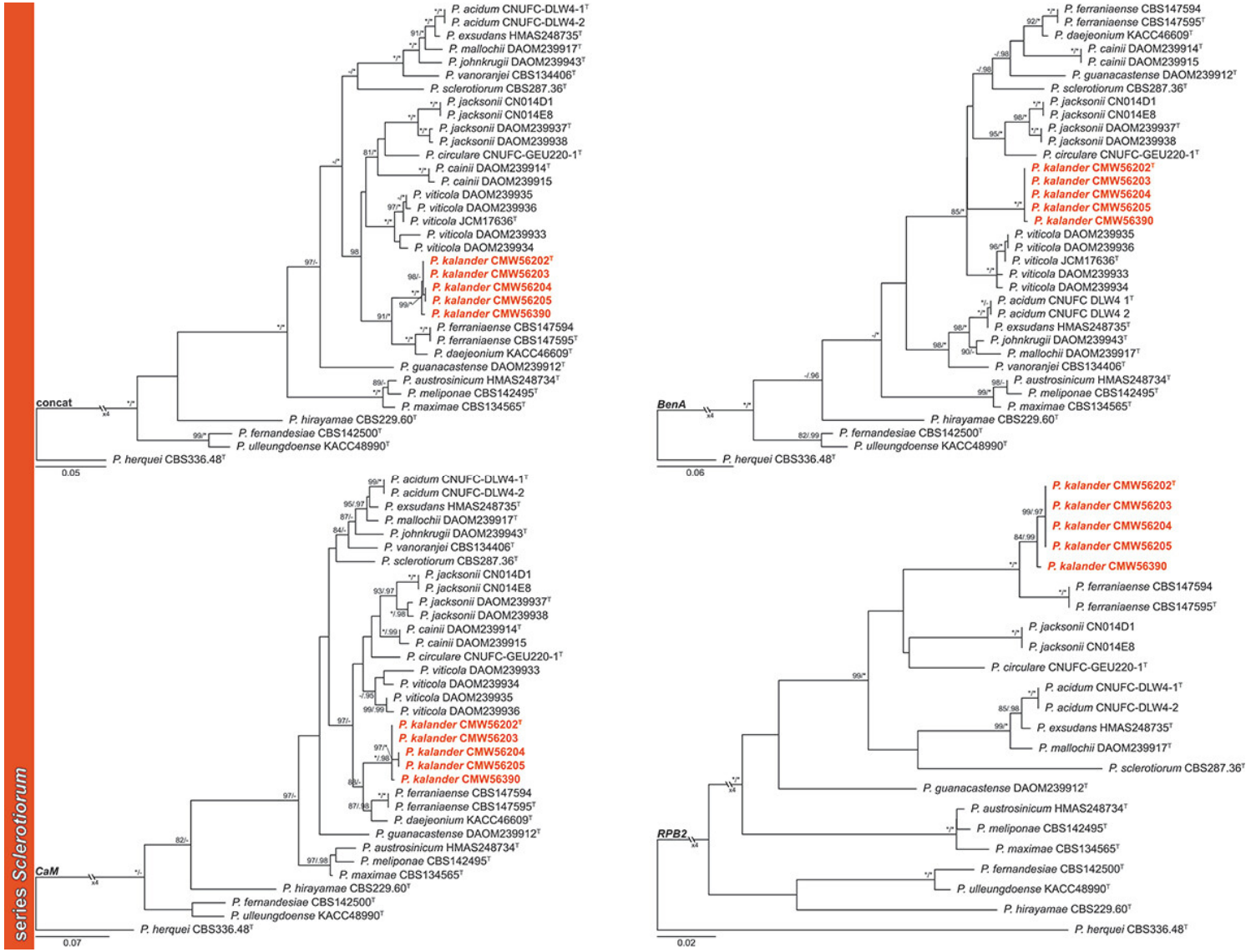


Figure 1. Phylogenetic trees of *Penicillium* section *Canescentia* series *Atroveneta* based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. Strains of the new species, *P. claroviride*, are shown in colored bold text. Trees were rooted to *P. canescens*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).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concat
P. acidum CNUFC-DLW4-1T
P. acidum CNUFC-DLW4-2
P. exsudans HMAS248735T
P. mallochii DAOM239917T
P. johnkrugii DAOM239943T
P. vanoranjei CBS134406T
P. sclerotiorum CBS287.36T
P. jacksonii CN014D1
P. jacksonii CN014E8
P. jacksonii DAOM239937T
P. jacksonii DAOM239938
P. circulare CNUFC-GEU220-1T
P. cainii DAOM239914T
P. cainii DAOM239915
P. viticola DAOM239935
P. viticola DAOM239936
P. viticola JCM17636T
P. viticola DAOM239933
P. viticola DAOM239934
P. kalander CMW56202T
P. kalander CMW56203
P. kalander CMW56204
P. kalander CMW56205
P. kalander CMW56390
P. ferraniaense CBS147594
P. ferraniaense CBS147595T
P. daejeonium KACC46609T
P. guanacastense DAOM239912T
P. austrosinicum HMAS248734T
P. meliponae CBS142495T
P. maximae CBS134565T
P. hirayamae CBS229.60T
P. fernandesiae CBS142500T
P. ulleungdoense KACC48990T
P. herquei CBS336.48T
0.05
BenA
P. ferraniaense CBS147594
P. ferraniaense CBS147595T
P. daejeonium KACC46609T
P. cainii DAOM239914T
P. cainii DAOM239915
P. guanacastense DAOM239912T
P. sclerotiorum CBS287.36T
P. jacksonii CN014D1
P. jacksonii CN014E8
P. jacksonii DAOM239937T
P. jacksonii DAOM239938
P. circulare CNUFC-GEU220-1T
P. kalander CMW56202T
P. kalander CMW56203
P. kalander CMW56204
P. kalander CMW56205
P. kalander CMW56390
P. viticola DAOM239935
P. viticola DAOM239936
P. viticola JCM17636T
P. viticola DAOM239933
P. viticola DAOM239934
P. acidum CNUFC DLW4 1T
P. acidum CNUFC DLW4 2
P. exsudans HMAS248735T
P. johnkrugii DAOM239943T
P. mallochii DAOM239917T
P. vanoranjei CBS134406T
P. austrosinicum HMAS248734T
P. meliponae CBS142495T
P. maximae CBS134565T
P. hirayamae CBS229.60T
P. fernandesiae CBS142500T
P. ulleungdoense KACC48990T
P. herquei CBS336.48T
0.06
CaM
P. acidum CNUFC-DLW4-1T
P. acidum CNUFC-DLW4-2
P. exsudans HMAS248735T
P. mallochii DAOM239917T
P. johnkrugii DAOM239943T
P. vanoranjei CBS134406T
P. sclerotiorum CBS287.36T
P. jacksonii CN014D1
P. jacksonii CN014E8
P. jacksonii DAOM239937T
P. jacksonii DAOM239938
P. cainii DAOM239914T
P. cainii DAOM239915
P. circulare CNUFC-GEU220-1T
P. viticola DAOM239933
P. viticola DAOM239934
P. viticola DAOM239935
P. viticola DAOM239936
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P. kalander CMW56203
P. kalander CMW56204
P. kalander CMW56205
P. kalander CMW56390
P. ferraniaense CBS147594
P. ferraniaense CBS147595T
P. daejeonium KACC46609T
P. guanacastense DAOM239912T
P. austrosinicum HMAS248734T
P. meliponae CBS142495T
P. maximae CBS134565T
P. hirayamae CBS229.60T
P. fernandesiae CBS142500T
P. ulleungdoense KACC48990T
P. herquei CBS336.48T
0.07
RPB2
P. kalander CMW56202T
P. kalander CMW56203
P. kalander CMW56204
P. kalander CMW56205
P. kalander CMW56390
P. ferraniaense CBS147594
P. ferraniaense CBS147595T
P. jacksonii CN014D1
P. jacksonii CN014E8
P. circulare CNUFC-GEU220-1T
P. acidum CNUFC-DLW4-1T
P. acidum CNUFC-DLW4-2
P. exsudans HMAS248735T
P. mallochii DAOM239917T
P. sclerotiorum CBS287.36T
P. guanacastense DAOM239912T
P. austrosinicum HMAS248734T
P. meliponae CBS142495T
P. maximae CBS134565T
P. fernandesiae CBS142500T
P. ulleungdoense KACC48990T
P. hirayamae CBS229.60T
P. herquei CBS336.48T
0.02

Figure 2. Phylogenetic trees of *Penicillium* section *Sclerotiorum* series *Sclerotiorum* based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. Strains of the new species, *P. kalander*, are shown in colored bold text. Trees were rooted to *P. herquei*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

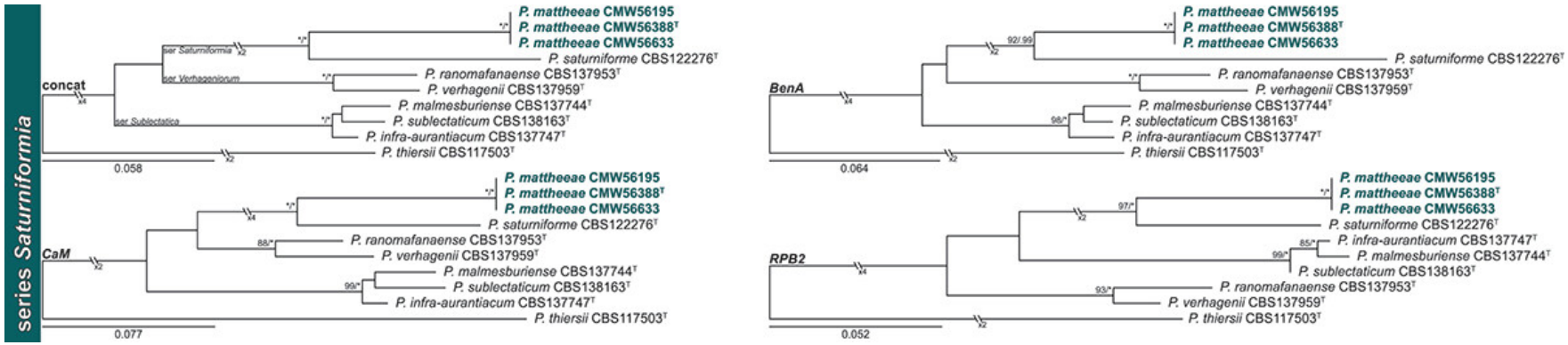
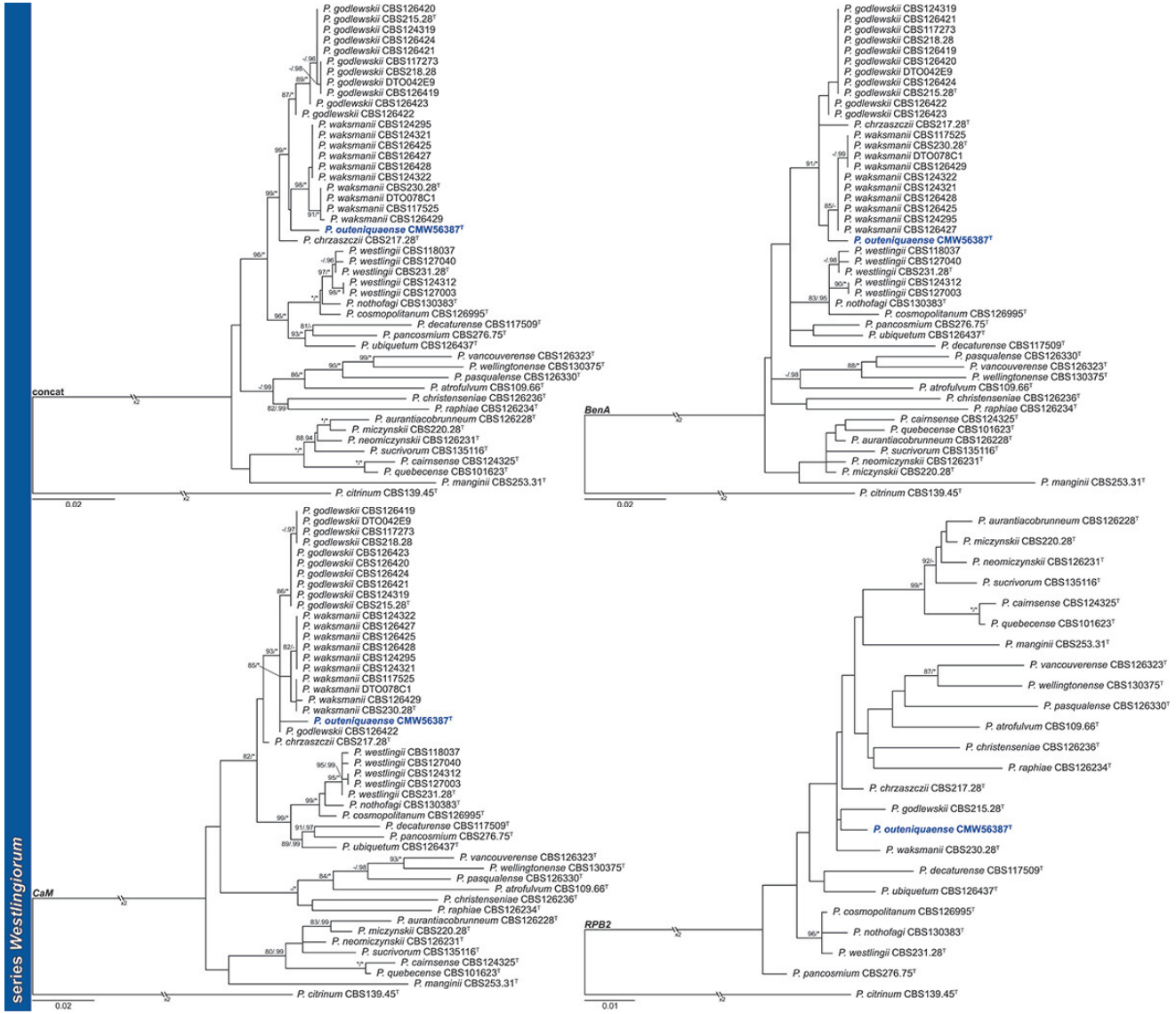


Figure 3. Phylogenetic trees of *Penicillium* section *Aspergilloides* series *Saturniformia* and closely related series, based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. Strains of the new species, *P. mattheeae*, are shown in colored bold text. Trees were rooted to *P. thiersii*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).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concat
BenA
CaM
RPB2
P. godlewskii CBS126420
P. godlewskii CBS215.28T
P. godlewskii CBS124319
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P. godlewskii CBS126421
P. godlewskii CBS117273
P. godlewskii CBS218.28
P. godlewskii DTO042E9
P. godlewskii CBS126419
P. godlewskii CBS126423
P. godlewskii CBS126422
P. waksmanii CBS124295
P. waksmanii CBS124321
P. waksmanii CBS126425
P. waksmanii CBS126427
P. waksmanii CBS126428
P. waksmanii CBS124322
P. waksmanii CBS230.28T
P. waksmanii DTO078C1
P. waksmanii CBS117525
P. waksmanii CBS126429
P. outeniquaense CMW56387T
P. chrzaszczii CBS217.28T
P. westlingii CBS118037
P. westlingii CBS127040
P. westlingii CBS231.28T
P. westlingii CBS124312
P. westlingii CBS127003
P. nothofagi CBS130383T
P. cosmopolitanum CBS126995T
P. decaturense CBS117509T
P. pancosmium CBS276.75T
P. ubiquetum CBS126437T
P. vancouverense CBS126323T
P. wellingtonense CBS130375T
P. pasqualense CBS126330T
P. atrofulvum CBS109.66T
P. christenseniae CBS126236T
P. raphiae CBS126234T
P. aurantiacobrunneum CBS126228T
P. miczynskii CBS220.28T
P. neomiczynskii CBS126231T
P. sucrivorum CBS135116T
P. cairnsense CBS124325T
P. quebecense CBS101623T
P. manginii CBS253.31T
P. citrinum CBS139.45T
0.02
P. godlewskii CBS124319
P. godlewskii CBS126421
P. godlewskii CBS117273
P. godlewskii CBS218.28
P. godlewskii CBS126419
P. godlewskii CBS126420
P. godlewskii DTO042E9
P. godlewskii CBS126424
P. godlewskii CBS215.28T
P. godlewskii CBS126422
P. godlewskii CBS126423
P. chrzaszczii CBS217.28T
P. waksmanii CBS117525
P. waksmanii CBS230.28T
P. waksmanii DTO078C1
P. waksmanii CBS126429
P. waksmanii CBS124322
P. waksmanii CBS124321
P. waksmanii CBS126428
P. waksmanii CBS126425
P. waksmanii CBS124295
P. waksmanii CBS126427
P. outeniquaense CMW56387T
P. westlingii CBS118037
P. westlingii CBS127040
P. westlingii CBS231.28T
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P. westlingii CBS127003
P. nothofagi CBS130383T
P. cosmopolitanum CBS126995T
P. pancosmium CBS276.75T
P. ubiquetum CBS126437T
P. decaturense CBS117509T
P. pasqualense CBS126330T
P. vancouverense CBS126323T
P. wellingtonense CBS130375T
P. atrofulvum CBS109.66T
P. christenseniae CBS126236T
P. raphiae CBS126234T
P. cairnsense CBS124325T
P. quebecense CBS101623T
P. aurantiacobrunneum CBS126228T
P. sucrivorum CBS135116T
P. neomiczynskii CBS126231T
P. miczynskii CBS220.28T
P. manginii CBS253.31T
P. citrinum CBS139.45T
0.02
P. godlewskii CBS126419
P. godlewskii DTO042E9
P. godlewskii CBS117273
P. godlewskii CBS218.28
P. godlewskii CBS126423
P. godlewskii CBS126420
P. godlewskii CBS126424
P. godlewskii CBS126421
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P. waksmanii CBS126428
P. waksmanii CBS124295
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P. waksmanii CBS117525
P. waksmanii DTO078C1
P. waksmanii CBS126429
P. waksmanii CBS230.28T
P. outeniquaense CMW56387T
P. godlewskii CBS126422
P. chrzaszczii CBS217.28T
P. westlingii CBS118037
P. westlingii CBS127040
P. westlingii CBS124312
P. westlingii CBS127003
P. westlingii CBS231.28T
P. nothofagi CBS130383T
P. cosmopolitanum CBS126995T
P. decaturense CBS117509T
P. pancosmium CBS276.75T
P. ubiquetum CBS126437T
P. vancouverense CBS126323T
P. wellingtonense CBS130375T
P. pasqualense CBS126330T
P. atrofulvum CBS109.66T
P. christenseniae CBS126236T
P. raphiae CBS126234T
P. aurantiacobrunneum CBS126228T
P. miczynskii CBS220.28T
P. neomiczynskii CBS126231T
P. sucrivorum CBS135116T
P. cairnsense CBS124325T
P. quebecense CBS101623T
P. manginii CBS253.31T
P. citrinum CBS139.45T
0.02
P. aurantiacobrunneum CBS126228T
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P. sucrivorum CBS135116T
P. cairnsense CBS124325T
P. quebecense CBS101623T
P. manginii CBS253.31T
P. vancouverense CBS126323T
P. wellingtonense CBS130375T
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P. atrofulvum CBS109.66T
P. christenseniae CBS126236T
P. raphiae CBS126234T
P. chrzaszczii CBS217.28T
P. godlewskii CBS215.28T
P. outeniquaense CMW56387T
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P. decaturense CBS117509T
P. ubiquetum CBS126437T
P. cosmopolitanum CBS126995T
P. nothofagi CBS130383T
P. westlingii CBS231.28T
P. pancosmium CBS276.75T
P. citrinum CBS139.45T
0.01

Figure 4. Phylogenetic trees of *Penicillium* section *Citrina* series *Westlingiorum* based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. The strain of the new species, *P. outeniquaense*, is shown in colored bold text. Trees were rooted to *P. citrinum*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

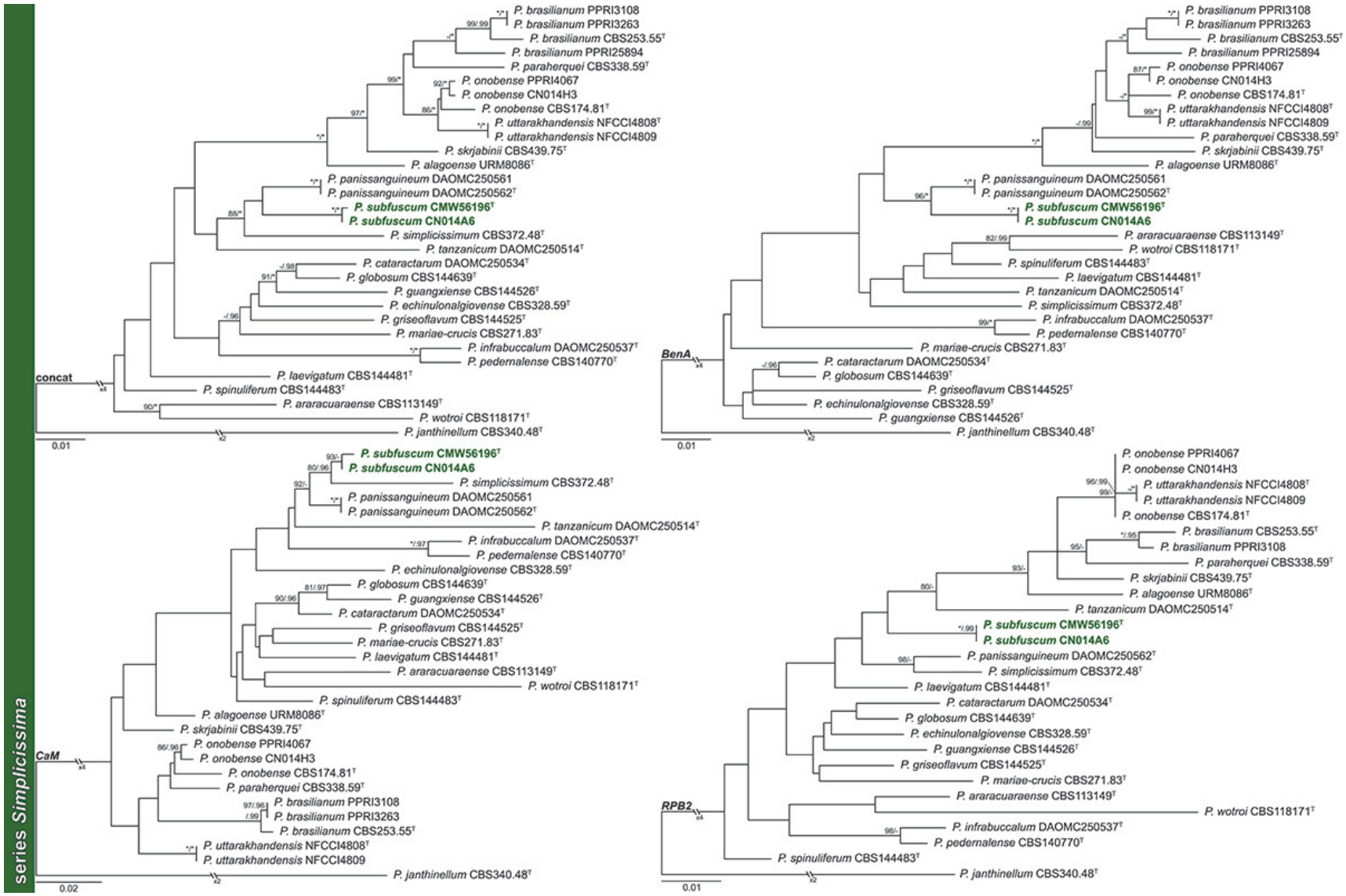
series Simplicissima
concat
P. brasilianum PPRI3108
P. brasilianum PPRI3263
P. brasilianum CBS253.55T
P. brasilianum PPRI25894
P. paraherquei CBS338.59T
P. onobense PPRI4067
P. onobense CN014H3
P. onobense CBS174.81T
P. uttarakhandensis NFCCI4808T
P. uttarakhandensis NFCCI4809
P. skrjabinii CBS439.75T
P. alagoense URM8086T
P. panissanguineum DAOMC250561
P. panissanguineum DAOMC250562T
P. subfuscum CMW56196T
P. subfuscum CN014A6
P. simplicissimum CBS372.48T
P. tanzanicum DAOMC250514T
P. cataractarum DAOMC250534T
P. globosum CBS144639T
P. guangxiense CBS144526T
P. echinulonalgiovense CBS328.59T
P. griseoflavum CBS144525T
P. mariae-crucis CBS271.83T
P. infrabuccalum DAOMC250537T
P. pedernalense CBS140770T
P. laevigatum CBS144481T
P. spinuliferum CBS144483T
P. araracuaraense CBS113149T
P. wotroi CBS118171T
P. janthinellum CBS340.48T
0.01
BenA
P. brasilianum PPRI3108
P. brasilianum PPRI3263
P. brasilianum CBS253.55T
P. brasilianum PPRI25894
P. onobense PPRI4067
P. onobense CN014H3
P. onobense CBS174.81T
P. uttarakhandensis NFCCI4808T
P. uttarakhandensis NFCCI4809
P. paraherquei CBS338.59T
P. skrjabinii CBS439.75T
P. alagoense URM8086T
P. panissanguineum DAOMC250561
P. panissanguineum DAOMC250562T
P. subfuscum CMW56196T
P. subfuscum CN014A6
P. araracuaraense CBS113149T
P. wotroi CBS118171T
P. spinuliferum CBS144483T
P. laevigatum CBS144481T
P. tanzanicum DAOMC250514T
P. simplicissimum CBS372.48T
P. infrabuccalum DAOMC250537T
P. pedernalense CBS140770T
P. mariae-crucis CBS271.83T
P. cataractarum DAOMC250534T
P. globosum CBS144639T
P. griseoflavum CBS144525T
P. echinulonalgiovense CBS328.59T
P. guangxiense CBS144526T
P. janthinellum CBS340.48T
0.01
CaM
P. subfuscum CMW56196T
P. subfuscum CN014A6
P. simplicissimum CBS372.48T
P. panissanguineum DAOMC250561
P. panissanguineum DAOMC250562T
P. tanzanicum DAOMC250514T
P. infrabuccalum DAOMC250537T
P. pedernalense CBS140770T
P. echinulonalgiovense CBS328.59T
P. globosum CBS144639T
P. guangxiense CBS144526T
P. cataractarum DAOMC250534T
P. griseoflavum CBS144525T
P. mariae-crucis CBS271.83T
P. laevigatum CBS144481T
P. araracuaraense CBS113149T
P. wotroi CBS118171T
P. spinuliferum CBS144483T
P. alagoense URM8086T
P. skrjabinii CBS439.75T
P. onobense PPRI4067
P. onobense CN014H3
P. onobense CBS174.81T
P. paraherquei CBS338.59T
P. brasilianum PPRI3108
P. brasilianum PPRI3263
P. brasilianum CBS253.55T
P. uttarakhandensis NFCCI4808T
P. uttarakhandensis NFCCI4809
P. janthinellum CBS340.48T
0.02
RPB2
P. onobense PPRI4067
P. onobense CN014H3
P. uttarakhandensis NFCCI4808T
P. uttarakhandensis NFCCI4809
P. onobense CBS174.81T
P. brasilianum CBS253.55T
P. brasilianum PPRI3108
P. paraherquei CBS338.59T
P. skrjabinii CBS439.75T
P. alagoense URM8086T
P. tanzanicum DAOMC250514T
P. subfuscum CMW56196T
P. subfuscum CN014A6
P. panissanguineum DAOMC250562T
P. simplicissimum CBS372.48T
P. laevigatum CBS144481T
P. cataractarum DAOMC250534T
P. globosum CBS144639T
P. echinulonalgiovense CBS328.59T
P. guangxiense CBS144526T
P. griseoflavum CBS144525T
P. mariae-crucis CBS271.83T
P. araracuaraense CBS113149T
P. wotroi CBS118171T
P. infrabuccalum DAOMC250537T
P. pedernalense CBS140770T
P. spinuliferum CBS144483T
P. janthinellum CBS340.48T
0.01

Figure 5. Phylogenetic trees of *Penicillium* section *Lanata-Divaricata* series *Simplicissima* based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. Strains of the new species, *P. subfuscum*, are shown in colored bold text. Trees were rooted to *P. janthinellum*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

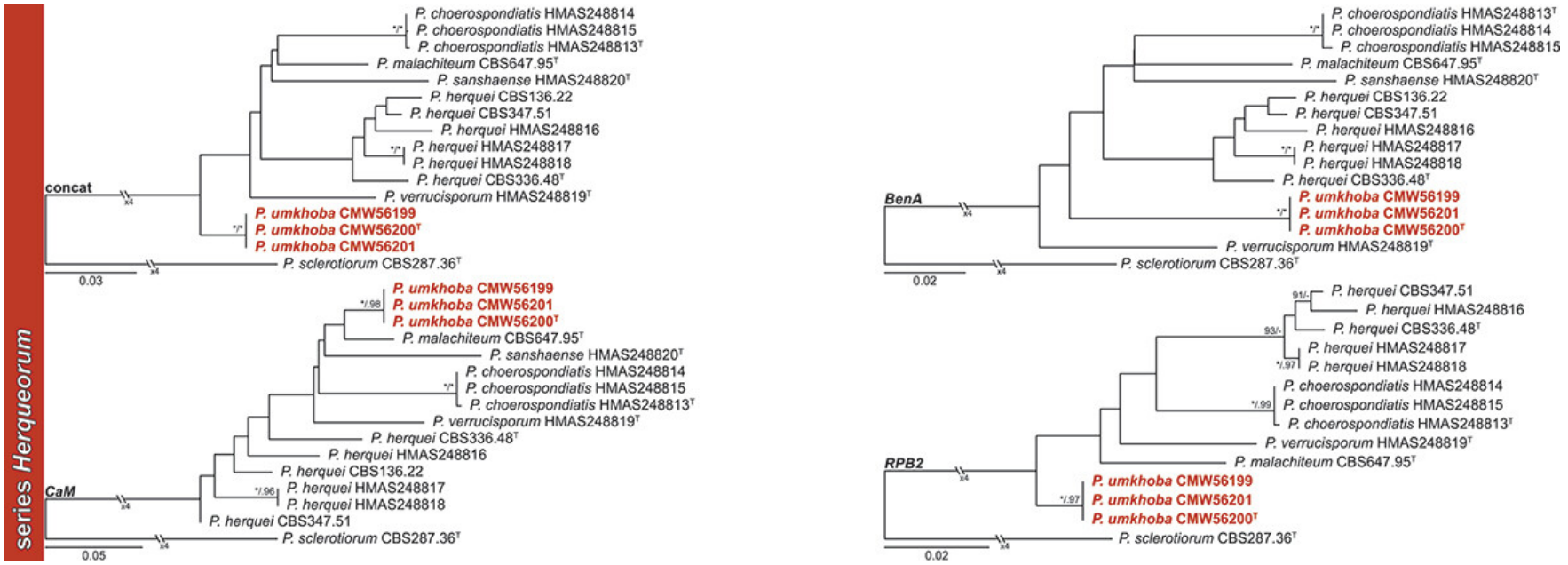

Figure 6. Phylogenetic trees of *Penicillium* section *Sclerotiorum* series *Herqueorum* based on a concatenated dataset, *BenA*, *CaM* and *RPB2*. Strains of the new species, *P. umkhoba*, are shown in colored bold text. Trees were rooted to *P. sclerotiorum*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated at relevant branches (T = ex-type; * = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp).

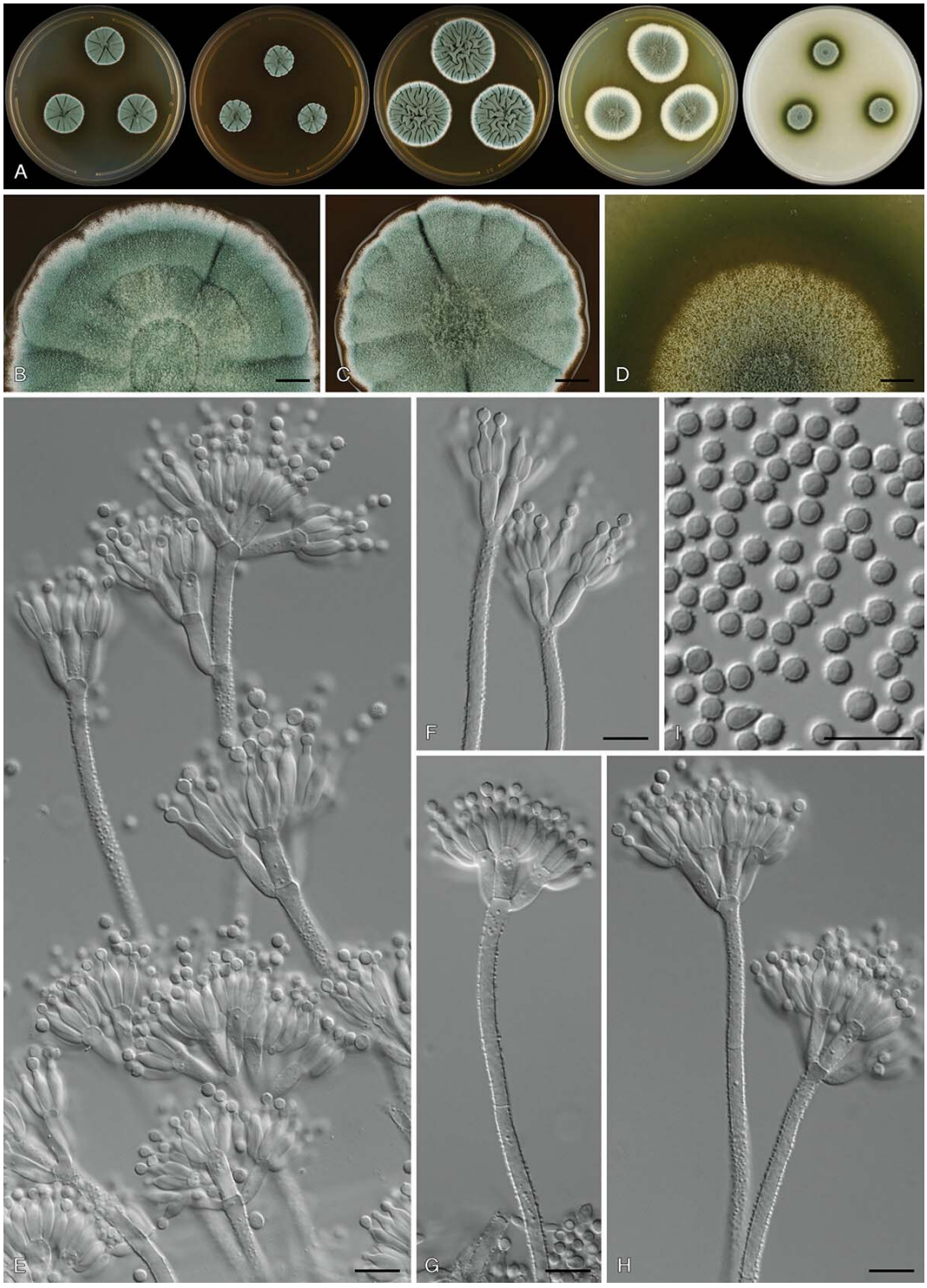

Figure 7. Morphological characters of *Penicillium claroviride*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; C. texture on MEA; D. texture of OA. E–H. conidiophores; I. conidia. Bar = 1 mm (B, C), 250 µm (D), 10 µm (E–I).

TAXONOMY

Penicillium claroviride Visagie & Yilmaz, sp. nov. FIG. 7

MycoBank: MB844184

In: subgenus *Penicillium* section *Canescentia* series *Atroveneta* (Houbraken and others 2020).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz* (**holotype** PREM: 63221 (dried specimen in metabolically inactive state), culture ex-type CMW: 56197 = CBS: 147458 = CN014D2). DNA reference sequences: ITS = MT949909, *BenA* = MT957414, *CaM* = MT957456, *RPB2* = MT957482.

Additional strains examined: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz*, culture CMW: 56198 = CBS: 47418 = CN014D3.

Etymology: Latin, *claroviride*, green = *viride*; bright = *clarus*, named after the bright green soluble pigments produced by colonies on OA.

Description: Colony diam, 7d, in mm: CYA 20–21; CYA10C 13–15; CYA15C 24–26; CYA20C 28–30; CYA30C no growth; CYA37C no growth; CYAS 22–23; MEA 15–16; YES 32–34; DG18 25–28; OA 12–14; CREA 7–9.

CYA 25 C, 7 d: Colonies low, radially and concentrically sulcate, crateriform; margins low, entire, narrow; mycelia white; texture velutinous; sporulation dense; conidia *en masse* Greyish Green (25C4–5); soluble pigments yellowish brown; exudates absent; reverse Olive (3D6–F4). MEA 25 C, 7 d: Colonies low, radially sulcate, slightly crateriform; margins low, entire, narrow; mycelia

white; texture velutinous; sporulation dense; conidia *en masse* Greyish Green (25C4–5); soluble pigments absent; exudates absent; reverse Olive (3F4). YES 25 C, 7 d: Colonies raised, radially sulcate, crateriform; margins low, entire, narrow; mycelia white; texture velutinous; sporulation dense; conidia *en masse* Greyish Green (25C3–5); soluble pigments absent; exudates absent; reverse Olive Brown (4D5). DG18 25 C, 7 d: Colonies slightly raised, radially and concentrically sulcate; margins low, entire, narrow; mycelia white and yellow, inconspicuous; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish Green (25D5–E5); soluble pigments yellow; exudates absent; reverse Greyish Yellow (2B5–C5). OA 25 C, 7 d: Colonies low, plane; margins low, entire, wide; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Pale to Greyish Turquoise (24A3–C5); soluble pigments intense, dark green; exudates clear, minute droplets; reverse Dark Green (27F7), Greyish Green (29D5). CREA 25 C, 7 d: Colonies dense growth, acid produced, weak.

Conidiophores symmetrically biverticillate, terverticillicate rare; *stipes* rough walled to almost verrucose, (95–)115–265 × 3–4 µm; *branches* 15–55 µm; *metulae* 4–6 per stipe/branch, appressed, 10.5–16 × 3.5 µm; *phialides* ampulliform, 6–10 per metulae, 8–12 × 2.5–4 µm ($\bar{x}$ = 9.8±1.0 × 3.1±0.4), average metula:phialide = 1.3; *conidia* rough walled, globose, some subglobose, 2.5–3.5 µm, ($\bar{x}$ = 3.0±0.4), average width:length = 0.95, n = 50.

Distinguishing features: *Penicillium claroviride* is closely related to *P. nucicola* (FIG. 1), both producing symmetrically biverticillate and appressed conidiophores that are typical of series *Atroveneta*. Similar conidiophores are produced by species classified in section *Aspergilloides* series *Saturniformia* and section *Sclerotiorum* series *Herqueorum*. Distinguishing between these groups are difficult, but series *Saturniformia* species produce strictly biverticillate

conidiophores, series *Atroveneta* species consistently produce a proportion of terverticillate conidiophores, while both lack the bright yellow to orange mycelia of series *Herqueorum* species. Several characters distinguish *P. claroviride* and *P. nucicola* from others in series *Atroveneta*, most notably their colonies on OA that produce intense dark green soluble pigments. Comparing these two species, *P. claroviride* has darker sporulation, while it grows slightly slower on YES (32–34 vs 37–40 mm), OA (12–14 vs 17–20 mm) and CREA (7–9 vs 13–15 mm). Additionally, *P. claroviride* conidia are slightly larger than those of *P. nucicola* (2.5–3.5 ($\overline{x}$ = 3.0±0.4) vs 2.5–3 ($\overline{x}$ = 2.8±0.2) µm) (Visagie and others 2016).

Penicillium kalander Visagie & Yilmaz, sp. nov. FIG. 8

MycoBank: MB844185

In: subgenus *Aspergilloides* section *Sclerotiorum* series *Sclerotiorum* (Houbraken and others 2020).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz* (**holotype** PREM: 63223 (dried specimen in metabolically inactive state), culture ex-type CMW: 56202 = CN014E1). DNA reference sequences: ITS = MT949914, *BenA* = MT957421, *CaM* = MT957461, *RPB2* = MT957487.

Additional strains examined: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz*, cultures CMW: 56203 = CN014E2, CMW: 56204 = CN014E3, CMW: 56205 = CBS: 147422 = CN014E4, CMW: 56390 = CBS: 147423 = CN014E5.

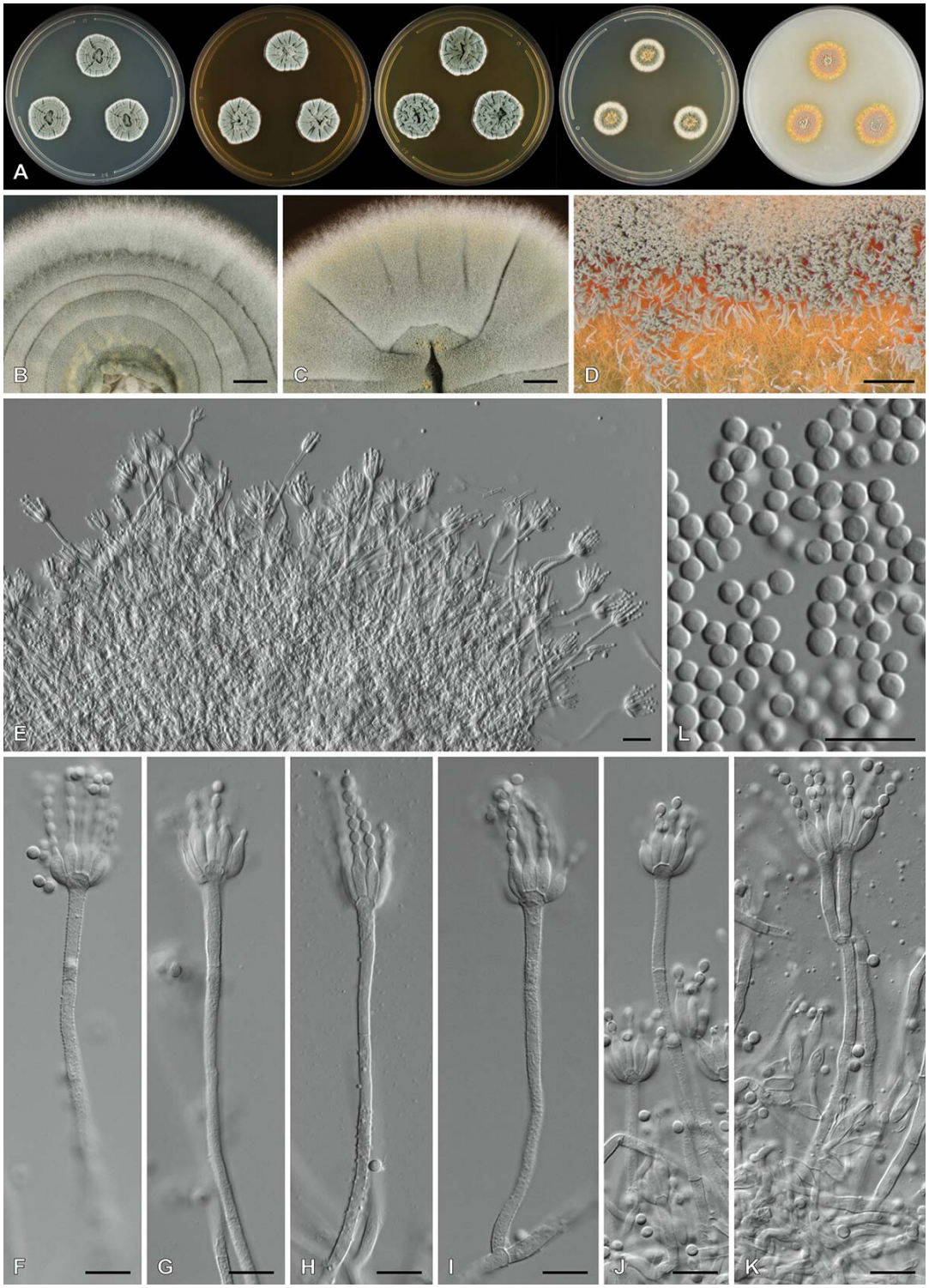

Figure 8. Morphological characters of *Penicillium kalander*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; C, D. texture on MEA; E–K. conidiophores; L. conidia. Bar = 1 mm (B, D), 500 µm (C), 20 µm (E), 10 µm (F–L).

Etymology: Latin, *kalander*, from the Afrikaans name for the Outeniqua yellowwood (*Afrocarpus falcatus* (= *Podocarpus falcatus*)).

Description: Colony diam, 7d, in mm: CYA 20–24; CYA10C 7–10; CYA15C 17–22; CYA20C 25–30; CYA30C no growth; CYA37C no growth; CYAS 18–20; MEA 24–27 (–31); YES 15–20; DG18 18–21; OA 17–20; CREA 4–5.

CYA 25 C, 7 d: Colonies moderately deep, radially concentrically sulcate, crateriform; margins low, entire, narrow; mycelia white, becoming orange with time; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25A3–B3, 25C3–D4); soluble pigments absent; exudates absent, sometimes yellow; reverse Greyish Yellow (2B3–C4). MEA 25 C, 7 d: Colonies moderately deep, radially sulcate, crateriform; margins low, entire, narrow; mycelia white, becoming bright orange with time; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25A3–B3, 25C3–D4); soluble pigments absent; exudates absent, sometimes yellow; reverse Greyish Yellow (2B3–C4). YES 25 C, 7 d: Colonies deep, radially and concentrically sulcate, crateriform; margins low, entire, narrow; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25A3–B3, 25C3–D4); soluble pigments absent; exudates absent; reverse Greyish Yellow (2B3–C4). DG18 25 C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, entire, narrow; mycelia white, deep yellow to orange centrally; texture floccose; sporulation moderately dense; conidia *en masse* Greyish Green (25D5); soluble pigments absent; exudates absent; reverse Pale Yellow (2A3), Orange to Brownish Orange (6B8–C8). OA 25 C, 7 d: Colonies low, plane; margins low to subsurface, irregular, wide; mycelia orange; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish Turquoise to Green

(24C4–25C4); soluble pigments deep yellow to orange; exudates absent; reverse Greyish Orange to Reddish Brown (5B5–8D8). CREA 25 C, 7 d: Colonies dense growth, acid produced by some strains, weak.

Conidiophores monoverticillate, biverticillate rare, non-vesiculate; *stipes* rough walled, 50–130 × (2–)2.5–3.5(–4) µm; *vesicles* 4–5(–7) µm; *metulae* rarely observed, 2 per stipe, 5.5–33 × 2.5–3.5 µm; *phialides* ampulliform, 8–16 per stipe, 8.5–11(–12.5) × 2.5–4 µm ($\overline{x}$ = 10.5±1.0 × 3.2±0.3); *conidia* smooth walled, globose, 2.5–3.5 µm, ($\overline{x}$ = 2.9±0.3), average width/length = 0.97, n = 50.

Distinguishing features: *Penicillium kalander* is classified in section *Sclerotiorium* series *Sclerotiorum* as a close relative of *P. cainii, P. circulare, P. daejeonium, P. ferraniaense, P. jacksonii* and *P. viticola* (FIG. 2). The new species grows slower on CYA and YES (20–24 mm; 15–20 mm) than *P. circulare* (36–41 mm; 33–36 mm), *P. daejeonium* (36–41 mm; 33–36 mm), *P. jacksonii* (30–33 mm; 30–32 mm) and *P. viticola* (26–36 mm; 29–33 mm) (Rivera and others 2012; Rivera and Seifert 2011; Sang and others 2013; Visagie and others 2013). Compared to *P. cainii, Penicillium kalander* has similar growth rates on CYA but grows slower on YES (15–20 vs 31–24 mm) (Rivera and others 2012; Rivera and Seifert 2011). *Penicillium kalander* grows slightly slower than *P. ferraniaense* (25–28 mm; 21–23 mm) on CYA and YES, but our new species does not grow on CYA at 30 C and produces only globose conidia, compared to *P. ferraniaense* that grows 16–20 mm on CYA at 30 C and produces globose, subglobose to broadly ellipsoidal conidia (Crous and others 2021). This group of species produces conidiophores with rough walled stipes, except for *P. circulare* and *P. daejeonium,* which have smooth walled (sometimes finely roughened) stipes.

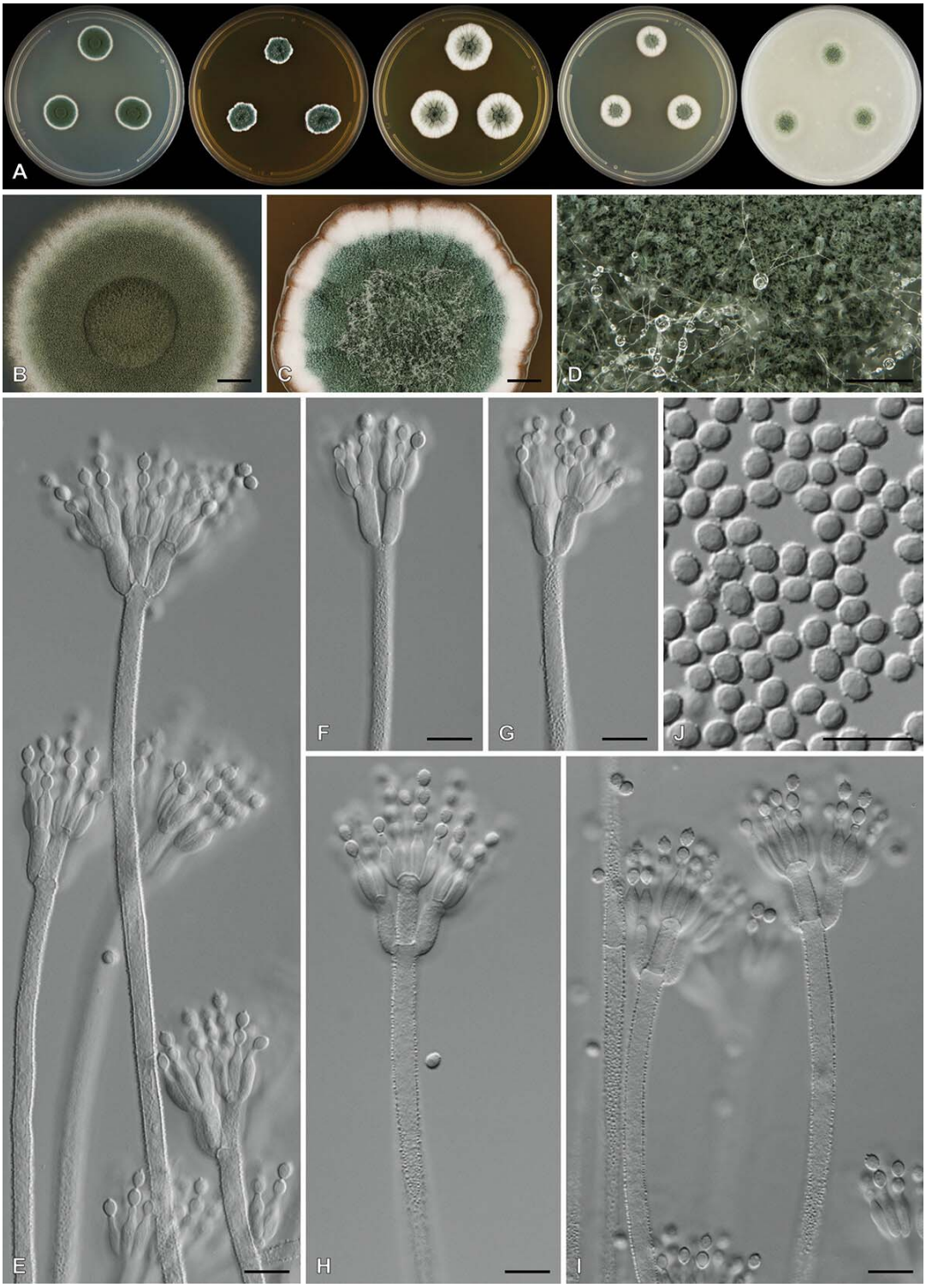

Figure 9. Morphological characters of *Penicillium mattheeae*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; C, D. texture on MEA; E–I. conidiophores; J. conidia. Bar = 1 mm (B, C), 250 µm (D), 10 µm (E–J).

Penicillium mattheeae Visagie & Yilmaz, sp. nov. FIG. 9

MycoBank: MB844186

In: subgenus *Aspergilloides* section *Aspergilloides* series *Saturniformia* (Houbraken and others 2020).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz* (**holotype** PREM: 63219 (dried specimen in metabolically inactive state), culture ex-type CMW: 56388 = CBS: 147415 = CN014C5). DNA reference sequences: ITS = MT949904, *BenA* = MT957408, *CaM* = MT957451, *RPB2* = MT957477.

Additional strains examined: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, December 2018, Dec 2018, *M.J. Wingfield, C.M. Visagie & N. Yilmaz*, cultures CMW: 56195 = CBS: 147416 = CN014C6, CMW: 56633 = CBS: 147417 = CN014C7.

Etymology: Latin, *mattheeae*, named after Dalene Matthee, authoress of the classical South African stories ‘Kringe in 'n bos’, ‘Fiela se Kind’, ‘Toorbos’ and ‘Moerbei Bos’, novels about the woodcutter’s lives and gold rush set in the Knysna forest area.

Description: Colony diam, 7d, in mm: CYA 15–20; CYA10C 7–9; CYA15C 15–17; CYA20C 21–23; CYA30C no growth; CYA37C no growth; CYAS microcolonies, 1–2; MEA 15–17; YES 19–24; DG18 15–17; OA 16–19; CREA 4–5.

CYA 25 C, 7 d: Colonies moderately deep, concentrically sulcate; margins low, entire, narrow; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dark Green (26E6–F6); soluble pigments absent; exudates absent; reverse Greyish Yellow (2B3).

MEA 25 C, 7 d: Colonies moderately deep, radially and concentrically slightly sulcate; margins

low, entire, narrow; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dark Green (25E7–F7); soluble pigments absent; exudates absent; reverse Light Brown to Brown (6D5–E5). YES 25 C, 7 d: Colonies moderately deep, radially slightly sulcate; margins low, entire, narrow; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Dull Green (26D4); soluble pigments absent; exudates absent; reverse Pale Yellow (3A3), Greyish Yellow (4B3). DG18 25 C, 7 d: Colonies low, radially sulcate; margins low, entire, narrow; mycelia white; texture floccose; sporulation sparse; conidia *en masse* Greenish White (25A2); soluble pigments absent; exudates absent; reverse Pale to Greyish Yellow (2A3–B4). OA 25 C, 7 d: Colonies low, plane; margins subsurface, entire, wide; mycelia white; texture floccose and velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dark Green (25E7–F7); soluble pigments absent; exudates clear; reverse white to Greyish Green (1C3). CREA 25 C, 7 d: Colonies weak growth, acid not produced.

Conidiophores symmetrically biverticillate; *stipes* rough walled, 200–660 × 3.5–5 µm; *metulae* 3–5 per stipe, appressed, 11–15(–16) × 3.5–5 µm; *phialides* ampulliform, 5–8 per metula, 8–11.5 × 2.5–4 µm ($\overline{x}$ = 9.7±0.9 × 3.4±0.3), average metula:phialide = 1.4; *conidia* spirally rough walled, broadly ellipsoidal, 3–4 × 2.5–3.5 µm, ($\overline{x}$ = 3.7±0.2 × 2.8±0.2), average width/length = 0.77, n = 50.

Distinguishing features: *Penicillium mattheeae* is classified in section *Aspergilloides* series *Saturniformia* (FIG. 3). The only other species in the series is *P. saturniforme.* Both species produce symmetrically biverticillate appressed conidiophores, making them morphologically distinct from all other members of section *Aspergilloides* which generally produce monoverticillate conidiophores, noting that divaricate conidiophores are produced by *P.*

fortuitum, P. improvisum, P. ranomafanaense and *P. verhagenii* (Crous and others 2018; Houbraken and others 2014; Visagie and others 2016). Series *Saturniformia* conidiophores resemble those observed in section *Canescentia* series *Atroveneta* species and section *Sclerotiorum* series *Herqueorum* species. Distinguishing between these phylogenetically distant groups are difficult but see comparison provided in the distinguishing features for *Penicillium claroviride* above. *Penicillium mattheeae* can be distinguished from *P. saturniforme* based on the new species' longer stipes (200–660 vs 80–200 µm), lack of cleistothecia and slower growth on CYA (15–20 vs 27–29 mm), MEA (15–17 vs 21–24 mm) and YES (19–24 vs 38–42 mm) (Wang and Zhuang 2009).

Penicillium outeniquaense Visagie & Yilmaz, sp. nov. FIG. 10

MycoBank: MB844187

In: subgenus *Aspergilloides* section *Citrina* series *Westlingiorum* (Houbraken and others 2020).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, M.J. Wingfield, C.M. Visagie & N. Yilmaz (**holotype** PREM: 63218 (dried specimen in metabolically inactive state), culture ex-type CMW: 56387 = CBS: 147414 = CN014C2). DNA reference sequences: ITS = MT949903, *BenA* = MT957405, *CaM* = MT957450, *RPB2* = MT957476.

Etymology: Latin, *outeniquaense*, named after the Outeniqua mountains, location of the sampling site where this species was found.

Description: Colony diam, 7d, in mm: CYA 25–27; CYA10C 9–10; CYA15C 18–19; CYA20C 25–26; CYA30C germination; CYA37C no growth; CYAS 25–27; MEA 18–19; YES 28–30; DG18 20–21; OA 18–19; CREA 18–19.

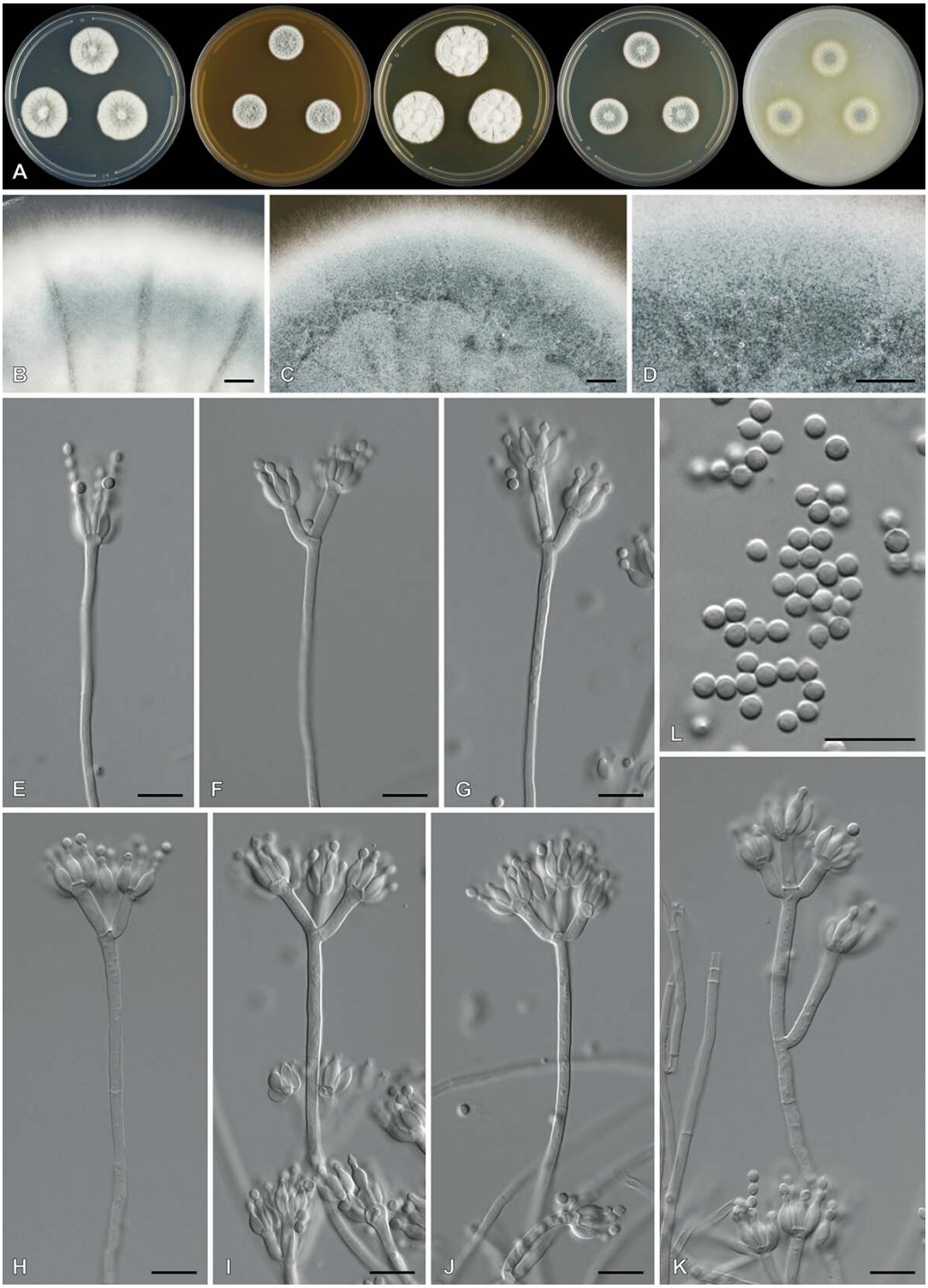

Figure 10. Morphological characters of *Penicillium outeniquaense*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; C, D. texture on MEA; E–K. conidiophores; l. conidia. Bar = 1 mm (B–D), 10 µm (E–L).

CYA 25 C, 7 d: Colonies moderately deep, radially slightly sulcate; margins low, entire, narrow; mycelia white; texture floccose; sporulation sparse to moderately dense; conidia *en masse* Pale Green to Greyish Green (25A3, 25C3–26C3); soluble pigments absent; exudates absent; reverse Orange White to Orange Grey (5A2–B2). MEA 25 C, 7 d: Colonies moderately deep, randomly sulcate; margins low, entire, narrow; mycelia white; texture floccose; sporulation sparse moderately dense; conidia *en masse* Greyish Green (25B4–D4); soluble pigments absent; exudates absent; reverse Brownish Orange to Brown (5C6–E8). YES 25 C, 7 d: Colonies moderately deep, randomly sulcate; margins low, entire, narrow; mycelia white; texture floccose; sporulation very sparse; conidia *en masse* not determined; soluble pigments absent; exudates absent; reverse Pale Yellow to Greyish Yellow (4A3–B6). DG18 25 C, 7 d: Colonies low, radially sulcate; margins low, entire, narrow; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* Light Green to Greyish Green (25A4–D4); soluble pigments absent; exudates absent; reverse Pale Yellow to Greyish Yellow (2A3–B3–4). OA 25 C, 7 d: Colonies low, plane; margins low, entire, wide; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* Greyish Turquoise (24D3–4); soluble pigments Olive to Yellow; exudates absent; reverse Pale to Greyish Yellow (1A3–B4). CREA 25 C, 7 d: Colonies good growth, acid not produced.

Conidiophores biverticillate, mono- or terverticillate rare; *stipes* smooth walled, 100–350 × 2–3 µm; *branches* 16–25 µm; *metulae* (2–)3–6 per stipe/branch, divergent, 9–20 × 2.5–3.5 µm; *phialides* ampulliform, 6–12 per metula, 7–8(–8.5) × 2.5–3 µm ($\bar{x}$ = 7.6±0.5 × 2.9±0.1), average metula:phialide = 1.7; *conidia* finely rough walled, globose, 2–2.5 µm, ($\bar{x}$ = 2.5±0.1), average width/length = 0.96, n = 38.

Distinguishing features — Penicillium outeniquaense is closely related to *P. chrzaszczii, P. godlewskii* and *P. waksmanii* in section *Citrina* series *Westlingiorum* (FIG. 4). Compared to *P. chrzaszczii*, *P. outeniquaense* grows slightly slower on MEA (18–19 vs 21–28 mm), has a higher CYAS:CYA ratio (1:1 vs 0.95:1), germinates on CYA30C and lacks the yellow soluble pigments typical of the former species (Houbraken and others 2011). Compared to *P. godlewskii*, conidia of the new species can germinate on CYA30C (no growth in the former) and has a CYAS:CYA ratio of 1:1 vs 1:1.4 (Houbraken and others 2011). Compared to *P. waksmanii*, conidia of *P. outeniquaense* germinate on CYA30C, colonies generally grow slower on MEA (18–19 vs 18–24(–30) mm), while its CYA reverse is orange-white to orange grey in contrast to the brown reverse of *P. waksmanii* (Houbraken and others 2011).

Penicillium subfuscum Visagie & Yilmaz, sp. nov. FIG. 11

MycoBank: MB844188

In: subgenus *Aspergilloides* section *Lanata-Divaricata* series *Simplicissima* (Houbraken and others 2020).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, M.J. Wingfield, C.M. Visagie & N. Yilmaz (**holotype** PREM: 63220 (dried specimen in metabolically inactive state), culture ex-type CMW: 56196 = CBS: 147455 = CN014C9). DNA reference sequences: ITS = MT949907, *BenA* = MT957412, *CaM* = MT957454, *RPB2* = MT957480.

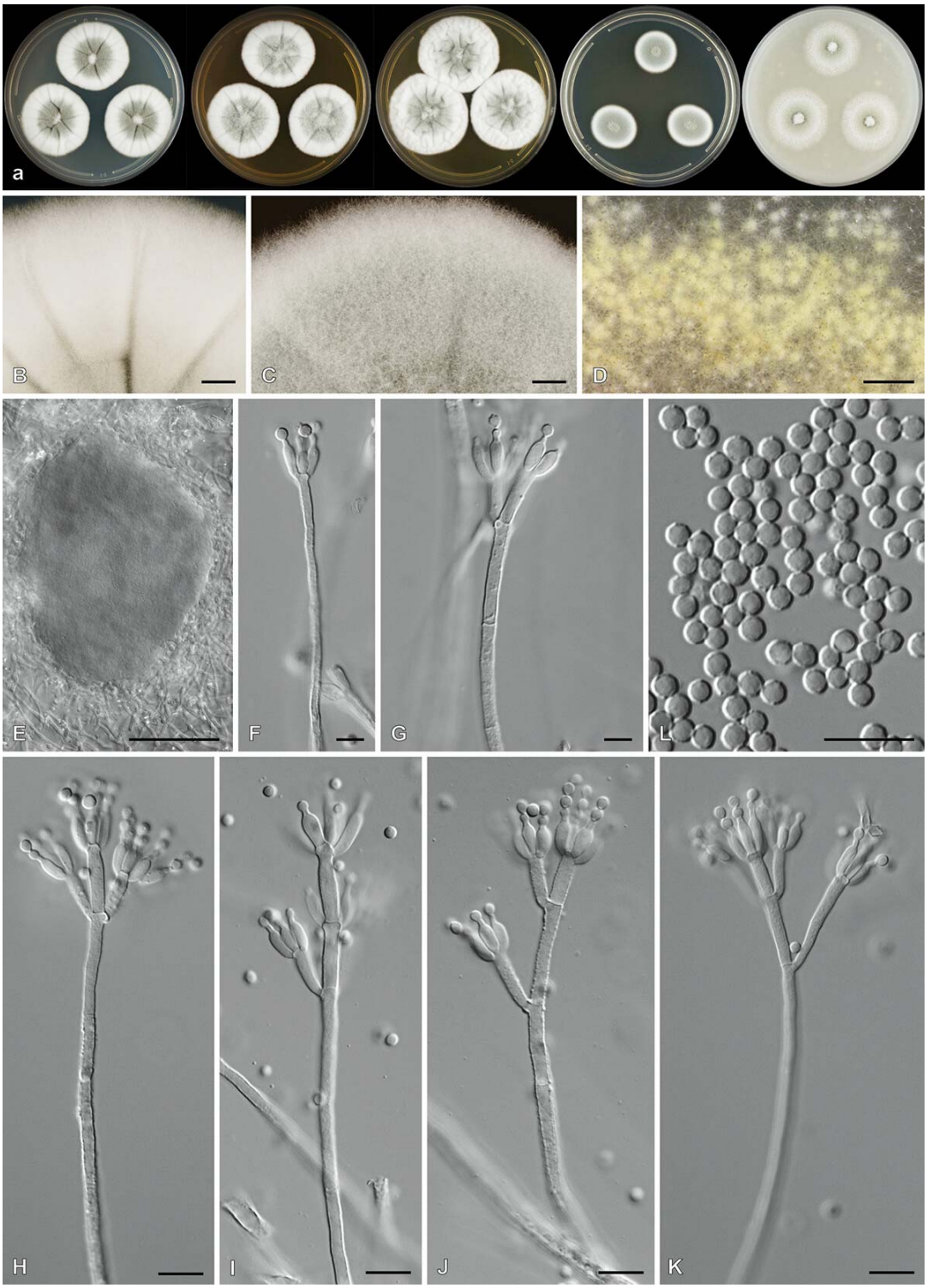

Figure 11. Morphological characters of *Penicillium subfuscum*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; c. texture on MEA; D. texture on OA; E. soft sclerotia from OA; F–K. conidiophores; L. conidia. Bar = 1 mm (B–D), 50 µm (E), 10 µm (F–L).

Additional strains examined: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, M.J. Wingfield, C.M. Visagie & N. Yilmaz, culture CN014A6.

Etymology: Latin, *subfuscum*, '*sub'* = below; *'fuscum'* = brown, named for the brown colony reverse.

Description: Colony diam, 7d, in mm: CYA 37–40; CYA10C 5–7; CYA15C 20–21; CYA20C 30–33; CYA30C 24–25; CYA37C no growth; CYAS 29–32; MEA 34–37; YES 42–44; DG18 23–25; OA 30–32; CREA 32–33.

CYA 25 C, 7 d: Colonies moderately deep, radially slightly sulcate; margins low, entire, wide; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25B3–C4, 25E3–26E3); soluble pigments absent; exudates clear; reverse Pale to Light Yellow (3A3–4). MEA 25 C, 7 d: Colonies moderately deep, radially slightly sulcate, sclerotia produced after prolonged incubation; margins low, entire, wide; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25B3–C4, 25E3–26E3); soluble pigments absent; exudates clear; reverse Greyish Orange to Brown (5B4–D6). YES 25 C, 7 d: Colonies moderately deep, lightly sulcate; margins low, somewhat irregular, wide; mycelia white; texture floccose; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25E3–26E3); soluble pigments absent; exudates absent; reverse Pale to Light Yellow (3A3–4). DG18 25 C, 7 d: Colonies low, plane; margins low, entire, narrow; mycelia white; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (25E3–26E3); soluble pigments absent; exudates absent; reverse Yellowish White (1A2), Greenish Grey (1B2). OA 25 C, 7 d: Colonies low, plane, raised centrally, sclerotia produced after prolonged incubation; margins low, entire, wide; mycelia white; texture

floccose; sporulation moderately dense; conidia *en masse* Greyish to Dull Green (26C4–E4); soluble pigments absent; exudates clear; reverse Yellowish White to Yellowish Grey (3A2–B2). CREA 25 C, 7 d: Colonies weak growth, acid produced, weak.

Conidiophores divaricate and biverticillate, monoverticillate rare; *stipes* rough walled, sometimes finely rough walled, 120–500 × 2.5–3.5 µm; *branches* 12.5–37 µm; *metulae* 2–4 per stipe/branch, divergent, 9–18 × 2–4 µm; *phialides* ampulliform, 4–6 per metula, 7–9 × 2.5–3.5 µm ($\bar{x}$ = 8.2±0.5 × 2.9±0.2), average metula:phialide = 1.6; *conidia* rough walled, globose, 2.5–3.5 µm, ($\bar{x}$ = 3.4±0.2), average width/length = 0.98, n = 54; *sclerotia* soft, of interwoven hyphae, 45–145 µm.

Distinguishing features — Penicillium subfuscum is closely related to *P. panissanguineum* and *P. simplicissimum* in series *Simplicissima* (FIG. 5). Species in this series typically grow quickly on most media, while conidiophores are divaricate with roughened stipes. *Penicillium subfuscum* and *P. panissanguineum* does not grow on CYA at 37 C, distinguishing them from *P. simplicissimum.* The new species produces greyish orange to brown colony reverses on MEA and soft sclerotia, in contrast to the pinkish red reverse and lack of sclerotia in *P. panissanguineum* (Visagie and others 2016).

Penicillium umkhoba Visagie & Yilmaz, sp. nov. FIG. 12

MycoBank: MB844189

In: subgenus *Aspergilloides* section *Sclerotiorum* series *Herqueorum* (Houbraken and others 2020).

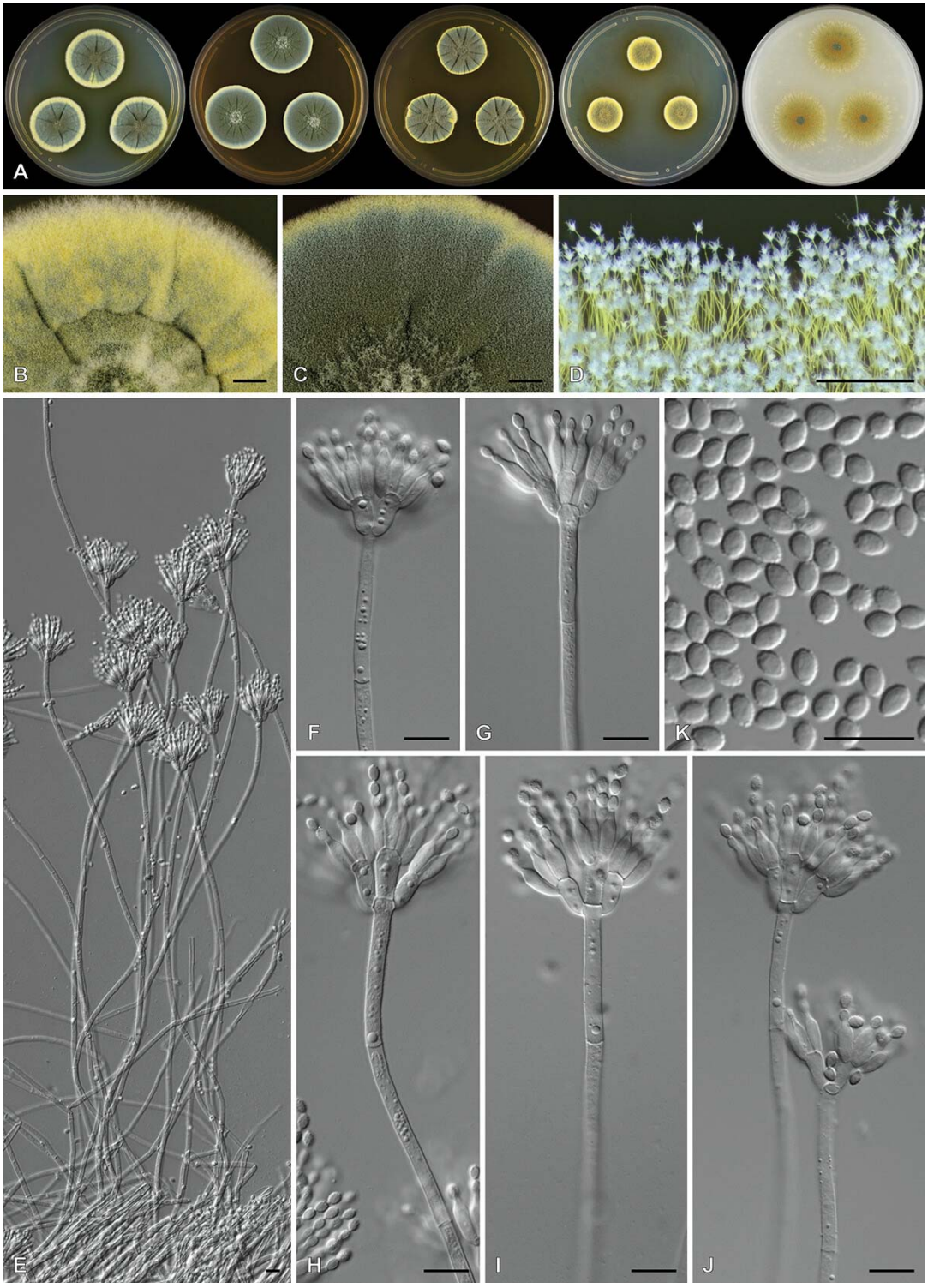

Figure 12. Morphological characters of *Penicillium umkhoba*; A. colonies from left to right on CYA, MEA, YES, DG18 and OA; B. texture on CYA; C, D. texture on MEA; E–J. conidiophores; K. conidia. Bar = 1 mm (B, C), 500 µm (D), 10 µm (E–K).

Typification: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, M.J. Wingfield, C.M. Visagie & N. Yilmaz (**holotype** PREM: 63222 (dried specimen in metabolically inactive state), culture ex-type CMW: 56200 = CBS: 147457 = CN014D5). DNA reference sequences: ITS = MT949912, *BenA* = MT957417, *CaM* = MT957459, *RPB2* = MT957485.

Additional strains examined: SOUTH AFRICA, WESTERN CAPE, Wilderness (-33.934155, 22.645636), from soil, Dec 2018, M.J. Wingfield, C.M. Visagie & N. Yilmaz, cultures CMW: 56199 = CBS: 147456 = CN014D4, CMW: 56201 = CBS: 147419 = CN014D6.

Etymology: Latin, *umkhoba*, from the Xhosa name for the Outeniqua yellowwood (*Afrocarpus falcatus* (= *Podocarpus falcatus*)).

Description: Colony diam, 7d, in mm: CYA 28–31; CYA10C 4–6; CYA15C 15–16; CYA20C 23–25; CYA30C 3–5; CYA37C no growth; CYAS microcolonies, 1–2; MEA 28–32; YES 24–26; DG18 16–19; OA 26–30; CREA 14–15.

CYA 25 C, 7 d: Colonies low, radially and concentrically sulcate, slightly crateriform; margins low, entire, narrow; mycelia yellow; texture velutinous; sporulation dense; conidia *en masse* Greyish Turquoise (24D4–5); soluble pigments yellow; exudates absent; reverse Dull Yellow (3B4), Olive Brown (4F7). MEA 25 C, 7 d: Colonies low, radially sulcate, raised centrally; margins low, entire, narrow; mycelia white and yellow; texture velutinous; sporulation dense; conidia *en masse* Greyish to Dull Green (25D4–F4); soluble pigments absent; exudates absent; reverse Brownish Orange (5C5), Light Brown (6D6), Dark Brown (6F6). YES 25 C, 7 d: Colonies deep, radially and concentrically sulcate, crateriform; margins low, entire, narrow; mycelia white, yellow; texture velutinous; sporulation moderately dense; conidia *en masse* Greyish Turquoise

(24D4–5); soluble pigments absent; exudates absent; reverse Light Yellow (4A4), Brown (7E5–7). DG18 25 C, 7 d: Colonies slightly raised, radially sulcate; margins low, entire, narrow; mycelia yellow, deep yellow to orange; texture velutinous; sporulation moderately dense; conidia *en masse* Olive to Olive Brown (2D5–4D5); soluble pigments absent, sometimes yellow, inconspicuous; exudates absent; reverse Dull Yellow (3B4), Olive Brown (4F7), Light Brown (6D7). OA 25 C, 7 d: Colonies low, plane; margins low, entire, wide; mycelia yellow to inconspicuously orange; texture velutinous; sporulation moderately dense; conidia *en masse* Dull to Dark Green (25E5–F5); soluble pigments yellow to orange; exudates clear; reverse Light Yellow to Greyish Yellow to Brownish Orange (4A4–C6–5C5). CREA 25 C, 7 d: Colonies weak growth, acid not produced.

Conidiophores symmetrically biverticillate; *stipes* smooth, sometimes very finely rough walled, 200–600 × 3–4 µm; *metulae* 4–6 per stipe, appressed, 8–11(–13.5) × 3–5 µm; *phialides* ampulliform, 4–8 per metula, 8–13 × 2.5–4 µm ($\overline{x}$ = 9.9±1.1 × 3.2±0.3), average metula:phialide = 1; *conidia* rough walled, ellipsoidal, slightly ovoid, 3.5–4.5 × 2.5–3 µm, ($\overline{x}$ = 3.9±0.3 × 2.6±0.1), average width/length = 0.65, n = 50.

Distinguishing features — Penicillium umkhoba is classified in section *Sclerotiorum* series *Herqueorum* together with *P. choerospondiatis, P. herquei, P. malachiteum, P. sanshaense* and *P. verrucisporum* (FIG. 6). Compared to *P. choerospondiatis*, the new species grows faster on CYA (28–31 vs 11–12.5 mm) and YES (24–26 vs 16–19 mm), and produces slightly larger conidia (3.5–4.5 × 2.5–3 µm vs 4.5–6.5 × 3.3–4.5 µm) (Wang and others 2017). Compared to *P. herquei*, *P. umkhoba* grows slower on YES (24–26 vs 30–40 mm) and CYAS (microcolonies vs 10–17 mm) (Visagie and others 2013). Compared to *P. malachiteum, P. umkhoba* grows slightly faster on

CYA (28–31 vs 20–25 mm), slightly slower on YES (24–26 vs 28–32 mm) and does not produce sclerotia (Visagie and others 2013). *Penicillium umkhoba* grows faster on CYA (28–31 vs 21–23 mm), slower on YES (24–26 vs 32–34 mm) and produces larger conidia (3.5–4.5 × 2.5–3 µm vs 3–3.5 × 2–2.5 µm) than P*. sanshaense* (Wang and others 2017). Compared *P. verrucisporum*, the new species grows slower on MEA (28–32 vs 36–37 mm) and YES (24–26 vs 43–44 mm), but produces larger conidia (3.5–4.5 × 2.5–3 µm vs 3–3.5 × 2.5–3 µm) (Wang and others 2017). Series *Herqueorum* species produce symmetrically biverticillate and appressed conidiophores, similar to species classified in section *Canescentia* series *Atroveneta* and section *Aspergilloides* series *Saturniformia*. Distinguishing between these groups are difficult but see comparison provided in the distinguishing features for *Penicillium claroviride* above.

DISCUSSION

The fynbos biome covering most of South Africa's Western Cape is considered a biodiversity hotspot (Myers and others 2000). The region is also considered a hotspot for *Penicillium* diversity with past fynbos surveys recovering many species, of which a high proportion were described as new species (Visagie and others 2021). This survey collected samples from three sites and because the fynbos is highly heterogenous (Mucina and Rutherford 2006) we believe it only started to uncover *Penicillium* diversity in the region. Very little work has been done to document *Penicillium* in the South Cape, but a strain (CBS: 328.71) isolated in the 1960s from soil collected in the Tsitsikamma Forest (near Knysna and Wilderness) was described as *P. tsitsikammaense* [MB 809976] by Houbraken and others (2014). This survey is the first in the region that used modern taxonomic approaches to characterize communities. In the process,

74 *Penicillium* strains were morphologically and phylogenetically characterized and resulted in 12 species identified and six new taxa described. Unpublished surveys made across South Africa in recent years resulted in the identification of a further ±30 new *Penicillium* species that will be described in the near future. Considering the large proportion of accepted species that will be described from South Africa, it has an important role to play in any international inventory of the genus.

The modernization of *Penicillium* taxonomy over the past decade has resulted in identifications that are now heavily reliant on DNA sequence data. This brings the advantage of relatively quick, robust, and reliable identifications when compared to traditional morphological approaches. However, this modern approach also led to a more aggressive species concept. This is not necessarily a bad approach but resulted in about 160 of the 513 currently accepted *Penicillium* species being monotypic. Even though far from ideal, our push to describe the missing diversity (Hawksworth and Lucking 2017) partly led to the normalization of introducing new species even if based only on one strain, as is done in the case of *P. outeniquaense* above. During this study, we also managed to isolate a strain of *P. onobense* (previously only known from its ex-type strain CBS: 174.81) and we were able to add *BenA*, *CaM* and *RPB2* to our knowledge of this species. However, much work is needed to further capture infraspecies diversity within *Penicillium* from as many different substrates and geographic regions as possible. This will help to better define species boundaries and make future identifications easier, while we will learn more about species distribution and potential ecological significance.

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LITERATURE CITED

Akaike H. 1974. A new look at the statistical model identification. IEEE Transations on Automatic Control 19:716–723.

Barron GL. 1968. The Genera of Hyphomycetes from Soil. University of Minnesota, USA: Williams & Wilkins.

Biourge P. 1923. Les moisissures du groupe Penicillium Link: Étude monographique.

Christensen M, Frisvad J, Tuthill D. 2000. *Penicillium* species diversity in soil and some taxonomic and ecological notes. In: Samson RA, Pitt J, eds. Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification. Amsterdam: Harwood Academic Publishers. p. 309–320.

Crous PW, Cowan DA, Maggs-Kölling G, Yilmaz N, Thangavel R, Wingfield MJ, Noordeloos M, Dima B, Brandrud T, Jansen G et al. . 2021. Fungal Planet description sheets: 1182–1283. Persoonia 46:313–528.

Crous PW, Luangsa-ard JJ, Wingfield MJ, Carnegie AJ, Hernández-Restrepo M, Lombard L, Roux J, Barreto RW, Baseia IG, Cano-Lira JF et al. . 2018. Fungal Planet description sheets: 785–867. Persoonia - Molecular Phylogeny and Evolution of Fungi 41:238–417.

Domsch KH, Gams W, Anderson T-H. 1980. Compendium of soil fungi. Eching: IHW-Verlag. p. 672.

Frisvad JC. 1989. The connection between the Penicillia and Aspergilli and mycotoxins with special emphasis on misidentified isolates. Archives of Environmental Contamination and Toxicology 18:452–467.

Hawksworth DL, Lucking R. 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiol Spectr 5(4).

Houbraken J, Frisvad JC, Samson RA. 2011. Taxonomy of Penicillium section Citrina. Studies in Mycology 70:53–138.

Houbraken J, Kocsube S, Visagie CM, Yilmaz N, Wang X-C, Meijer M, Kraak B, Hubka V, Samson RA, Frisvad JC. 2020. Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): an overview of families, genera, subgenera, sections, series and species. Studies in Mycology 95:5–169.

Houbraken J, Samson RA. 2011. Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. Studies in Mycology 70:1–51.

Houbraken J, Visagie CM, Frisvad JC. 2021. Recommendations To Prevent Taxonomic Misidentification of Genome-Sequenced Fungal Strains. Microbiology Resource Announcements 10(48):e0107420.

Houbraken J, Visagie CM, Meijer M, Frisvad JC, Busby PE, Pitt JI, Seifert KA, Louis-Seize G, Demirel R, Yilmaz N et al. . 2014. A taxonomic and phylogenetic revision of *Penicillium* section *Aspergilloides*. Studies in Mycology 78:373–451.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30:772–780.

Kornerup A, Wanscher JH. 1967. Methuen Handbook of Colour. London, England: Methuen & Co Ltd.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3):772–773.

Mucina L, Rutherford MC. 2006. The vegetation of South Africa, Lesotho and Swaziland.

Myers N, Mittermeier RA, Mittermeier CG, daFonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32:268–274.

Pitt JI. 1980. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Acdemic Press. p. 1–634.

Ramírez C. 1982. Manual and Atlas of the Penicillia. Amsterdam: Elsevier Biomedical Press. p. 1–874.

Raper KB, Thom C. 1949. A manual of the Penicillia. Baltimore: The Williams & Wilkins company. p. 1–875.

Rivera KG, Díaz J, Chavarría-Díaz F, Garcia M, Urb M, Thorn RG, Louis-Seize G, Janzen DH, Seifert KA. 2012. Penicillium mallochii and P. guanacastense, two new species isolated from Costa Rican caterpillars. Mycotaxon 119:315–328.

Rivera KG, Seifert KA. 2011. A taxonomic and phylogenetic revision of the Penicillium sclerotiorum complex. Stud Mycol 70(1):139-58.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.

Sang H, An T-J, Kim CS, Choi YP, Deng J-X, Paul NC, Sung G-H, Yu SH. 2013. Penicillium daejeonium sp. nov., a new species isolated from a grape and schisandra fruit in Korea. The Journal of Microbiology 51:536–539.

Thom C. 1930. The Penicillia. Baltimore: Williams & Wilkins. p. 1–644.

Visagie CM, Frisvad JC, Houbraken J, Visagie A, Samson RA, Jacobs K. 2021. A re-evaluation of Penicillium section Canescentia, including the description of five new species. Persoonia 46:163–187.

Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. 2014. Identification and nomenclature of the genus *Penicillium*. Studies in Mycology 78:343–371.

Visagie CM, Houbraken J, Rodriques C, Silva Pereira C, Dijksterhuis J, Seifert KA, Jacobs K, Samson RA. 2013. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family. Persoonia 31:42–62.

Visagie CM, Renaud JB, Burgess KM, Malloch DW, Clark D, Ketch L, Urb M, Louis-Seize G, Assabgui R, Sumarah MW et al. . 2016. Fifteen new species of *Penicillium*. Persoonia 36:247–280.

Wang L, Zhuang W-Y. 2009. Eupenicillium saturniforme, a New Species Discovered from Northeast China. Mycopathologia 167:297–305.

Wang X-C, Chen K, Zeng Z-Q, Zhuang W-Y. 2017. Phylogeny and morphological analyses of Penicillium section Sclerotiora (Fungi) lead to the discovery of five new species. Scientific Reports 7:8233.