A new *Penicillium* section *Citrina* species and series from India

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Abstract

This study introduces a new *Penicillium* species isolated from soil in Yavatmal, India. *Penicillium sanjayi sp. nov.* is classified in *Penicillium* section *Citrina*. The new species is delimited using phenotypic characters and sequences of the nuclear ribosomal internal transcribed spacer (ITS) rDNA regions, partial beta-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) region. Phylogenetic analyses consistently resolved the new species in a well-delineated clade with its close relative *P. vascosobrinhoanum* (originally published as *P. vascosobrinhous*), distinct from all other series of section *Citrina*. As a result, we introduce the series *Vascosobrinhoana* for this unique lineage. Key distinguishing characteristics such as greyish ruby to ruby centre of colonies (obverse) on malt extract agar (MEA), presence of cream colour sclerotia on oatmeal agar (OA), growth rates on standardised media, growth at 30 °C but lack of growth at 37 °C, additional microscopic characters such as solitary or rarely with a subterminal branch other than predominant monoverticillate penicilli, and conspicuously roughened to verruculose conidial ornamentation distinguish the new species *P. sanjayi* from other monoverticillate section Citrina species.

Keywords: *Aspergillaceae*; Fungal diversity; Genealogical concordance phylogenetic species recognition (GCPSR); Taxonomy

Introduction

Penicillium is well known and one of the most common of all fungi, often dominating communities from diverse habitats like air, soil, natural vegetation, indoor environments, and various food products. It has a worldwide distribution and an enormous economic impact on human life. *Penicillium* species gain attention from many research fields, including food, indoor, medical, and biotechnology. The modern taxonomic approaches for the identification, taxonomy, and nomenclature of *Penicillium* was established in recent studies (Houbraken and Samson 2011; Visagie et al. 2014a, b; Houbraken et al. 2020) leading to the genus having one of the most advanced taxonomies of all fungi. A list of accepted species and associated reference data are well maintained and updated, with 494 *Penicillium* species in current use. Furthermore, the genus was segregated into two subgenera, 32 sections and 100 series (Houbraken et al. 2020). This species list, reference data, and well-

defined subgeneric classification mean that it has never been easier to identify and discover new *Penicillium* species.

Members of *Penicillium* section *Citrina* are abundant with a worldwide distribution especially in soil (Pitt and Hocking 2009; Houbraken et al. 2010, 2011a, 2020; Samson et al. 2010; Visagie et al. 2014a). Traditionally, the group was centred on *P. citrinum* that has restricted growth on Czapek's agar (CZ) or Czapek yeast autolysate agar (CYA) and mostly produces biverticillate conidiophores with relatively small conidia (Raper and Thom 1949; Pitt 1980; Ramirez 1982). Houbraken et al. (2010) and Houbraken et al. (2011b) revised the section and accepted 39 species (introducing 17 they described as new) using a consilient concept of species based on a combination of phenotypic characters, extrolite profiles, and sequence data from ITS, *BenA* and *CaM*. In subsequent studies, three new section *Citrina* species were introduced: *P. sucrivorum* from fynbos soil collected in South Africa (Visagie et al. 2014a), *P. dokdoense* from soil collected on Dokdo Island in the East Sea of Korea (Phookamsak et al. 2019), and *P. vascosobrinhoanum* as an endophyte of *Melocactus zehntneri* (*Cactaceae*) (Barbosa et al. 2020).

During a survey of *Penicillium* occurring in India, soil samples were collected during the pre-monsoon season of 2017 and 2018 from the dry vegetational zones of Maharashtra State, India. During isolations, several strains displayed slow-growing greyish ruby colonies on CYA with subsequent sequence analyses resolving strains as a unique lineage in section *Citrina*. This study introduces a new species using a consilient concept of species, employing morphological and phylogenetic comparisons.

Materials and methods

Isolation

Isolation of *Penicillium* strains was done following serial dilution and particle filtration techniques. The isolated strains were sub-cultured onto malt extract agar (MEA; HIMEDIA Laboratories Pvt. Ltd, Mumbai, India) containing the antibiotic streptomycin sulfate (100 mg/L) CMS220-5G (HIMEDIA Laboratories Pvt. Ltd, Mumbai, India). Fungarium specimens were deposited in the Ajrekar Mycological Herbarium (AMH) at the Agharkar Research Institute, Pune, India. The ex-type and reference strains were accessioned and preserved in the National Fungal Culture Collection of India (NFCCI; WDCM-932), at the Agharkar Research Institute, Pune, India.

Morphology

Visagie et al. (2014b) were followed for morphological characterization. This included the methods and media used for examining colony characters, the suggestions made for inoculating and incubating cultures, and microscopic examination. The cultures were inoculated in eight prescribed media and incubated in a Bio Multi Incubator (model LH-30-8CT, Japan). Colony characters were noted after 7 days of incubation on creatine sucrose agar (CREA), Czapek yeast autolysate agar (CYA), CYA with 5% NaCl (CYAS), Czapek's agar (CZ), dichloran 18% glycerol agar (DG18), MEA (malt extract 20 g/l, peptone 1 g/l, dextrose (glucose) 20 g/l, agar 20 g/l; HIMEDIA Laboratories Pvt. Ltd, Mumbai, India), oatmeal agar

(OA), and yeast extract sucrose (YES) agar. Plates were also incubated for extended periods to check if ascomata will mature and produce ascospores. Colour codes and names used in descriptions are from Kornerup and Wanscher (1978). Microscopic observations were made with an Olympus (model CX-41, Japan) dissecting microscope and a Zeiss (AXIO Imager 2, Germany) compound microscope equipped with a Nikon Digital Sight DS-Fi1 and AxioCam MRc5 cameras driven by AxioVision Rel 4.8 software (AXIO Imager 2, Germany).

DNA extraction, amplification, and phylogenetic analyses

Colonies were grown on MEA plates for 5 days, and genomic DNA extracted from them following the rapid salt extraction method of Aljanabi and Martinez (1997). ITS was amplified using primer pairs ITS5 and ITS4 (White et al. 1990), *BenA* with primer pairs Bt2a and Bt2b (Glass and Donaldson 1995) with 50 °C as annealing temperature, and *CaM* using primer pairs CMD5 and CMD6 (Hong et al. 2006) with 55 °C as annealing temperature. For the amplification of *RPB2*, primer pairs *RPB2*-5F and *RPB2*-7CR (Liu et al. 1999) were used with touch-up PCR conditions: 5 cycles with annealing temperature 48 °C followed by five cycles at 50 °C and a final 25 cycles at 52 °C. PCR products were purified using the StrataPrep PCR Purification Kit (Agilent Technologies, TX, USA) and sequenced using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing reactions were run on an ABI PRISM® 3100 genetic analyzer (Applied Biosystems, USA). Sequence contigs were assembled in Geneious Prime v. 2021.0.3 (BioMatters Ltd., Auckland, New Zealand).

A sequence dataset for section *Citrina* was compiled (Table 1) mainly based on ex-type reference sequences as published by Houbraken et al. (2020). This dataset was aligned with our newly obtained sequences using MAFFT v. 7.453 (Katoh and Standley 2013) by selecting the G-INS-i option. Datasets were partitioned based on the gene region and their introns and exons, with the most appropriate nucleotide substitution model selected for each using PartitionFinder v. 2.1 (Lanfear et al. 2017) based on the Akaike information criterion (Akaike 1974). Phylogenetic analyses were performed using both maximum likelihood (ML) and Bayesian tree inference (BI). ML analyses were performed in IQtree v. 2.1.2 (Nguyen et al. 2015) with support in nodes calculated with bootstrap analyses of 1000 repeats. BI analyses were performed in MrBayes v. 3.2.7 (Ronquist et al. 2012) using three sets of four chains (1 cold and 3 heated) and were stopped using the stoprule option at an average standard deviation for split frequencies of 0.01. The Interactive tree of life (iTOL) v. 6 (Letunic and Bork 2016) was used to visualise trees and was further edited in Affinity Publisher 1.9.3 (Serif (Europe) Ltd, Nottingham, UK). ML trees were used to present phylogenetic results with both bootstrap values and posterior probabilities shown on supported branches. Newly generated DNA sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Table 1 Strains used for phylogenetic comparisons.

Species	**Strain**	**Series**	**GenBank ITS**	**GenBank *BenA***	**GenBank *CaM***	**GenBank *RPB2***
Penicillium anatolicum	CBS 479.66 = IBT 30764 (ex-type)	*Euglauca*	AF033425	JN606849	JN606571	JN606593
Penicillium argentinense	CBS 130371 = IBT 30761 (ex-type)	*Euglauca*	JN831361	JN606815	JN606549	MN969105
Penicillium atrofulvum	CBS 109.66 = DTO 31B2 = FRR 799 = IBT 30032 = IBT 29667 (ex-type)	*Westlingiorum*	JN617663	JN606677	JN606387	JN606620
Penicillium aurantiacobrunneum	CBS 126228 = IBT 18753 (ex-type)	*Westlingiorum*	JN617670	JN606702	MN969238	MN969106
Penicillium cairnsense	CBS 124325 = IBT 29042 (ex-type)	*Westlingiorum*	JN617669	JN606693	MN969240	MN969108
Penicillium christenseniae	CBS 126236 = IBT 23355 (ex-type)	*Westlingiorum*	JN617674	JN606680	MN969243	JN606624
Penicillium chrzaszczii	CBS 217.28 = FRR 903 = MUCL 29167 = NRRL 1741 = NRRL 903 (ex-type)	*Westlingiorum*	GU944603	JN606758	MN969244	JN606628
Penicillium citrinum	CBS 139.45 = ATCC 1109 = ATCC 36382 = CECT 2269 = FRR 1841 = IMI 091961 = IMI 092196 = LSHBAd 95 = LSHBP 25 = LSHBP 6 = MUCL 29781 = NRRL 1841 = NRRL 1842 (ex-type)	*Citrina*	AF033422	GU944545	MN969245	JF417416
Penicillium copticola	CBS 127355 = IBT 30771 (ex-type)	*Copticolarum*	JN617685	JN606817	JN606553	JN606599
Penicillium corylophilum	CBS 312.48 = TCC9784 = ATHUM2890 = CECT 2270 = FRR 802 = IMI 039754 = MUCL 28671 = MUCL 29073 = MUCL 29131 = NRRL 802 = QM 7510 (ex-type)	*Corylophila*	AF033450	JX141042	KP016780	KP064631
Penicillium cosmopolitanum	CBS 126995 = IBT 30681 (ex-type)	*Westlingiorum*	JN617691	JN606733	MN969249	MN969113
Penicillium decaturense	CBS 117509 = NRRL 28152 = IBT 27117 (ex-type)	*Westlingiorum*	GU944604	JN606685	MN969252	JN606621
Penicillium dokdoense	MRC:SF:013606 (ex-type)	*Copticolarum*	MG906868	MH243037	MH243031	—
Penicillium euglaucum	CBS 323.71 = IBT 30767 (ex-type)	*Euglauca*	JN617699	JN606856	JN606564	JN606594
Penicillium gallaicum	CBS 167.81 = ATCC 42232 = IJFM 5597 (ex-type)	*Galliaca*	JN617690	JN606837	JN606548	JN606609

Penicillium galliacum	CBS 164.81 = DTO 34G2 = IJFM 7026 = IMI 253797 = VKM F-2193 = IBT 22014	*Galliaca*	KC411681	JN606836	JN606547	—
Penicillium galliacum	CBS 418.69 = DTO 23A9 = NRRL 3759 = IBT 30046 = IMI 140303 = FRR 519	*Galliaca*	EF634448	JN606845	JN606567	—
Penicillium godlewskii	CBS 215.28 = ATCC 10449 = ATCC 48714 = FRR 2111 = IFO 7724 = IMI 040591 = MUCL 29243 = NRRL 2111 = QM 7566 = VKMF-1826 (ex-type)	*Westlingiorum*	JN617692	JN606768	MN969258	JN606626
Penicillium gorlenkoanum	CBS 408.69 = FRR 511 = IMI 140339 = VKMF-1079 (ex-type)	*Citrina*	GU944581	GU944520	MN969259	JN606601
Penicillium hetheringtonii	CBS 122392 = IBT 29057 (ex-type)	*Citrina*	GU944558	GU944538	MN969263	JN606606
Penicillium manginii	CBS 253.31 = NRRL 2134 (ex-type)	*Westlingiorum*	GU944599	JN606651	MN969274	JN606618
Penicillium miczynskii	CBS 220.28 = ATCC 10470 = DSM2437 = FRR 1077 = IFO 7730 = IMI 040030 = MUCL 29228 = NRRL 1077 = QM 1957 (ex-type)	*Westlingiorum*	GU944600	JN606706	MN969277	JN606623
Penicillium neomiczynskii	CBS 126231 = IBT 23560 (ex-type)	*Westlingiorum*	JN617671	JN606705	MN969278	MN969128
Penicillium nothofagi	CBS 130383 = IBT 23018 = DTO 76C2 (ex-type)	*Westlingiorum*	JN617712	JN606732	JN606507	MN969129
Penicillium pancosmium	CBS 276.75 = DAOM 147467 = IBT 29991 (ex-type)	*Westlingiorum*	JN617660	JN606790	MN969284	MN969130
Penicillium pasqualense	CBS 126330 = IBT 14235 (ex-type)	*Westlingiorum*	JN617676	JN606673	MN969286	—
Penicillium paxilli	CBS 360.48 = ATCC 10480 = FRR 2008 = IMI 040226 = NRRL 2008 = QM 725 (ex-type)	*Paxillorum*	GU944577	JN606844	JN606566	JN606610
Penicillium quebecense	CBS 101623 = IBT 29050 (ex-type)	*Westlingiorum*	JN617661	JN606700	JN606509	JN606622
Penicillium raphiae	CBS 126234 = IBT 22407 (ex-type)	*Westlingiorum*	JN617673	JN606657	MN969292	JN606619
Penicillium roseopurpureum	CBS 226.29 = ATCC 10492 = ATHUM2895 = FRR 2064 = IMI 040573 = MUCL 28654 = MUCL 29237 = NRRL 2064 = NRRL 2064A (ex-type)	*Roseopurpurea*	GU944605	JN606838	JN606556	JN606613
Penicillium sanguifluum	CBS 127032 = IBT 29041 (ex-type)	*Roseopurpurea*	JN617681	JN606819	JN606555	MN969135
Penicillium sanjayi	**NFCCI 5017 (ex-type)**	***Vascosobrinhoana***	**MZ571358**	**MZ558484**	**MZ558492**	**MZ558482**
Penicillium sanjayi	**NFCCI 5018**	***Vascosobrinhoana***	**MZ571359**	**MZ558485**	**MZ558493**	**MZ558483**

Results

Phylogenetic analyses

Based on a BLAST search of *BenA* in NCBI's GenBank nucleotide database, the closest matches were *Penicillium godlewskii* (GenBank JX140955; Identities = 365/421 (87%), gaps = 12/421 (2%)), *Penicillium atrofulvum* (GenBank JN606677; Identities = 353/405 (87%), gaps = 15/405 (3%)), and *Penicillium neomiczynskii* (GenBank JN606705; Identities = 351/404 (87%), gaps = 14/404 (3%)).

Aligned datasets of ITS, *BenA*, *CaM*, and *RPB2* were respectively 543, 480, 765, and 912 bp long. ITS was excluded from the concatenated dataset. The most appropriate nucleotide substitution models for each partition were as follows: TRN+G for *CaM*_codon2, *BenA*_codon1, *BenA*_codon3; JC+I for *CaM*_codon1, *RPB2*_codon2, *BenA*_codon2; SYM+I+G for *CaM*_introns, *BenA*_introns; TRN+I+G for *CaM*_codon3, *RPB2*_codon1; TVM+I+G for *RPB2*_codon3; and GTR+I+G for ITS. Single gene and multigene phylogenetic analyses consistently resolved our strains as a unique node with *P. vascosobrinhoanum* its closest relative (Figs. 1 and 2). *Penicillium vascosobrinhoanum* was previously considered to belong to series *Euglauca* (Houbraken et al. 2020). Based on each of our analyses, however, *P. sanjayi* and *P. vascosobrinhoanum* resolve as close relatives distinct of all currently accepted series and we thus consider it to represent a new series. Backbone support in all phylogenies was rather poor, but branches holding series themselves were well supported. The new series seems to be most closely related to series *Roseopurpurea*, and this certainly is reflected in their morphologies with species producing monoverticillate conidiophores, a character atypical of the section.

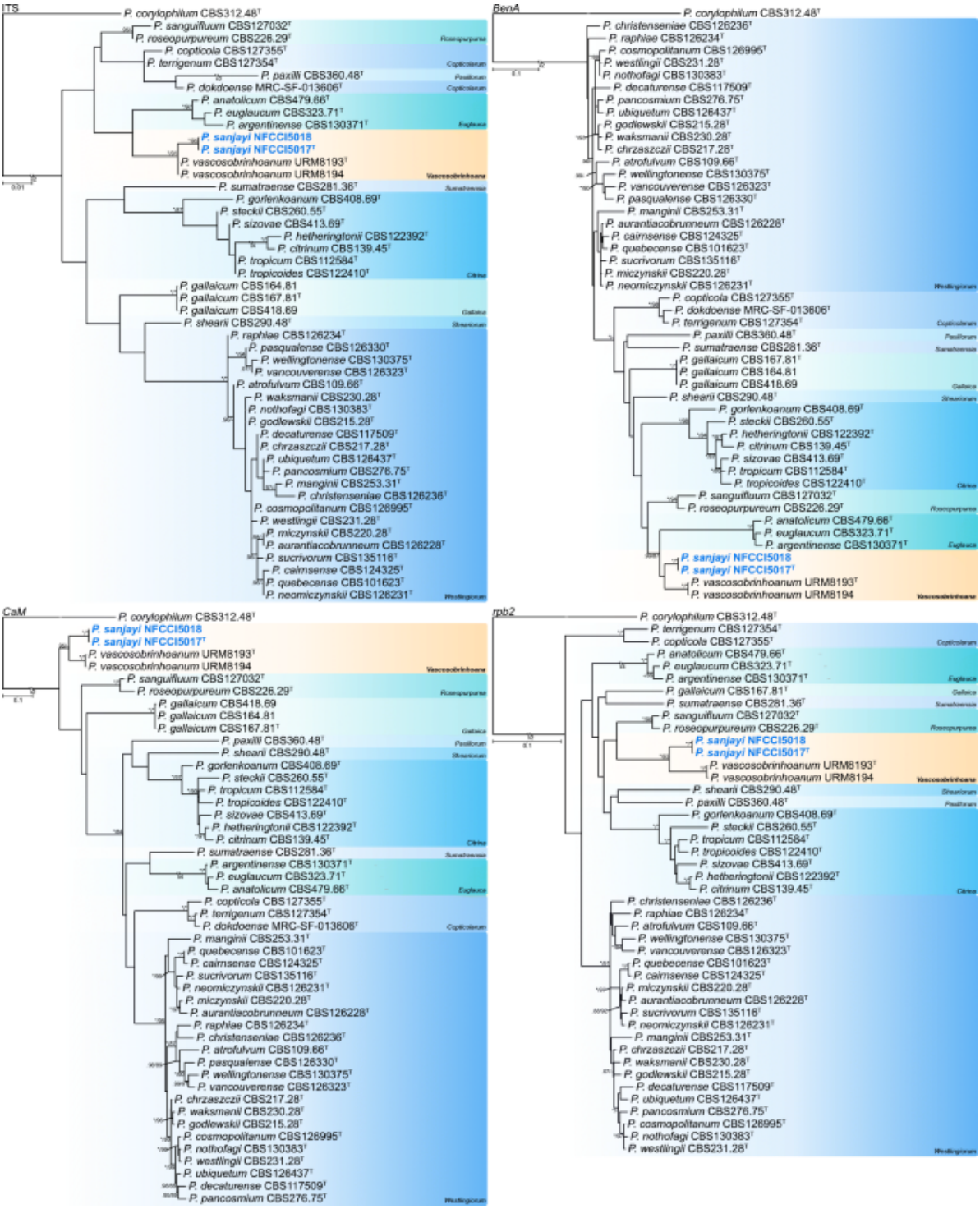

Fig. 1. Phylogenetic trees of section *Citrina* based on ITS, *BenA*, *CaM*, and *RPB2* sequences. Strains of the new species are shown in bold blue text. The series classification is shown in coloured boxes. Branch support in nodes higher than 80% bs and/or 0.95 pp is indicated at relevant branches (^{T} = ex-type; * = 100% bs or 1.00 pp; - = support lower than 80% bs and/or 0.95 pp). Trees are rooted with *P. corylophilum* (CBS 312.48^T)

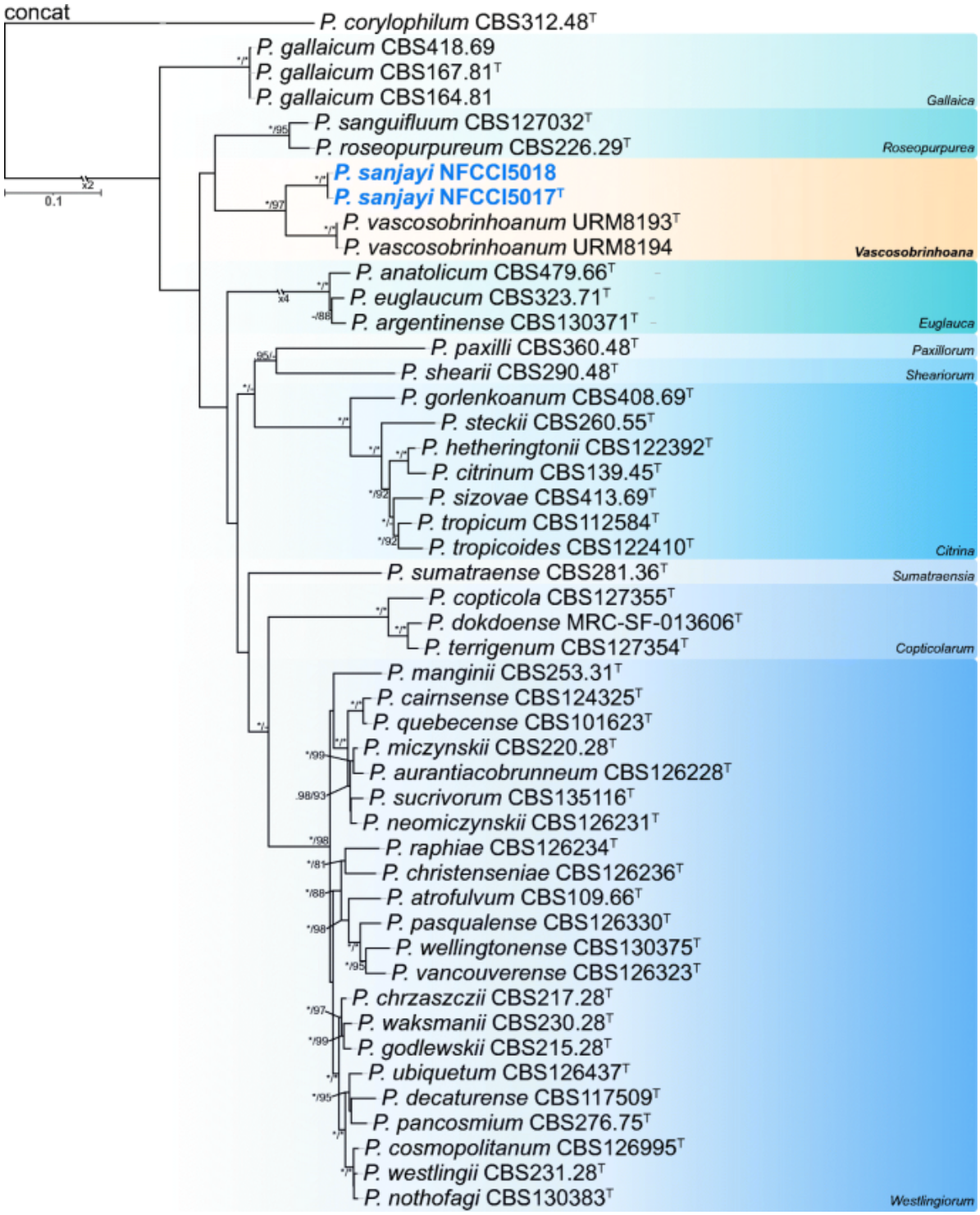

Fig. 2. Phylogenetic tree of section *Citrina* based on a concatenated dataset of *BenA*, *CaM*, and *RPB2* sequences. Strains of the new species are shown in bold blue text. The series classification is shown in coloured boxes. Branch support in nodes higher than 80% bs and/or 0.95 pp is indicated at relevant branches (^{T} = ex-type; * = 100% bs or 1.00 pp; - = support lower than 80% bs and/or 0.95 pp). Trees are rooted with *P. corylophilum* (CBS 312.48^T)

Taxonomy

Series *Vascosobrinhoana* Rajeshk., Visagie, N. Ashtekar & Yilmaz, ***ser. nov.***

MycoBank MB#840643

Etymology: Named after the type species of the series, *Penicillium vascosobrinhoanum*

Type: *Penicillium vascosobrinhoanum* [published as '*vascosobrinhous*'] R.N. Barbosa & J.D.P. Bezerra, Acta Botanica Brasilica 34 (2): 412 (2020) [MB 833816].

Diagnosis: Series *Vascosobrinhoana* belongs to subgenus *Aspergilloides* section *Citrina.* The series is phylogenetically distinct, but its relationship with others remains unresolved. Morphologically it is most similar to series *Gallaica* and *Roseopurpurea* with these containing monoverticillate species. Colonies generally grow moderately fast, grow at 30 °C, and some species grow at 37 °C; soluble pigments reddish brown on CYA, yellow on MEA; sporulation is moderate; conidiophores monoverticillate, occasionally with an additional branch or solitary phialides; stipes smooth; conidia globose to subglobose, smooth or roughened to verruculose; sexual morph not observed; sclerotia present for some species, cream.

Penicillium sanjayi Rajeshk., Visagie, N. Ashtekar & Yilmaz ***sp. nov.*** **[Figs. 3and 4]**

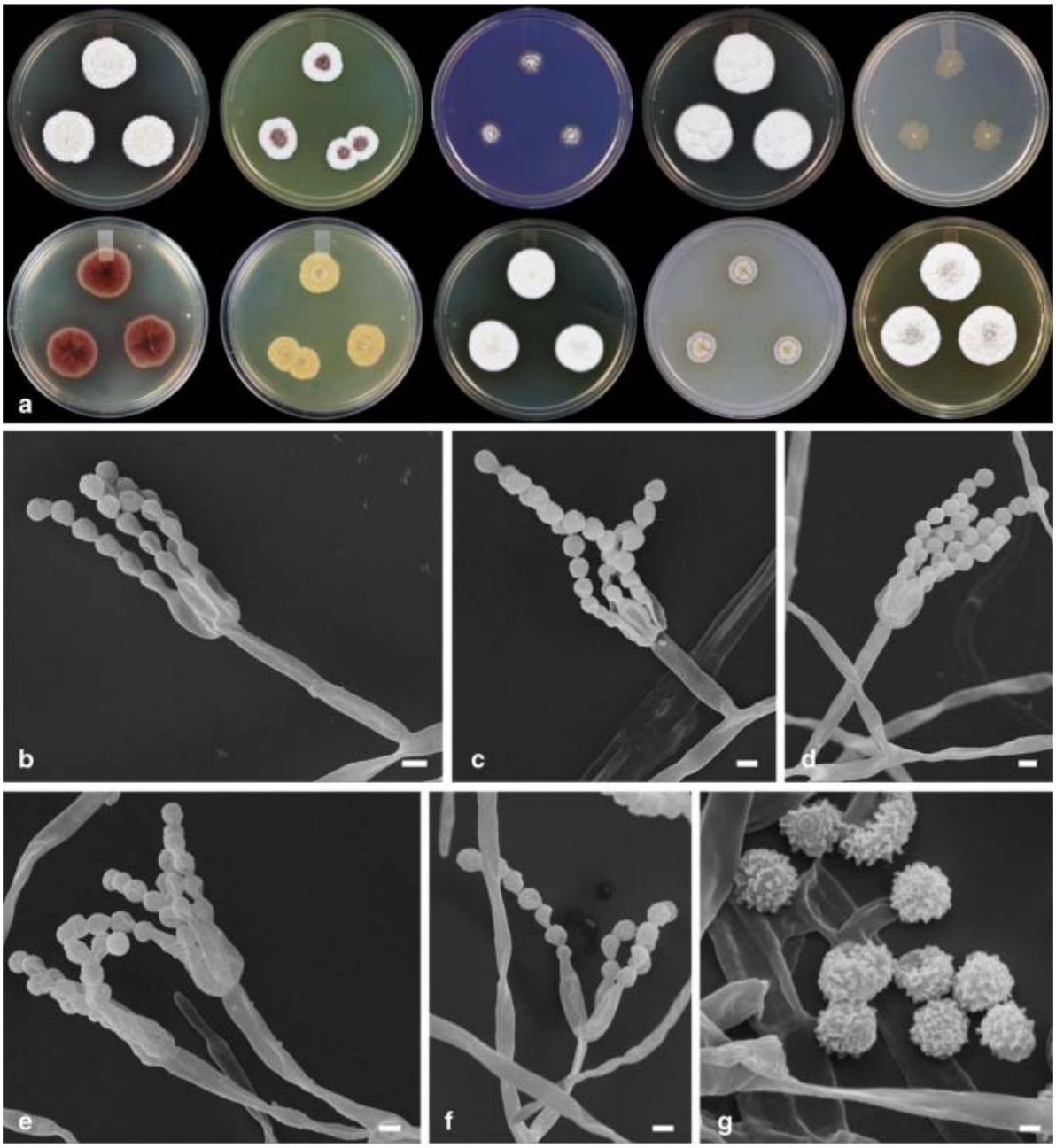

Fig. 3. ***Penicillium sanjayi*** (NFCCI 5017). **a** Colonies from left to right (top row) on CYA, MEA, CREA, CYAS, CZA, (bottom row) CYA reverse, MEA reverse, DG18, OA, and YES. **b**–**f** Monoverticillate penicilli. **g** Conidia. *Scale bar* (**b**–**f**) 2 µm; (**g**) 1 µm

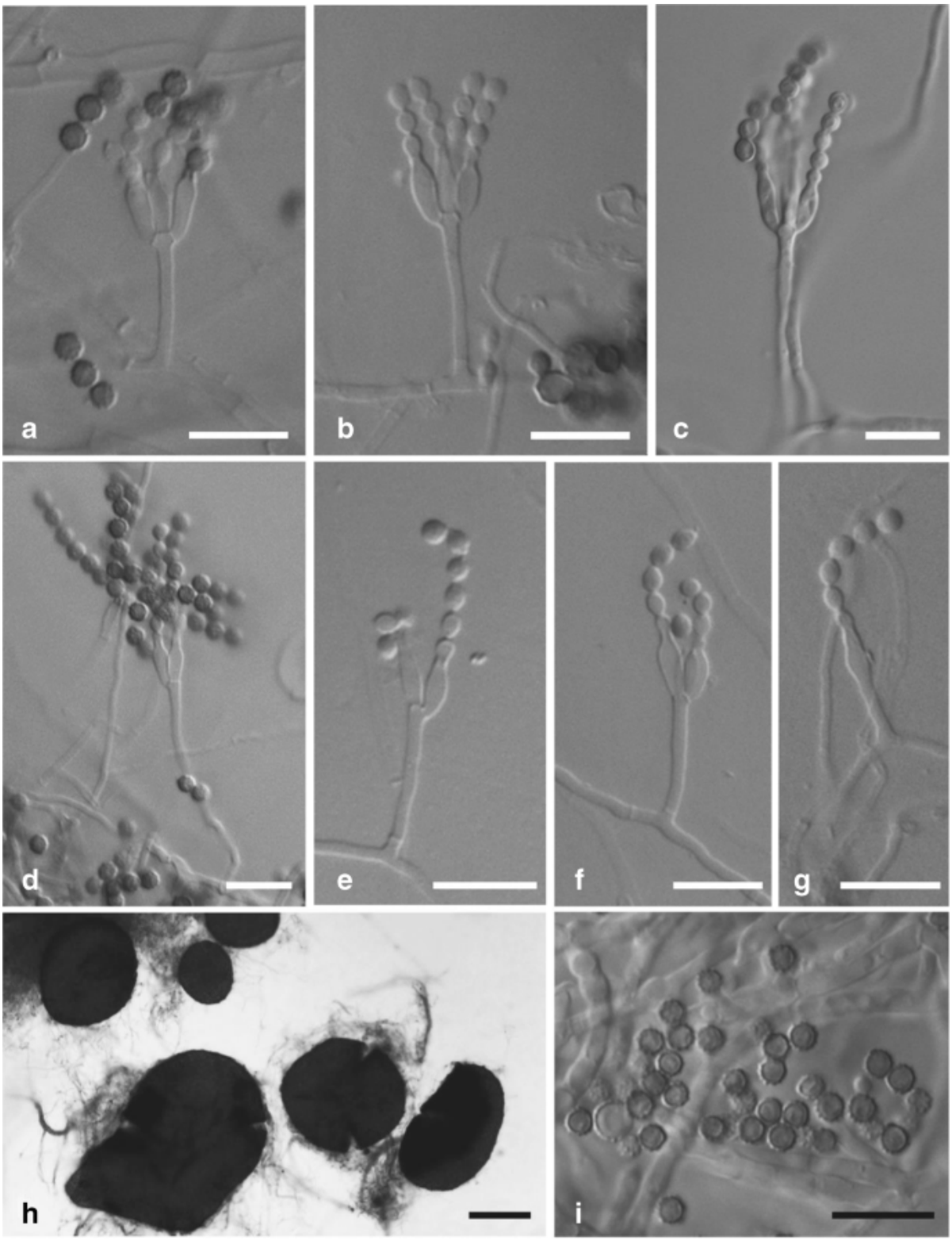

Fig. 4. ***Penicillium sanjayi*** (NFCCI 5017). **a**–**f** Monoverticillate penicilli. **g** Solitary phialide. **h** Sclerotia. **i** Conidia. *Scale bar* (**a**–**f**, **g**) 10 µm; (**h**) 100 µm

MycoBank MB#840643

Classification: series *Vascosobrinhoana*, section *Citrina*, subgenus *Aspergilloides*, *Penicillium*, *Aspergillaceae*, *Eurotiales*, *Eurotiomycetes*.

Etymology: Latin, ***sanjayi***, Named after the Indian mycologist, Dr. Sanjay K. Singh, Coordinator, National Fungal Culture Collection of India (NFCCI) at MACS Agharkar Research Institute, Pune, India.

Colony diam (*25 °C*, *7 d*, *in mm*): CREA 12–13; CYA 25–27; CYA 30 °C 10–12; CYA 37 °C no growth; CYAS 30–39; CZ 14–16; DG18 24–26; MEA 16–20; MEA 30 °C 13–15; MEA 37 °C no growth; OA 14–15; YES 29–31.

Colony characters (*25 °C*, *7 d*): *CREA*: Colonies poor growth, acid production absent. *CYA*: Colonies moderately deep, radially sulcate; texture velutinous; mycelia white, pale greyish ruby (12C3) at the centre; sporulation density low, colour greyish; exudates hyaline; soluble pigments reddish brown, reverse brownish red (10D7) at centre, pale towards margin. *CYAS*: Colonies plane, texture velutinous, slightly raised or umbonate; mycelia white (1A1); sporulation density low; exudates absent; soluble pigments absent, reverse colourless, margin regular. *CZ*: Colonies plane, poor growth, scanty aerial mycelia, reddish-blonde (5C3), margin irregular; sporulation poor; exudates absent; soluble pigments absent, reverse colourless. *MEA*: Colonies moderately deep, radially sulcate; texture velutinous; mycelia initially white (1A1), greyish ruby to ruby (12C5–12E8) at the centre, white (1A1) at margin, margin wavy; sporulation density low, exudates hyaline; soluble pigments yellow, reverse greyish brown (6D3) at centre, pale towards margin. *DG18*: Colonies plane, texture velutinous; mycelia white (1A1) with pale reddish grey (12C2) tinge at centre and pale towards the margin; margin regular; sporulation density low; exudates absent; soluble pigments absent, reverse pale reddish blonde (5C3) at centre, pale towards margin. *OA*: Colonies moderately shallow, radially sulcate; texture velutinous; mycelia golden-blonde (5C4) to reddish-blonde (5C3) at centre and pale greyish ruby (12C3) towards the periphery; sclerotia present, cream; sporulation density low, colour pale grey; margin wavy or irregular; exudates hyaline (aggregated at centre); soluble pigments greyish brown (6D3); reverse reddish-brown (6C3) at centre, greyish brown (6D3) towards periphery. *YES*: Colonies moderately deep, radially sulcate; texture velutinous; mycelia white, pale greyish ruby (12C3) at the centre, white (1A1) towards the periphery; sporulation density low, colour greyish; exudates absent; soluble pigments absent, margin irregular, reverse reddish-brown (6C3) to greyish brown (6D3) centre and pale towards the periphery.

Conidiophores monoverticillate, rarely as solitary phialides or with a subterminal branch. *Stipes* 15–50 × 2.0–2.8 µm. *Branches* when present 30–60 × 2.3–2.7 µm. *Phialides* in verticils of 2 to 7, ampulliform, 6–7 × 1.5–2.5 µm. *Conidia* globose to subglobose, 2–2.5 µm, with walls roughened to verruculose. Sclerotia present, globose to irregular, cream, 100–350 µm diam.

Typus: INDIA, Maharashtra, Yavatmal, (20.391944N 78.132222E, ± 445 msl) from soil, 23 May 2017, coll. Rajeshkumar KC & N. Ashtekar (**holotype AMH 10349**, preserved as

metabolically inactive dried specimen), **ex-type** culture **NFCCI 5017**; GenBank Numbers: ITS MZ571358, *BenA* MZ558484, *CaM* MZ558492, *RPB2* MZ558482.

Additional material examined: INDIA, Maharashtra, Pratapgadh, (17.933611N, 73.580278E, ±1050 msl) from soil, 09 April 2018, coll. N. Ashtekar & Rajeshkumar KC (**AMH 10350**, additional culture **NFCCI 5018**); GenBank Numbers: ITS MZ571359, *BenA* MZ558485, *CaM* MZ558493, *RPB2* MZ558483.

Distinguishing characteristics: Phylogenies resolve *P. sanjayi* as a close relative of *P. vascosobrinhoanum* within a distinct clade we introduce above as series *Vascosobrinhoana*. *Penicillium sanjayi* typically produce monoverticillate conidiophores, an uncommon character in section *Citrina.* Other monoverticillate species include *P. euglaucum* (series *Euglauca*), *P. gallaicum* (series *Gallaica*), *P. roseopurpureum* (series *Roseopurpurea*), *P. sanguifluum* (series *Roseopurpurea*), and *P. vascosobrinhoanum* (series *Vascosobrinhoana*). Of these, *P. euglaucum*, *P. gallaicum*, and *P. vascosobrinhoanum* can grow at 37 °C. *Penicillium sanjayi* (25–27 mm) generally grows faster than both *P. roseopurpureum* (7–16 mm) and *P. sanguifluum* (15–26 mm) on CYA, produces sclerotia, and produces roughened conidia compared to the smooth to finely roughened conidia of the latter two species (Houbraken et al. 2011a).

Discussion

In this study, we introduce a novel species as *Penicillium sanjayi*, isolated from soil from the Yavatmal and Pratapgadh area of Maharashtra in India. Single and multigene phylogenetic analyses consistently resolved our strains as a unique node with *P. vascosobrinhoanum* its closest relative (Figs. 1 and 2). *Penicillium vascosobrinhoanum* was introduced by Barbosa et al. (2020). In their study, single gene phylogenies resolved it as a distinct lineage between the *P. roseopurpureum* and *P. euglaucum* clades (respectively named series *Roseopurpurea* and *Euglauca* in Houbraken et al. (2020) but that a multigene phylogeny resolved it in a poorly supported clade with series *Euglauca*. As a result, Houbraken et al. (2020) placed *P. vascosobrinhoanum* in series *Euglauca*, but concluded that the phylogenetic relationship within section *Citrina* was unresolved largely due to poor backbone support in the phylogenies. This was also observed in our single gene and multigene phylogenies (Figs. 1 and 2). One consistent feature from our phylogenies was that *P. sanjayi* and *P. vascosobrinhoanum* resolve as close relatives in a clade distinct of all currently accepted series.

Subgeneric classifications have a long history in *Penicillium* and were discussed in detail by Houbraken et al. (2020). They also introduced a new series classification to add to the well-established subgenera and sections (Houbraken & Samson 2011). The currently accepted 494494 *Penicillium* species are classified into 2 subgenera, 32 sections, and 100 series. Section *Citrina* was divided into nine series (series *Citrina*, *Copticolarum*, *Euglauca*, *Galliaca*, *Paxillorum*, *Roseopurpurea*, *Sheariorum*, *Sumatraensia*, and *Westlingiorum*). This subgeneric classification was proposed based on multigene phylogenetic analyses. In practice, this has made working with a speciose genus more manageable, while many of the series do provide insight into what a species morphology or secondary metabolite profiles would look like (Houbraken et al. 2020). Furthermore, we believe that a subgeneric classification can be

useful in culture-independent detection techniques that characterize communities using ITS and high-throughput sequencing techniques. It is well reported that ITS does not distinguish between many *Penicillium* species. However, ITS will be useful to place an operational taxonomic unit (OTU) into series which is valuable information compared to a genus-level classification.

Comparing tree topologies obtained between different gene regions, or simply termed genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al. 2000), has become a widely used approach to more consistently recognize or determine species boundaries. The concept of GCPSR can also be applied to higher taxonomies, for example at series level. Our phylogenetic analyses revealed that the branch supporting *P. vascosobrinhoanum* and *P. sanjayi* is distinct from the series classification proposed in Houbraken et al. (2020) and we thus introduce the series *Vascosobrinhoana* for this clade. Section *Citrina* thus now contains 43 accepted species classified into 10 series. The majority of section *Citrina* species produce symmetrically biverticillate penicilli. Monoverticillate species are found in series *Euglauca* (*P. euglaucum*; note that *P. anatolicum* and *P. argentinense* produce biverticillate conidiophores), series *Gallaica* (*P. gallaicum*), and series *Roseopurpurea* (*P. roseopurpureum* and *P. sanguifluum*). Comparing these species (Table 2), *P. euglaucum*, *P. gallaicum*, and *P. vascosobrinhoanum* typically grow on CYA at 37 °C (Houbraken et al. 2011a; Barbosa et al. 2020). *Penicillium euglaucum* is distinguished by its yellow soluble pigment and production of a sexual state. *Penicillium gallaicum* produces sclerotia, a character absent in *P. vascosobrinhoanum*. *Penicillium roseopurpureum* and *P. sanguifluum* are distinct from *P. sanjayi* based on their generally more restricted growth on CYA at 25 and 30 °C. *Penicillium sanjayi* also produces roughened to verruculose conidia, compared to the smooth to finely roughened conidia of *P. roseopurpureum* and *P. sanguifluum* (Houbraken et al. 2011a). Comparing these latter two species, *P. roseopurpureum* does not grow on CYA at 30 °C, while *P. sanguifluum* does. Members of series *Roseopurpurea* produce the compound roseopurpurin (also known as anthraquionone carviolin) and related anthraquinones (Hind 1940; Posternak 1940). Also, *P. sanguifluum* produces compounds like aculeatusquinones, citreofuran, citridones, curvularins, neobrugarones, penilactone, roseopurpurins A-I (not related to roseopurpurin), sulfimarin, and trichodimerol (Aly et al. 2011; Shang et al. 2016). *Penicillium euglaucum* (series *Euglauca*) produces the key metabolite terrain (Houbraken et al. 2011a), while *P. gallaicum* (series *Gallaica*) produces citreoviridin, distinguishing them from their close relatives. Extrolite profiling of series *Vascosobrinhoana* is yet to be studied. Such chemotaxonomic studies will be promising due to the endophytic nature of *P. vascosobrinhoanum* and the saprophytic nature of *P. sanjayi* identified from tropical ecogeographic zones of Brazil and India respectively.

Table 2 Synopsis of the comparative morphology of the new species and allied species (**series**: *Euglauca*, *Gallaica*, *Roseopurpurea*, *Vascosobrinhoana*).

Species	**Colony diameter (mm)**			**Conidiophore branching**	**Conidial ornamentations**	**Cleistothecia /Sclerotia**	**References**
	CYA at 25°C	**CYA at 30°C**	**CYA at 37°C**				
Penicillium anatolicum	18–30	23–32	0–5	Predominantly biverticillate rarely divaricate	Finely roughened	Cleistothecia	Stolk 1968, Houbraken et al. 2011
Penicillium argentinense	21–27	22–30	no growth	Monoverticillate or biverticillate	Smooth	Cleistothecia	Houbraken et al. 2011
Penicillium euglaucum	23–29	21–30	(0–)5–15	Monoverticillate when young biverticillate in age	Finely roughened	Cleistothecia	Beyma 1940, Houbraken et al. 2011
Penicillium gallaicum	19–25	18–25	0–5	Monoverticillate occasionally biverticillate	Smooth	Sclerotia	Ramírez et al. 1980, Houbraken et al. 2011
Penicillium roseopurpureum	7–16	no growth	no growth	Monoverticillate when young biverticillate in age	Smooth or very finely roughened	Absent	Dierckx 1901, Houbraken et al. 2011
Penicillium sanguifluum	(15–)18–26	Micro-colony –13	no growth	Monoverticillate	Smooth or finely roughened	Absent	Biourge 1923, Houbraken et al. 2011
Penicillium sanjayi	25–27	10–12	no growth	Predominantly monoverticillate or solitary phialide or rarely biverticillate	Roughened to verruculose	Sclerotia	Present study
Penicillium vascosobrinhoana	20–22	25–27	5–9	Monoverticillate	Smooth	Not observed	Barbosa et al. 2020

The natural forests of India, especially the Western Ghats, are known for its high fungal diversity (Rajeshkumar and Singh 2012; Rajeshkumar and Sharma 2013; Rajeshkumar et al. 2018, 2019a, b, 2021). In a recent compilation of the Fungi of India (Sankaran and Minter 2021; personal communication based on a Cybertruffle project), 3700 genera and 23,500 species have been recorded from India. This includes 77 *Penicillium* species. Among these, five section *Citrina* species have been reported, namely *P. citrinum*, *P. miczynskii*, *P. paxilli*, *P. roseopurpureum*, and *P. waksmanii*. They were recorded from 14 states and a Union Territory. During the current survey, we recovered *P. citrinum* and *P. steckii* (GenBank accessions: MZ571358–MZ571363; MZ558480–MZ558497). This is a first report of *P. steckii* from India and it was isolated from soil collected from the Mulshi area of Maharashtra. The most dominant species *P. citrinum* has been reported from across India on various host substrates such as leafs, stems, fruits, seeds, spore-cases (sporangia) of fern, pollen, a pneumatophore (*Sonneratia acida*), and the phylloplane of several plants (Ghosh and Dutta 1960; Rai 1976; Luke and Devi 1979; Roy et al. 1980; Chauhan et al. 1980; Rai and Pathak 1981; Reddy et al. 1982; Devi and Wadhwani 1983; Manoharachary and Khalis 1983; Chauhan and Gupta 1984; Chauhan and Gupta 1986; Bilgrami et al. 1991; Sadhu et al. 1992; Bamba and Sumbali 1999). Other reported species include the following: *Penicillium miczynskii* has only been recorded from a soil sample from Satara, Maharashtra (Usekar 1977); *P. paxilli* from the northern states of India such as Delhi, Uttar Pradesh, and West Bengal, associated with *Allium sativum* and air (Roy et al. 1977); *P. roseopurpureum* has been recorded from Indian states, viz. Delhi, Kerala, Maharashtra, Uttar Pradesh, and West Bengal from dung from *Axis axis*, *Boselaphus tragocamelus*, captive *Macropus* sp., *Taurotragus oryx*, and plant hosts such as *Corchorus* sp. and *Oryza sativa*, textile waste, soil, and air (Saksena and Mehrotra 1952; Mukerjee 1966; Rai et al. 1969; Saxena et al. 1969; Usekar 1977; Bilgrami et al. 1991); and *P. waksmanii* was recorded from Orissa, Punjab, and Uttar Pradesh associated with *Brassica oleracea* and *Sorghum* sp. (seed) and soil (Ghosh and Dutta 1962; Verma and Khan 1965). *Penicillium* species reported from India were mostly identified based on morphological observations and we believe that the diversity of this genus has been grossly underestimated. Once surveys start to use modern taxonomic approaches, we believe that we will discover many new species. Surveys of Indian fungi looking to build collections and using modern taxonomic approaches to correctly identify them are considered important as these bioresources are critical for applied research from different eco-geographic zones looking to exploit the natural properties of these fungi. Further investigation is also needed to study the ecological importance of these species.

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Ethics declarations

Ethics approval and consent to participate

This is an original research work and submitted to *Mycological Progress* for publication. Authors adhered to discipline-specific rules for acquiring, selecting, and processing data. No data or theories by others are presented in this study.

Consent for publication

All authors agreed to publish this manuscript in *Mycological Progress*.

Competing interests

The authors declare no competing interests.

Contributions

Conceptualization: Rajeshkumar KC. Methodology: Rajeshkumar KC, Cobus Visagie, Neriman Yilmaz, Nikhil Ashtekar. Formal analysis and investigation: Cobus Visagie, Neriman Yilmaz (final tree analyses), Rajeshkumar KC, and Nikhil Ashtekar (collection, data, lab work, taxonomy, preliminary analyses). Writing—original draft preparation: Rajeshkumar KC, Cobus Visagie, Nikhil Ashtekar, Neriman Yilmaz. Writing—review and editing: Rajeshkumar KC, Cobus Visagie, Neriman Yilmaz. Funding acquisition: Rajeshkumar KC. Resources: Rajeshkumar KC. Supervision: Rajeshkumar KC, Cobus Visagie.

Data availability

All sequence data generated for this study can be accessed via GenBank. Taxonomy details are deposited in MycoBank (nomenclature is verified with Curator).

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