

doi.org/10.3114/fuse.2024.13.10

A new *Paecilomyces* from wooden utility poles in South Africa

C.M. Visagie^{1*}, E.M. Cruywagen², T.A. Duong¹

¹Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

²Crop Protection, Agricultural Research Council - Vegetable, Industrial and Medicinal Plants, Private Bag X293, Pretoria, 0001, Pretoria, South Africa

*Corresponding author: cobus.visagie@fabi.up.ac.za

Key words:

Byssochlamys

Eurotiales

GCPSR

multigene phylogeny

new taxon

Thermoascaceae

Abstract: During a survey of fungi on electricity utility poles in South Africa, a diverse range of fungi were discovered. *Paecilomyces* was frequently isolated, with five species identified using ITS and β -tubulin (*BenA*) sequences. These were *P. brunneolus*, *P. dactylethromorphus*, *P. lecythidis*, *P. paravariotii* and a potential new species. The genomes of 30 of these strains were subsequently sequenced and used in a phylogenomic analysis with 45 previously published genomes of the genus. Phylogenetic analyses were also conducted using ITS, *BenA*, calmodulin (*CaM*), RNA polymerase II second largest subunit (*RPB2*), RNA polymerase II largest subunit (*RPB1*), the genes coding for the theta subunit of the TCP-1 chaperonin complex (*Cct8*), and for a putative ribosome biogenesis protein (*Tsr1*). Both phylogenomic and phylogenetic analyses supported the 15 *Paecilomyces* species currently accepted and confirmed the novelty of the new species, which we describe as *P. lignorum*. The latter is the sister species of *P. brunneolus* and belongs to a clade also containing *P. variotii* and *P. paravariotii*. Morphologically, the new species produces longer ellipsoidal conidia and grows more restricted on malt extract agar at 30 °C compared to its closest relatives.

Citation: Visagie CM, Cruywagen EM, Duong TA (2024). A new *Paecilomyces* from wooden utility poles in South Africa. *Fungal Systematics and Evolution* 13: 163–181. doi: 10.3114/fuse.2024.13.10

Received: 15 April 2024; **Accepted:** 16 May 2024; **Effectively published online:** 12 June 2024

Corresponding editor: B. Slippers

INTRODUCTION

Paecilomyces typically thrive as saprophytic fungi across a diverse range of environments. They are characterized by their branched (verticillate) conidiophores, which bear divergent whorls of branches and phialides. These phialides feature inflated bases tapering into long and distinct necks and bend away from the main axis, and produce yellow-brown conidia in basipetal chains (Brown & Smith 1957, Samson 1974, Houbraken *et al.* 2020). *Byssochlamys* was long regarded as the sexual morph of *Paecilomyces*, producing almost naked ascospores, with globose asci and ellipsoidal, smooth-walled ascospores (Brown & Smith 1957, Stolk & Samson 1971). Samson (1974) monographed *Paecilomyces*, introduced a sectional classification and accepted 31 species. Section *Paecilomyces* included the meso-thermophilic species that produce yellow-brown colonies and those that produce a sexual morph was associated with *Byssochlamys*, *Talaromyces* and *Thermoascus*. On the other hand, section *Isarioidea* included the species associated with insects and often are insect-pathogenic, including *P. farinosus* (\equiv *Isaria farinosa*) following the reclassification of the species proposed by Brown & Smith (1957).

More recent efforts that generated DNA sequences and phylogenetic analyses resulted in several taxonomic changes. Luangsa-Ard *et al.* (2004) first showed that *Paecilomyces* was polyphyletic across *Eurotiomycetidae* and *Sordariomycetidae*, with species belonging to *Eurotiales* (the generic type *P. variotii*

and other thermophilic species), *Hypocreales* (*P. farinosus*, *P. lilacinus* and other mesophilic species) and *Sordariales* (*P. inflatus*). Luangsa-ard *et al.* (2005) subsequently provided a phylogenetic review of *Paecilomyces* section *Isarioidea* based on ITS and β -tubulin (*BenA*) and found most species to be closely related to *P. farinosus* in *Hypocreales* and for which they took up the old generic name *Isaria*, providing new combinations to species where appropriate. Luangsa-Ard *et al.* (2011) then introduced the new genus *Purpureocillium* to accommodate *Paecilomyces lilacinus* in *Ophiocordycipitaceae* (*Hypocreales*), while *Paecilomyces inflatus* was reclassified as *Phialemonium inflatum* in *Cephalothecaceae* (*Sordariales*) by Perdomo *et al.* (2013). Houbraken & Samson (2011) divided the family *Trichocomaceae* (*Eurotiales*) into three families, namely *Aspergillaceae* (for *Aspergillus*, *Penicillium* and related genera), *Thermoascaceae* (for *Thermoascus* and *Paecilomyces*) and *Trichocomaceae* (for *Talaromyces*, *Trichocoma* and related genera). This limited *Paecilomyces* to the definition of Samson *et al.* (2009) who accepted nine species, five that produced a *Byssochlamys* sexual morph and four that produced a *Paecilomyces* asexual morph. Following “One Fungus = One Name” *Paecilomyces* was the preferred name for the clade (Houbraken & Samson 2011, Houbraken *et al.* 2020), furthermore supported by the former that have priority over *Byssochlamys* (Reblova *et al.* 2016). Ten *Paecilomyces* species were included

in the accepted species list published for *Eurotiales* (Houbraken *et al.* 2020). This number has since risen to 15 with *P. clematidis* (Spetik *et al.* 2022), *P. penicilliformis* (Crous *et al.* 2020) and *P. paravariotii* (Urquhart & Idnurm 2023) described more recently. Furthermore, *P. lecythidis* and *P. maximus*, which Samson *et al.* (2009) considered synonyms of *P. formosus*, was shown to be distinct species following a revision published by Urquhart & Idnurm (2023).

Paecilomyces has a global distribution and is commonly found in various indoor and environmental settings. Their thermophilic and/or xerophilic traits, render them effective spoilage agents in heat-processed, acidic foods (Pitt & Hocking 2009, Samson *et al.* 2019). In the food industry, they can produce mycotoxins like patulin (*e.g.* *P. dactylethromorphus*), byssochlamic acid (*P. divaricatus*, *P. fulvus*, *P. lagunculariae* and *P. niveus*) or viriditoxin (*P. variotii*) (Samson *et al.* 2009). Additionally, species are known to grow on and damaged materials such as wood, leather, paper and textiles (Brown & Smith 1957), have been isolated from human and animal samples (Heshmatnia *et al.* 2017, Anderson *et al.* 2022), and from plants and trees (Heidarian *et al.* 2018, Sabernasab *et al.* 2019, Rostami & Jamali 2023). Accurately linking species with their growing substrates is, however, difficult as identifications were often based on morphology, which can be problematic.

A survey was conducted in South Africa to determine the fungi present on wooden utility poles. From a total of 423 fungal isolates obtained in the survey (de Meyer 2007), 171 belonged to the genus *Paecilomyces*. Strains were found to belong to five species, including a putative new species. The aim of this study was, therefore, to compare strains of the putative new species with others using a consilient approach that included morphology, DNA sequences and whole genomes sequences.

MATERIALS AND METHODS

Strains

Strains used in this study were isolated in a survey conducted in 2005 to catalogue the fungi associated with wooden utility poles in South Africa. Strains were revived from the CMW research collection [Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa]. All strains were deposited in the CMW-IA culture collection housed at FABI, with representative reference strains also deposited at CBS (Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands). Strain accession numbers and its associated metadata is summarized in Table 1.

DNA extraction, sequencing and phylogenetic analyses

Genomic DNA was extracted from colonies grown for 7 d at 25 °C on malt extract agar (MEA: Oxoid CM0059) using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, CA, USA). The DNA was stored at -20 °C until used for PCR. The rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS) and partial β -tubulin (*BenA*) gene region was amplified with primer pairs V9G & LS266 for ITS (Masclaux *et al.* 1995, de Hoog & Gerrits van den Ende 1998) and T10 & Bt2b for *BenA* (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). The PCR was performed in 25 μ L reactions using the Kapa ReadyMix kit (Kapa Biosystems, Woburn, USA). Reactions consisted of 12.5 μ L 2 \times ReadyMix,

10.5 μ L MiliQ H₂O, 1 μ L template DNA (2.5–25 ng) and 0.5 μ L of each primer (20 μ M). Sequencing of the PCR products was conducted in both directions using BigDye Terminator v. 3.1 Ready Reaction Mix (PerkinElmer, Warrington, UK) and the same primers used for PCR. Sequencing reactions were analyzed at the University of Pretoria sequencing facility on an ABI PRISM 3500XL auto-sequencer (Applied Biosystems, CA, USA). Contigs were assembled in Geneious Prime v. 2024.0.2. (<https://www.geneious.com>). The calmodulin (*CaM*), RNA polymerase II second largest subunit (*RPB2*), RNA polymerase II largest subunit (*RPB1*), the genes coding for the theta subunit of the TCP-1 chaperonin complex (*Cct8*), and for a putative ribosome biogenesis protein (*Tsr1*) were extracted from genome assemblies (see Tables 1, 2).

A DNA reference sequence database (including ITS, *BenA*, *CaM*, *RPB2*, *RPB1*, *Tsr1* and *Cct8*) was prepared largely based on the accepted species list published for *Eurotiales* by Houbraken *et al.* (2020) and a recent paper by Urquhart & Idnurm (2023). Strains used for phylogenetic comparisons, its associated metadata and GenBank accession numbers are listed in Table 1.

Each dataset was aligned with MAFFT v. 7.520 with the G-INS-I option selected (Kato & Standley 2013). Datasets were trimmed and concatenated in Geneious. Maximum Likelihood analyses were performed with IQ-TREE v. 2.2.2.6 on the concatenated and single gene datasets. The dataset was partitioned taking into consideration gene regions, introns and exons. The general time reversible model with gamma distribution and invariable sites (GTR+G+I) was applied to all partitions (Abadi *et al.* 2019). To calculate node support, a nonparametric bootstrap analysis with 1 000 repeats was performed in each analysis. Trees were visualized in TreeViewer v. 2.2.0 (<https://treeviewer.org>) and edited for publication in Affinity Publisher v. 2.4.0 (Serif (Europe), Nottingham, UK). Alignments and tree files were uploaded to the University of Pretoria's research data repository hosted on Figshare (doi: 10.25403/UPresearchdata.25578108).

DNA extraction and genome sequencing and assembly

Strains were grown for 14 d at 25 °C on malt and yeast extract agar (MYA: 2 % malt extract, 0.5 % yeast extract and 0.5 % agar). Mycelium was frozen at -80 °C, then lyophilized and pulverized *via* bead beating. Genomic DNA was then extracted using the Zymobiomics Soil Kit (Zymo Research, CA, USA). Sequencing libraries were prepared with ~100 ng genomic DNA as template and processed using the KAPA HyperPlus Kit using a PCR-free workflow (Roche, Switzerland), followed by seven rounds of amplification to increase library yields. Sequencing libraries were then pooled and size selected for 300–800 bp fragments using a QIAGEN GeneRead Size Selection Kit (QIAGEN, Germany). The size selected libraries were then sequenced on a NovaSeq Sequencing System (Illumina, USA) to obtain paired-end reads of 2 \times 151 bp. Adapters and low-quality regions were removed using Trimmomatic v. 0.36 (Bolger *et al.* 2014). Genomes were assembled using SPAdes v. 3.15.4 (Bankevich *et al.* 2012) using default parameters. Only contigs larger than 500 were retained in the final assemblies. Genome statistics and completeness were assessed using BUSCO v. 5.6.1 with the eurotiales_odb10 dataset (Manni *et al.* 2021). Assemblies were deposited to GenBank at the National Center for Biotechnology Information (NCBI). The genome statistics and genome accessions are listed in Table 2.

Table 1. Strains used for phylogenetic analyses of *Paecilomyces*.

Species	Strains	Country	Isolation source	ITS	GenBank accessions						
					Bena	CaM	RPB2	RPB1	Tsr1	Cct8	
<i>Monascus floridanus</i>	BYSS01	USA: Dayton	F-24 Jet A fuel	—	NIXA01	NIXA01	NIXA01	NIXA01	NIXA01	NIXA01	NIXA01
	CBS 142228 = FLAS-F54662 = CGMCC 3.5843 = BCRC 33310 = UAMH 4180 (ex-type)	USA	Sand pine roots	KY635848	KY611933	KY611972	MT419924	MT433380	MT433417		
<i>P. brunneolus</i>	CBS 370.70 = DTO 093-D7 = IFO 7563 = CECT 20341 = NBRC 7563 = JCM 12800 (ex-type)	Canada	Dry non-fat milk	EU037050	EU037068	EU037033	MN969152	—	—	—	—
	CMW-IA 005752 = CBS 118901 = CMW 18183 = CN175H9	South Africa: Kimberly	Wooden utility pole	—	PP197755	PP197786	PP197817	PP197848	PP197910	PP197879	
<i>P. clematidis</i>	CBS 148466 = MEND-F-0560 (ex-type)	Czech Republic: Lednice	Root of <i>Clematis</i>	MZ923760	MZ927740	MZ927738	OL323216	—	—	—	—
	MEND-F-0561	Czech Republic: Lednice	Root of <i>Clematis</i>	MZ923761	MZ927741	MZ927739	OL323217	—	—	—	—
<i>P. dactylethromorphus</i>	AF001	USA	Biofilm in oil tank	—	PNEM01	PNEM01	PNEM01	PNEM01	PNEM01	PNEM01	PNEM01
	CBS 251.55 = DTO 280-D1 = ATCC 11971 = IMI 65752 = MUC1.9649 (ex-type)	Brazil	Acetic acid solution	FJ389951	FJ390002	FJ389960	—	—	—	—	—
	CBS 323.34 = IMI 058411 = LSHB Pa4 = MUC1.9653	Japan	Unknown	FJ389947	FJ390005	FJ389962	—	—	—	—	—
	CBS 368.70 = IMI 082348	United Kingdom: Surrey	Medicine containing quinine	FJ389948	FJ390001	FJ389972	—	—	—	—	—
CBS 492.84	Denmark	Garden cress (<i>Lepidium sativum</i>)	FJ389949	FJ390003	FJ389973	—	—	—	—	—	
<i>P. divaricatus</i>	CBS 990.73A = IMI 058416 = LSHB P219 = LSHB Pa18 = NRRL 1123	Japan	Unknown	FJ389950	FJ390004	FJ389971	—	—	—	—	—
	CMW-IA 005736 = CBS 118903 = CMW 18161 = CN175G4	South Africa: St Lucia	Wooden utility pole (<i>Eucalyptus</i>)	PP191144	PP197732	PP197763	PP197794	PP197825	PP197887	PP197856	
	CMW-IA 005737 = CBS 124448 = CMW 18162	South Africa: St Lucia	Wooden utility pole (<i>Eucalyptus</i>)	PP191145	PP197733	PP197764	PP197795	PP197826	PP197888	PP197857	
	CMW-IA 005738 = CBS 124331 = CMW 18163	South Africa: St Lucia	Wooden utility pole (<i>Eucalyptus</i>)	PP191146	PP197734	PP197765	PP197796	PP197827	PP197889	PP197858	
	FRR 5262	Australia: Sydney	Jet fuel	—	JAPVCE01	JAPVCE01	JAPVCE01	JAPVCE01	JAPVCE01	JAPVCE01	JAPVCE01
	FRR 699	Australia: Yeerongpilly	Moldy stored sorghum	—	JAPVCF01	JAPVCF01	JAPVCF01	JAPVCF01	JAPVCF01	JAPVCF01	JAPVCF01
CBS 110429	Mexico	Pectin	FJ389932	FJ389991	FJ389954	—	—	—	—	—	

Table 1. (Continued).

Species	Strains	Country	Isolation source	GenBank accessions								
				ITS	BenA	CaM	RPB2	RPB1	Tsr1	Cct8		
<i>P. formosus</i>	CBS 284.48 = DTO 093-D8 = ATCC 10121 = ATCC 18502 = DSM 1961 = IAM5001 = IMI 40025 = NBRC 100534 = NRRL 1115 = QM 6764 = VTT D-83214 = Thom 34 (ex-type)	USA	Mucilage bottle with library paste	FJ389931	FJ389992	FJ389953	—	—	—	—	—	—
	CBS 296.93 = No. B-7	Uzbekistan: Taskent	Human bone marrow	FJ389928	FJ389994	FJ389961	—	—	—	—	—	—
	CBS 990.73B = DTO 093-D2 = ATCC 10865 = IMI 58427 = LSHB Pa31 = LSHB X26 = NRRL 1282 (ex-type)	Japan	Unknown	FJ389929	FJ389993	FJ389978	MN969154	—	—	—	—	—
	FRR 3793	Australia: Sydney	Beer	—	JAPVCB01	JAPVCB01	JAPVCB01	JAPVCB01	JAPVCB01	JAPVCB01	JAPVCB01	JAPVCB01
	NBRC 109023 = No5	Japan: Nagasu port	Soil	—	BAUL01	BAUL01	BAUL01	BAUL01	BAUL01	BAUL01	BAUL01	BAUL01
	WS011	China: Guangzhou	Asian citrus psyllid (<i>Diaphorina citri</i>)	—	JACXGS01	JACXGS01	JACXGS01	JACXGS01	JACXGS01	JACXGS01	JACXGS01	JACXGS01
	CBS 113954 = ATCC 36841 = NRRL 32567	Unknown	Unknown	FJ389942	FJ389985	FJ389975	—	—	—	—	—	—
	CBS 132.33 = IMI 58.421 (ex-type)	United Kingdom	Bottled fruit	FJ389939	FJ389988	FJ389957	—	—	—	—	—	—
	CBS 135.62	Switzerland: Wädenswil	Fruit juice	FJ389943	FJ389989	FJ389976	—	—	—	—	—	—
	CBS 146.48 = ATCC 10099 = IFO 31767 = IFO 6758 = IMI 040021 = LSHB BB134 = LSHB Pa24 = NRRL 1125	United Kingdom	Bottled fruit	FJ389940	FJ389986	FJ389959	—	—	—	—	—	—
CBS 604.71 = IFO 31768 = NRRL 2975	Unknown	Unknown	FJ389941	FJ389997	FJ389967	—	—	—	—	—	—	
FRR 3794	Australia: Sydney	Beer	—	JAPVCG01	JAPVCG01	JAPVCG01	JAPVCG01	JAPVCG01	JAPVCG01	JAPVCG01	JAPVCG01	
CBS 110378	Unknown	Unknown	FJ389946	FJ390006	FJ389979	—	—	—	—	—	—	
CBS 373.70 = IMUJPe 2195 (ex-type)	Brazil	Wood of <i>Laguncularia racemosa</i>	FJ389944	FJ389995	FJ389965	MN969204	—	—	—	—	—	
CBS 696.95 = JCM 12806	The Netherlands	Pasteurized strawberries	FJ389945	FJ389996	FJ389970	—	—	—	—	—	—	
CBS 372.70 = IMUR 2191 (ex-type)	Brazil	Wood of <i>Lecythis unisitata</i>	FJ389926	FJ389990	FJ389964	—	—	—	—	—	—	

Table 1. (Continued).

Species	Strains	Country	Isolation source	ITS	GenBank accessions								
					Bena	CaM	RPB2	RPB1	Tsr1	Cct8			
	CBS 628.66 = CEB 3292.2 = IMI 114930	France: Lyon	Quebracho-tanned sheep leather	FJ389927	FJ389983	FJ389969	—	—	—	—	—	—	—
	CMW-IA 000550 = CBS 124330 = CMW 18167 = CN175G7	South Africa: Stellenbosch	Wooden utility pole (<i>Eucalyptus</i>)	PP191149	PP197737	PP197768	PP197799	PP197830	PP197892	PP197861	—	—	—
	CMW-IA 000560 = CBS 118899 = CMW 18169 = CN175G8	South Africa: Kimberley	Wooden utility pole (<i>Pinus</i>)	PP191150	PP197738	PP197769	PP197800	PP197831	PP197893	PP197862	—	—	—
	CMW-IA 004822 = CBS 124304 = CMW 18165 = CN175G5	South Africa: Stellenbosch	Wooden utility pole (<i>Pinus</i>)	PP191147	PP197735	PP197766	PP197797	PP197828	PP197890	PP197859	—	—	—
	CMW-IA 005020 = CBS 124305 = CMW 18166 = CN175G6	South Africa: Stellenbosch	Wooden utility pole (<i>Eucalyptus</i>)	PP191148	PP197736	PP197767	PP197798	PP197829	PP197891	PP197860	—	—	—
	CMW-IA 005739 = CBS 118897 = CMW 18170 = CN175G9	South Africa: Kimberley	Wooden utility pole (<i>Eucalyptus</i>)	PP191151	PP197739	PP197770	PP197801	PP197832	PP197894	PP197863	—	—	—
	DTO 045-11	United Kingdom	Pseudo-outbreak in hospital, blood culture	GU968651	GU968684	—	—	—	—	—	—	—	—
	DTO 049-D6 = NCPF 2837	United Kingdom	Brain abscess	GU968655	GU968691	—	—	—	—	—	—	—	—
	DTO 063-E3 = UMCN 1274	The Netherlands: Nijmegen	Human bronchoalveolar lavage fluid	GU968664	GU968678	—	—	—	—	—	—	—	—
	DTO 063-F1 = UMCN V49-58	The Netherlands: Zwolle	Human sputum	GU968670	GU968686	—	—	—	—	—	—	—	—
	DTO 063-F4 = UMCN V56-25	The Netherlands: Nijmegen	Human sputum	GU968673	GU968688	—	—	—	—	—	—	—	—
	FRR 3797	Australia: Sydney	Cardboard	—	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01	JAPVCD01
	FRR 4481	Unknown	Unknown	—	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01	JAPVCC01
<i>P. lignorum</i>	CMW-IA 005740 = CMW 18171 = CN175H1	South Africa: Groblersdal	Wooden utility pole (<i>Eucalyptus</i>)	PP191152	PP197740	PP197771	PP197802	PP197833	PP197895	PP197864	—	—	—
	CMW-IA 005741 = CMW 18172	South Africa: Groblersdal	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197747	PP197778	PP197809	PP197840	PP197902	PP197871	—	—	—
	CMW-IA 005743 = CMW 18174	South Africa: Groblersdal	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197748	PP197779	PP197810	PP197841	PP197903	PP197872	—	—	—
	CMW-IA 005744 = CBS 124306 = CMW 18175	South Africa: Groblersdal	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197749	PP197780	PP197811	PP197842	PP197904	PP197873	—	—	—
	CMW-IA 005745 = CBS 124307 = CBS 118902 = CMW 18176 = CN175H2	South Africa: Plettenberg Bay	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197750	PP197781	PP197812	PP197843	PP197905	PP197874	—	—	—

Table 1. (Continued).

Species	Strains	Country	Isolation source	GenBank accessions							
				ITS	BenA	CaM	RPB2	RPB1	Tsr1	Cct8	
<i>P. maximus</i>	CMW-IA 005746 = CBS 124308 = CMW 18177 = CN175H3	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197751	PP197782	PP197813	PP197844	PP197906	PP197875	
	CMW-IA 005747 = CMW 18178 = CN175H4	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197752	PP197783	PP197814	PP197845	PP197907	PP197876	
	CMW-IA 005748 = CMW 18179 = CN175H5	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197753	PP197784	PP197815	PP197846	PP197908	PP197877	
	CMW-IA 005749 = CBS 124309 = CMW 18180 = CN175H6 (ex-type)	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	PP191153	PP197741	PP197772	PP197803	PP197834	PP197896	PP197865	
	CMW-IA 005750 = CBS 124310 = CMW 18181 = CN175H7	South Africa: Stellenbosch	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197754	PP197785	PP197816	PP197847	PP197909	PP197878	
	CMW-IA 005751 = CMW 18182 = CN175H8	South Africa: Stellenbosch	Wooden utility pole (<i>Eucalyptus</i>)	PP191154	PP197742	PP197773	PP197804	PP197835	PP197897	PP197866	
	CMW-IA 005753 = CMW 18184 = CN175I1	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197756	PP197787	PP197818	PP197849	PP197911	PP197880	
	CMW-IA 005754 = CMW 18185 = CN175I2	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	PP191155	PP197743	PP197774	PP197805	PP197836	PP197898	PP197867	
	CMW-IA 005755 = CMW 18186 = CN175I3	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	—	PP197757	PP197788	PP197819	PP197850	PP197912	PP197881	
	CMW-IA 005756 = CMW 18187 = CN175I4	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	PP191156	PP197744	PP197775	PP197806	PP197837	PP197899	PP197868	
	CMW-IA 005758 = CMW 18190 = CN175I7	South Africa: Pietermaritzburg	Wooden utility pole (<i>Eucalyptus</i>)	PP191157	PP197745	PP197776	PP197807	PP197838	PP197900	PP197869	
	CBS 113247 = IMUR 2192 (ex-type)	Thailand	Soil from pineapple field	FJ389921	FJ390009	FJ389980	—	—	—	—	
	CBS 371.70 = IMUR 2192	Brazil	Sugar apple (<i>Annona squamosa</i>)	FJ389920	FJ389982	FJ389963	—	—	—	—	
	FRR 2337	Australia: Sydney	Human lung	—	JAPVBZ01	JAPVBZ01	JAPVBZ01	JAPVBZ01	JAPVBZ01	JAPVBZ01	
	FRR 4140	Australia: Perth	Human eye	—	JAPVBY01	JAPVBY01	JAPVBY01	JAPVBY01	JAPVBY01	JAPVBY01	
	FRR 4742	Australia: Sydney	Strawberry puree	—	JAPVCA01	JAPVCA01	JAPVCA01	JAPVCA01	JAPVCA01	JAPVCA01	
	CBS 100.11 = ATCC 22260 (ex-type)	Unknown	Unknown	FJ389934	FJ389999	FJ389956	JF417414	JN121551	JF417381	JF417514	
	CBS 113245 = JCM 12805	Switzerland	Pasteurized fruit juice	FJ389936	FJ389998	FJ389974	—	—	—	—	
CBS 133.37 = ATCC 24101	USA	Cow milk	FJ389935	FJ390000	FJ389958	—	—	—	—		
CBS 271.95	China: Shanghai	Mushroom bed	FJ389937	FJ389984	FJ389977	—	—	—	—		
CBS 606.71 = VKM F-1486	Ukraine: Kharkov	Oat grain	FJ389938	FJ389987	FJ389968	—	—	—	—		

Table 1. (Continued).

Species	Strains	Country	Isolation source	ITS	GenBank accessions							
					Bena	CaM	RPB2	RPB1	Tsr1	Cct8		
<i>P. paravaritii</i>	Cornellorchardsno7	USA: Ithaca	Unknown	—	QEIL01	QEIL01	QEIL01	QEIL01	QEIL01	QEIL01	QEIL01	QEIL01
	CMW-IA 000553 = CBS 118904 = CMW 18189 = CN17516	South Africa: Pietermaritzburg	Wooden utility pole (<i>Pinus</i>)	—	PP197759	PP197790	PP197821	PP197852	PP197914	PP197883	PP197883	
	CMW-IA 005757 = CBS 118829 = CMW 18188 = CN17515	South Africa: Pietermaritzburg	Wooden utility pole (<i>Pinus</i>)	—	PP197758	PP197789	PP197820	PP197851	PP197913	PP197882	PP197882	
	CMW-IA 005759 = CMW 18191 = CN17518	South Africa: St Lucia	Wooden utility pole	—	PP197760	PP197791	PP197822	PP197853	PP197915	PP197884	PP197884	
	CMW-IA 005760 = CMW 18192 = CN17519	South Africa: St Lucia	Wooden utility pole	—	PP197761	PP197792	PP197823	PP197854	PP197916	PP197885	PP197885	
	CMW-IA 005761 = CMW 18193 = CN176A1	South Africa: St Lucia	Wooden utility pole	—	PP197762	PP197793	PP197824	PP197855	PP197917	PP197886	PP197886	
	DTO 271-G3	South Africa	Ice tea	—	JAROOX01	JAROOX01	JAROOX01	JAROOX01	JAROOX01	JAROOX01	JAROOX01	
	FRR 5287 = DSTO 1357 (ex-type)	USA	Unknown	—	JANCMN01	JANCMN01	JANCMN01	JANCMN01	JANCMN01	JANCMN01	JANCMN01	
	CCF 5755 = CBS 146003 = EMSL 3392 (ex-type)	USA: Menasha	Air sample from pharmacy	LR679769	LR679768	LR778299	—	—	—	—	—	
	CCF 6350 = EMSL 4943	USA: Taunton	Fruit juice (peach-mango)	LR736038	LR778163	LR778165	—	—	—	—	—	
<i>P. tabacinus</i>	CBS 141098 = DTO 412-B7 = CCF 5290 (ex-type)	USA: Durham	Tobacco leaves	LT548280	MN969434	LT548288	MN969210	—	—	—	—	
	CCF 5290	USA: Durham	Tobacco leaves	LT626945	LT548286	LT548288	—	—	—	—	—	
<i>P. variotii</i>	CBS 101075 = ATCC 90900 = FRR 5219 = JCM 12815	Japan: Tokyo	Heat processed fruit beverage	—	RCNU01	RCNU01	RCNU01	RCNU01	RCNU01	RCNU01	RCNU01	
	CBS 102.74 = DTO 032-I8 = DTO 280-D5 = CECT2803 = NRRL 1116 (ex-type)	France	Unknown	EU037055	EU037073	EU037038	MN969153	—	—	—	—	
	CBS 144490	Australia: Melbourne	Culture contaminant	—	RHLL01	RHLL01	RHLL01	RHLL01	RHLL01	RHLL01	RHLL01	
	CBS 338.51 = JCM 12811	Switzerland	Fruit juice	FJ389930	FJ390007	FJ389955	—	—	—	—	—	
<i>P. variotii</i>	CMW-IA 001075 = CMW 60399 = CN002G9	Canada: Vancouver	House dust	PP191158	PP197746	PP197777	PP197808	PP197839	PP197901	PP197870	PP197870	
	DTO 021-C3	USA	Spoiled sports drink	—	JAROOU01	JAROOU01	JAROOU01	JAROOU01	JAROOU01	JAROOU01	JAROOU01	
	DTO 021-D3	USA	Heat shocked sucrose	—	JAROOV01	JAROOV01	JAROOV01	JAROOV01	JAROOV01	JAROOV01	JAROOV01	
	DTO 027-B3	USA	Spoiled sports drink	—	JAROOY01	JAROOY01	JAROOY01	JAROOY01	JAROOY01	JAROOY01	JAROOY01	
	DTO 027-B5	USA	Sucrose	—	JAROOZ01	JAROOZ01	JAROOZ01	JAROOZ01	JAROOZ01	JAROOZ01	JAROOZ01	

Species	Strains	Country	Isolation source	ITS	GenBank accessions								
					BenA	CaM	RPB2	RPB1	Tsr1	Cct8			
	DTO 027-B6	The Netherlands	Spoiled apple juice	—	JAROPA01	JAROPA01	JAROPA01	JAROPA01	JAROPA01	JAROPA01	JAROPA01	JAROPA01	JAROPA01
	DTO 027-B9	USA	Spoiled sports drink	—	JAROPB01	JAROPB01	JAROPB01	JAROPB01	JAROPB01	JAROPB01	JAROPB01	JAROPB01	JAROPB01
	DTO 032-I3	USA	High fructose corn syrup after heat shock	—	JAROOK01	JAROOK01	JAROOK01	JAROOK01	JAROOK01	JAROOK01	JAROOK01	JAROOK01	JAROOK01
	DTO 032-I4	USA	Spoiled bottle of sweetened tea	—	JAROPF01	JAROPF01	JAROPF01	JAROPF01	JAROPF01	JAROPF01	JAROPF01	JAROPF01	JAROPF01
	DTO 045-G8	USA	Drink	—	JAROPG01	JAROPG01	JAROPG01	JAROPG01	JAROPG01	JAROPG01	JAROPG01	JAROPG01	JAROPG01
	DTO 063-F5	The Netherlands	Human abscess	—	JAROPH01	JAROPH01	JAROPH01	JAROPH01	JAROPH01	JAROPH01	JAROPH01	JAROPH01	JAROPH01
	DTO 164-E3	The Netherlands	Blue berry ingredients	—	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1	JAROOLO1
	DTO 166-G4	The Netherlands	Pectin, heat treated	—	JAROOM01	JAROOM01	JAROOM01	JAROOM01	JAROOM01	JAROOM01	JAROOM01	JAROOM01	JAROOM01
	DTO 166-G5	The Netherlands	Pectin, heat treated	—	JAROON01	JAROON01	JAROON01	JAROON01	JAROON01	JAROON01	JAROON01	JAROON01	JAROON01
	DTO 169-C6	The Netherlands	Indoor environment	—	JAROOO01	JAROOO01	JAROOO01	JAROOO01	JAROOO01	JAROOO01	JAROOO01	JAROOO01	JAROOO01
	DTO 169-E5	The Netherlands	Indoor environment	—	JAROOP01	JAROOP01	JAROOP01	JAROOP01	JAROOP01	JAROOP01	JAROOP01	JAROOP01	JAROOP01
	DTO 195-F2	Belgium	Margarine	—	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01	JAROOQ01
	DTO 207-G8	The Netherlands	Fruit	—	JAROOR01	JAROOR01	JAROOR01	JAROOR01	JAROOR01	JAROOR01	JAROOR01	JAROOR01	JAROOR01
	DTO 212-C5	The Netherlands	Vanilla	—	JAROOS01	JAROOS01	JAROOS01	JAROOS01	JAROOS01	JAROOS01	JAROOS01	JAROOS01	JAROOS01
	DTO 217-A2	The Netherlands	Ice pop, heat treated	—	JAROOT01	JAROOT01	JAROOT01	JAROOT01	JAROOT01	JAROOT01	JAROOT01	JAROOT01	JAROOT01
	DTO 271-D3	Guatemala	Industry environment	—	JAROOW01	JAROOW01	JAROOW01	JAROOW01	JAROOW01	JAROOW01	JAROOW01	JAROOW01	JAROOW01
	DTO 280-E4	The Netherlands	Pectin	—	JAROPC01	JAROPC01	JAROPC01	JAROPC01	JAROPC01	JAROPC01	JAROPC01	JAROPC01	JAROPC01
	DTO 282-E5	Italy	Margarine	—	JAROPD01	JAROPD01	JAROPD01	JAROPD01	JAROPD01	JAROPD01	JAROPD01	JAROPD01	JAROPD01
	DTO 282-F9	United Kingdom	Wall covering, industry environment	—	JAROPE01	JAROPE01	JAROPE01	JAROPE01	JAROPE01	JAROPE01	JAROPE01	JAROPE01	JAROPE01
	FRR 1658	Unknown	Leather	—	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01	JANCMQ01
	FRR 2889	Unknown	Leather	—	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1	JANCMPO1
	FRR 3823	Unknown	Leather	—	JANCMO01	JANCMO01	JANCMO01	JANCMO01	JANCMO01	JANCMO01	JANCMO01	JANCMO01	JANCMO01
	IMV 00236	Ukraine	Wipes	—	MSJH02	MSJH02	MSJH02	MSJH02	MSJH02	MSJH02	MSJH02	MSJH02	MSJH02
	MTDF-01	China	Daqu liquor fermentation starter	—	RCHW01	RCHW01	RCHW01	RCHW01	RCHW01	RCHW01	RCHW01	RCHW01	RCHW01
<i>P. zollerniae</i>	CBS 374.70 = JCM 12808 (ex-type)	Brazil	Wood of <i>Zollernia ilicifolia</i>	FJ389933	FJ390008	FJ389966	—	—	—	—	—	—	—

Phylogenomic analysis

Publicly available *Paecilomyces* genomes were downloaded from NCBI and analyzed with those generated in the current study (see Table 2). The dataset included 77 genomes, with *Monascus floridanus* selected as an outgroup. A BUSCO analysis using the eurotiales_odb10 dataset was run on each genome to extract the shared complete single copy BUSCO genes into separate datasets. Each dataset was aligned with MAFFT using the ‘auto’ algorithm selection and then trimmed using trimAL v. 1.2 with the “-automated1” activated (Capella-Gutierrez *et al.* 2009). Maximum Likelihood analyses was conducted in IQ-TREE, with the most appropriate substitution model selected and applied using ModelFinder (Kalyaanamoorthy *et al.* 2017) and running an ultrafast bootstrapping approximation (1 000 repeats) using UFBoot2 (Hoang *et al.* 2018). Resulting trees were collected into a single file and branches with less than 10 % bootstrap support were collapsed. A species tree was then derived using ASTRAL v.5.7.7 (<https://github.com/smirarab/ASTRAL>). Branch lengths of this species tree was estimated using RAxML v. 8 (Stamatakis 2014), using the ASTRAL tree and a dataset of all BUSCO alignments concatenated using FASconCAT v. 1.0.2 (Kück & Longo 2014) as input. Finally, IQ-TREE was used to calculate the gene concordance factors (gcf) using the branch optimised species tree and all BUSCO gene trees as input. Trees were visualized in TreeViewer v. 2.2.0 (<https://treeviewer.org>) and edited for publication in Affinity Publisher v. 2.4.0 (Serif (Europe), Nottingham, UK). Alignments and tree files were uploaded to the University of Pretoria’s research data repository hosted on Figshare (doi: 10.25403/UPresearchdata.25578108).

Morphology

Strains were characterized following methods described in Samson *et al.* (2019). Briefly, strains were inoculated at three-equidistant points onto the growth media Czapek yeast autolysate agar (CYA), CYA supplemented with 5 % NaCl (CYAS), MEA, yeast extract sucrose agar (YES), dichloran-glycerol agar (DG18; Oxoid CM0729), oatmeal agar (OA), and creatine sucrose agar (CREA). Plates were incubated in the dark, unwrapped, at 25 °C, with additional CYA, YES, DG18 and OA incubated at 30 °C, and MEA and CREA at 30, 37, 40 and 45 °C. Colour names and codes used in the description follow Kornerup & Wanscher (1967).

A Sony alpha 7 III camera and Sony FE 90 mm f/2.8 Macro G OSS lens (Tokyo, Japan) was used to photograph colonies. Microscope photographs were taken using a Zeiss AXIO Imager. A2 compound and AXIO Zoom.V16 microscope equipped with an AxioCaM 512 colour camera, run by Zen Blue v. 3.2 software (Carl Zeiss CMP, Goettingen, Germany). Extended depth of field images was assembled in Helicon Focus v. 7.5.4 (HeliconSoft, Kharkiv, Ukraine). The photoplate was prepared in Affinity Photo v. 2.4.0 [Serif (Europe), Nottingham, UK].

RESULTS

Strains

A total of 30 *Paecilomyces* strains isolated from wooden utility poles were revived from the CMW culture collection. These strains represent a subset of the 171 strains originally isolated and

were selected based on their ITS-RFLP groupings to represent the diversity amongst the strains (de Meyer 2007). Strains belonged to five species, including *P. brunneolus*, *P. dactylethromorphus*, *P. lecythidis*, *P. paravariotii* and the new species described below as *P. lignorum*. Strains of the new species originated from four collection sites situated in the South African towns Groblersdal, Plettenberg Bay, Pietermaritzburg and Stellenbosch.

Phylogenetic and phylogenomic analyses

Aligned datasets for ITS, *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1* were 619, 547, 609, 1 116, 1 022, and 1 288 and 918 bp long, respectively. Phylogenetic resolution in single genes was low (Suppl. Fig. S1). This was clear for *P. variotii* and *P. paravariotii* where poor support was observed for *BenA*, *CaM* and *RPB2* trees. Due to poor backbone support obtained, single gene phylogenies cannot be considered incongruent. Considering identifications, all genes can distinguish between the species, with *BenA* proposed as a good DNA barcode in *Eurotiales* (Houbraken *et al.* 2020), noting that *CaM* is the proposed barcode for *Aspergillus* (Samson *et al.* 2014). Better support was obtained for the concatenated tree (based on *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1*) with all accepted species resolved in well-defined clades (Fig. 1). The new species is the closest relative of *P. brunneolus*, and belongs in a broader clade that also contain *P. variotii* and *P. paravariotii*.

Intraspecies variation between strains of the new species were low. The similarities with other *Paecilomyces* species were relatively low, with ITS differing by at least 40 bp, *BenA* by 8 bp, *CaM* by 21 bp, *RPB2* by 17 bp, *RPB1* by 18 bp, *Cct8* by 24 bp, and *Tsr1* by 10 bp.

The phylogenomic analysis included 77 genomes, 31 of which was newly generated in this study. Genomes are available for nine of the 15 accepted species. The BUSCO analysis revealed completeness score ranging from 93.5–94.7 % and identified 3 132 single copy BUSCO genes present in all 77 genomes (see Table 2 and doi: 10.25403/UPresearchdata.25578108 for genome statistics). The obtained species tree had a similar topology to that of the concatenated tree (Fig. 2). Our new species is most closely related to *P. brunneolus*, and they belong to a clade that include *P. variotii* and *P. paravariotii*. Support for the new species was high, with 98.4 % of the single copy genes supporting its branch. Although bootstrap support was low for *BenA*, *CaM*, *RPB2*, the markers typically used for phylogenetic analyses in *Eurotiales*, the *P. variotii* clade was supported by 83 % of the single copy genes and *P. paravariotii* by 88.4 %. A large degree of intraspecies variation was observed for *P. variotii*, *P. paravariotii*, *P. lecythidis*, *P. maximus* and *P. formosus* (Fig. 2).

As reported by Urquhart & Idnurm (2023), 8/46 publicly available genomes are incorrectly named in NCBI (Table 2). One of these (JAPVCB01) can be attributed to a recent revision showing that *P. lecythidis* and *P. maximus*, previously considered synonyms of *P. formosus* (Samson *et al.* 2009), were phylogenetically distinct species (Urquhart & Idnurm 2023). Our analyses confirm that these are well supported distinct species. Two genomes (JANCMN01 & JAROOX01) were deposited as *P. variotii*, but clearly belongs to the recently described *P. paravariotii* (Urquhart & Idnurm 2023). Two other genomes (BAUL01 & JAPVCB01) deposited as *P. variotii* should be renamed as *P. formosus*. The genomes deposited as *Byssochlamys* should be renamed as *P. dactylethromorphus* (PNEM01), *P. variotii* (MSJH02) and as *Monascus floridanus* (NIXA01).

Morphology

Generally, strains of the new species morphologically resembled each other, noting that a large degree of variation was observed in colony growth rates. These are noted in the formal description below.

Taxonomy

Paecilomyces lignorum Visagie, Cruywagen & Duong, *sp. nov.* MycoBank MB 853456. Fig. 3.

Etymology: Latin, *lignorum*, meaning “from wood”, named in reference to the new species isolated from wooden utility poles.

DNA barcodes: ITS = PP191153, *BenA* = PP197741, *CaM* = PP197772, *Cct8* = PP197865, *RPB1* = PP197834, *RPB2* = PP197803, *Tsr1* = PP197896.

Reference genomes: SAMN40250937[†], SAMN40250929, SAMN40250930, SAMN40250931, SAMN40250932, SAMN40250933, SAMN40250934, SAMN40250935, SAMN40250936, SAMN40250938, SAMN40250939, SAMN40250941, SAMN40250942, SAMN40250943, SAMN40250944, SAMN40250946.

Colony diam (in mm, after 7 d, at 25 °C): CYA 20–25; CYAS microcolonies; MEA 50–55; DG18 28–35; YES 10–22; OA 35–40; CREA 5–8; (at 30 °C): CYA 15–20; MEA 20–35; YES 20–45; DG18 40–50; OA 30–40; CREA 5–10; (at 37 °C): MEA 20–35; CREA 5–10; (at 40 °C): MEA microcolonies–10; CREA microcolonies–5; (at 45 °C): MEA no growth; CREA no growth.

Colony characters (after 7 d, at 25 °C): CYA colonies moderate, sulcate, slightly crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation sparse to moderate areas, conidia *en masse* Light Yellow (2A4) to Greyish Yellow to Golden (4C5–6); soluble pigments absent; exudates absent; reverse Light Yellow (4A3) and Yellowish Brown (5D5). MEA colonies moderate, lightly sulcate, raised at centre; margins low, wide, entire; mycelia white; texture velutinous; sporulation dense, conidia *en masse* Light Yellow (2A4) to Greyish Yellow to Golden to Olive Brown (4C5–6–D5); soluble pigments absent; exudates absent; reverse Olive Brown to Brown (4C5–5D5). YES colonies deep, plane in restricted growers, sulcate in fast growing; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* Light Yellow (2A4) to Greyish Yellow to Golden to Olive Brown (4C5–6–D5); soluble pigments absent; exudates absent; reverse Pale Yellow (3A3) to Olive Brown (4E8). DG18 colonies low, lightly sulcate; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* Light Yellow (2A4) to Greyish Yellow to Golden to Olive Brown (4C5–6–D5); soluble pigments absent; exudates absent; reverse Yellowish White to Greyish Yellow (4A2–B4). OA colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* Light Yellow (2A4) to Greyish Yellow to Golden to Olive Brown (4C5–6–D5); soluble pigments absent; exudates absent. CREA colonies moderate growth, acid production absent. A sexual state was not observed on any media after several months of incubation.

Conidiophores up to 100 µm tall, smooth, regular and irregular branching, consist of dense whorls of verticillate branches, each with 2 to 5 phialides; *branches* 7.5–14(–18) × 2.5–4 µm; *phialides* cylindrical to ellipsoidal base, tapering to a long cylindrical neck, 10–20 × 2.5–3.5 µm; *conidia* smooth, produced in long chains, ellipsoidal with truncate ends, sometimes only ellipsoidal, 4–6(–8) × 1.5–2.5(–3.5) µm (mean = 5.14 ± 0.87 × 2.09 ± 0.3), average width/length = 0.4, n = 70. *Chlamydospores* borne from stalk up to 40 µm long, single, smooth, globose to subglobose, with truncated end, 6.5–9.5 × 6–9.5 µm. *Sexual state* not observed.

Typus: South Africa, Kwazulu-Natal Province, Pietermaritzburg, from wood utility pole, 25 Mar. 2003, coll. and isol. by *E. de Meyer* [holotype PRU(M) 4585 preserved as dried culture; culture ex-type CMW-IA 005749 = CBS 124309 = CMW 18180 = CN175H6].

Additional strains examined: South Africa, Mpumalanga Province, Groblersdal, from wood utility pole, 5 Mar. 2003, coll. and isol. by *E. de Meyer*, culture CMW-IA 005740 = CMW 18171 = CN175H1; Kwazulu-Natal Province, Pietermaritzburg, from wood utility pole, 25 Mar 2003, coll. and isol. by *E. de Meyer*, cultures CMW-IA 005746 = CBS 124308 = CMW 18177 = CN175H3, CMW-IA 005747 = CMW 18178 = CN175H4, CMW-IA 005748 = CMW 18179 = CN175H5, CMW CMW-IA 005753 = CMW 18184 = CN175I1, CMW-IA 005754 = CMW 18185 = CN175I2, CMW-IA 005755 = CMW 18186 = CN175I3, CMW-IA 005756 = CMW 18187 = CN175I4, CMW-IA 005758 = CMW 18190 = CN175I7; Eastern Cape Province, Plettenberg Bay, from wood utility pole, 10 Jun. 2003, coll. and isol. by *E. de Meyer*, culture CMW-IA 005745 = CBS 124307 = CBS 118902 = CMW 18176 = CN175H2; Western Cape Province, Stellenbosch, from wood utility pole, 16 Oct. 2003, coll. and isol. by *E. de Meyer*, cultures CMW-IA 005750 = CBS 124310 = CMW 18181 = CN175H7, CMW-IA 005751 = CMW 18182 = CN175H8.

Notes: *Paecilomyces lignorum* is closely related to *P. brunneolus*, *P. paravariotii* and *P. variotii* (Figs 1, 2). The ellipsoidal conidia of the new species are longer than those of *P. brunneolus* [4–6(–8) vs (3.5–)4–5(–5.5) µm], while its colonies on MEA incubated at 30 °C grow more restricted (20–35 vs 35–45 mm) (Samson *et al.* 2009). These characters also distinguish *P. lignorum* from *P. paravariotii* (3.1–5.0 µm; ~45 mm based on CMW-IA 005757) (Urquhart *et al.* 2023) and *P. variotii* (3–6 µm; 60–90 mm) (Samson *et al.* 2019).

List of accepted *Paecilomyces* species

Paecilomyces brunneolus (N. Inagaki) Samson & Houbraken, *Persoonia* **22**: 21. 2009. [MB#512559] ≡ *Paecilomyces variotii* var. *brunneolus* N. Inagaki, *Trans. Mycol. Soc. Japan* **4**: 3. 1962. [MB#512559]. — Type: NHL (N. Inagaki, I-72). Ex-type: CBS 370.70 = DTO 093-D7 = IFO 7563. Reproduction: asexual. DNA barcodes: ITS = EU037050; *BenA* = EU037068; *CaM* = EU037033; *RPB2* = MN969152. Reference genome: SAMN40250940.

Paecilomyces clematidis Spetik *et al.*, *Phytotaxa* **559**: 242. 2022. [MB#843540]. — Type: BRNU 677844. Ex-type: CBS 148466 = MEND-F-0560. Reproduction: asexual. DNA barcodes: ITS = MZ923760; *BenA* = MZ927740; *CaM* = MZ927738; *RPB2* = OL332317. Reference genome: Not available.

Paecilomyces dactylethromorphus Bat. & H. Maia, *An. Soc. Biol. Pernambuco* **15**: 152. 1957. [MB#302183]. — Type: IMUR 235. Ex-type: CBS 251.55 = DTO 280-D1 = ATCC11971 = IMI

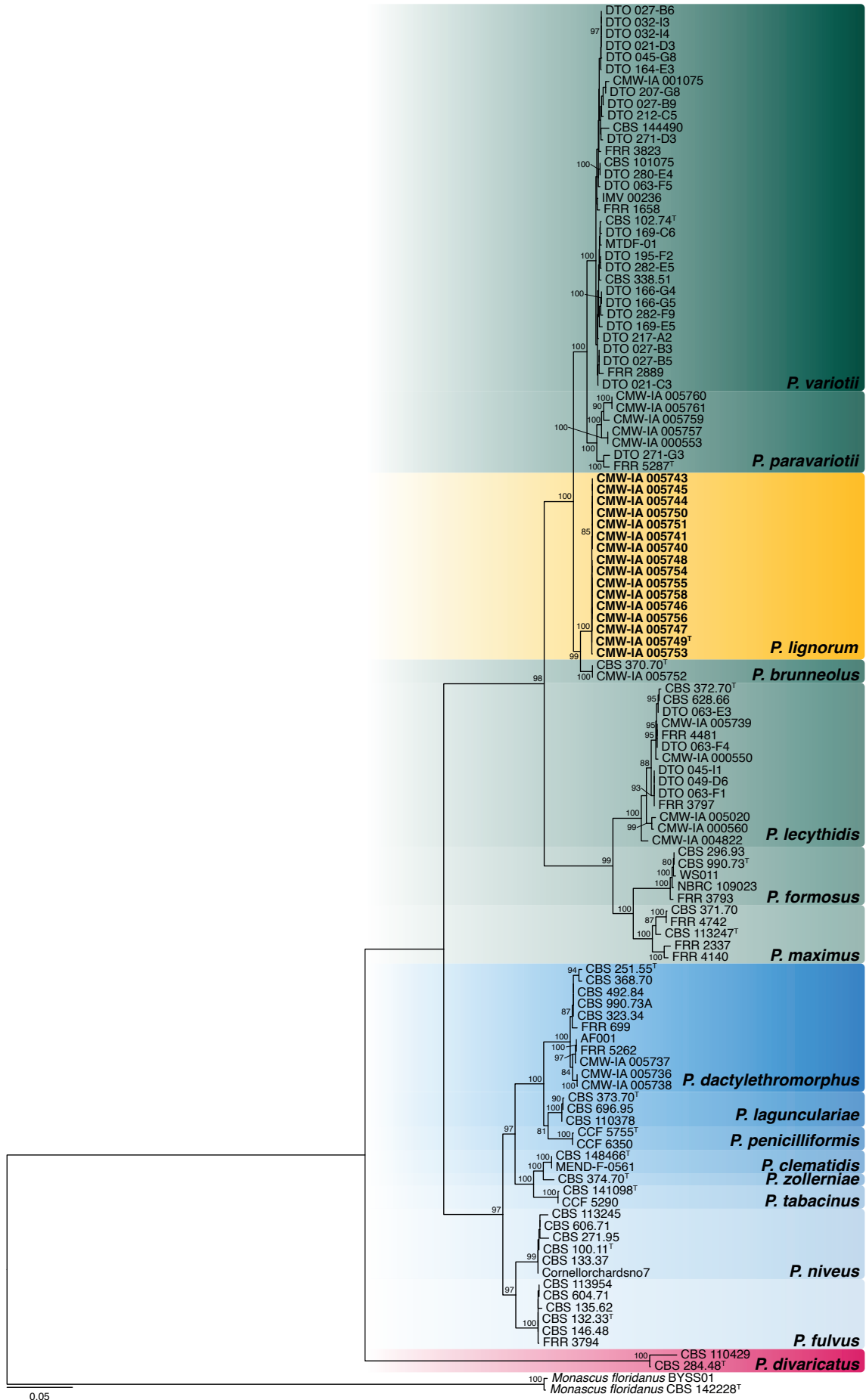


Fig. 1. Phylogenetic tree of *Paecilomyces* based on a concatenated dataset of *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1*. Strains of the new species is shown in bold text. Bootstrap support in nodes higher than 80 % are indicated at relevant branches (T = ex-type). The tree was rooted to *Monascus floricidus*.

65752 = MUCL 9649. Reproduction: asexual. DNA barcodes: ITS = FJ389951; *BenA* = FJ390002; *CaM* = FJ389960; *RPB2* = n.a. Reference genomes: JAPVCE01, JAPVCF01, PNEM01, SAMN40250926, SAMN40250927, SAMN40250928.

Paecilomyces divaricatus (Thom) Samson, Houbraken & Frisvad, *Persoonia* **22**: 21. 2009. [MB#512561]. ≡ *Penicillium divaricatum* Thom, *U.S.D.A. Bull. Bur. Animal Industr.* **118**: 72. 1910. [MB#512561]. — Type: CBS 284.48. Ex-type: CBS 284.48 = DTO 093-D8 = ATCC 10121 = ATCC 18502 = DSM 1961 = IAM5001 = IMI 40025 = NBRC 100534 = NRRL 1115 = QM6764 = VTT D-83214 = Thom 34. Reproduction: asexual. DNA barcodes: ITS = FJ389931; *BenA* = FJ389992; *CaM* = FJ389953; *RPB2* = n.a. Reference genome: Not available.

Paecilomyces formosus Urquhart, *J. Fungi* **9** (3, no. 285): 9. 2009. [MB#846977]. ≡ *Monilia formosa* Sakag., *et al.*, *Zentralbl. Bakteriell.*, **2**. Abt. 100: 302. 1939. [MB#252219]. (*nom. inval.*) = *Paecilomyces formosus* Sakag. *et al.* ex Houbraken & Samson, *Persoonia* **22**: 21. 2009. (*nom. inval.*) [MB#846977]. — Type: CBS 990.73B. Ex-type: CBS 990.73B = DTO 093-D2 = ATCC 10865 = IMI 58427 = LSHB Pa31 = LSHB X26 = NRRL 1282. Reproduction: protoheterothallic. DNA barcodes: ITS = FJ389929; *BenA* = FJ389993; *CaM* = FJ389978; *RPB2* = MN969154. Reference genomes: BAUL01, JACXGS01, JAPVCB01.

Paecilomyces fulvus Stolk & Samson, *Persoonia* **6**: 354. 1971. [MB#319107]. — Type: CBS 132.33. Ex-type: CBS 132.33 = IMI 058421. Reproduction: homothallic. DNA barcodes: ITS = FJ389939; *BenA* = FJ389988; *CaM* = FJ389957; *RPB2* = n.a. Reference genome: JAPVCG01.

Paecilomyces lagunculariae (C. Ram) Houbraken *et al.*, *Stud. Mycol.* **95**: 90. 2020. [MB#832559]. ≡ *Byssochlamys nivea* var. *lagunculariae* C. Ram, *Nova Hedwig.* **16**(1–2): 311. 1968. [MB#832559]. — Type: IMUFPe 2195. Ex-type: CBS 373.70. Reproduction: homothallic. DNA barcodes: ITS = FJ389944; *BenA* = FJ389995; *CaM* = FJ389965; *RPB2* = MN969204. Reference genome: Not available.

Paecilomyces lecythidis C. Ram., *Nova Hedwig.* **16**: 307. 1968. [MB#335530]. — Type: IMUFPe 2191. Ex-type: CBS 372.70 = IMUR 2191. Reproduction: protoheterothallic. DNA barcodes: ITS = FJ389926; *BenA* = FJ389990; *CaM* = FJ389964; *RPB2* = n.a. Reference genomes: JAPVCC01, JAPVCD01, SAMN36999128, SAMN40250922, SAMN40250924, SAMN40250925, SAMN36999129.

Paecilomyces lignorum Visagie, Cruywagen & Duong, published here [MB#335531]. — Type: PRU(M) 4585. Ex-type: CMW-IA 005749 = CBS 124309 = CMW 18180 = CN175H6. Reproduction: asexual. DNA barcodes: ITS = PP191153; *BenA* = PP197741; *CaM* = PP197772; *RPB2* = PP197803. Reference genomes: SAMN40250929, SAMN40250930, SAMN40250931, SAMN40250932, SAMN40250933, SAMN40250934, SAMN40250935, SAMN40250936, SAMN40250937, SAMN40250938, SAMN40250939, SAMN40250941, SAMN40250942, SAMN40250943, SAMN40250944, SAMN40250946.

Paecilomyces maximus C. Ram., *Nova Hedwig.* **16**: 306 (1968) [MB#335531]. — Type: IMUFPe No. 2192. Ex-type: CBS 113247 = IMUR 2192. Reproduction: protoheterothallic. DNA barcodes: ITS = FJ389921; *BenA* = FJ390009; *CaM* = FJ389980; *RPB2* = n.a. Reference genomes: JAPVBY01, JAPVBZ01, JAPVCA01.

Paecilomyces niveus Stolk & Samson, *Persoonia* **6**: 351. 1971. [MB#319117]. — Type: CBS 100.11. Ex-type: CBS 100.11 = ATCC 22260. Reproduction: homothallic. DNA barcodes: ITS = FJ389934; *BenA* = FJ389999; *CaM* = FJ389956; *RPB2* = JF417414. Reference genome: QEIL01.

Paecilomyces paravariotii Urquhart, *J. Fungi* **9** (3, no. 285): 9 (2023) [MB#846976]. — Type: MELUF 155137a (holotype). Ex-type: FRR 5287. Reproduction: asexual. DNA barcodes: ITS = JANCMN01; *BenA* = JANCMN01; *CaM* = JANCMN01; *RPB2* = JANCMN01. Reference genomes: JANCMN01, JAROOX01, SAMN40250921, SAMN40250945, SAMN40250947, SAMN40250948, SAMN40250949.

Paecilomyces penicilliformis Jurjević & Hubka, *Persoonia* **44**: 431 (2020) [MB#834874]. — Type: BPI 911216 (holotype). Ex-type: CCF 5755 = CBS 146003 = EMSL 3392. Reproduction: asexual. DNA barcodes: ITS = LR679769; *BenA* = LR679768; *CaM* = LR778299; *RPB2* = n.a. Reference genome: Not available.

Paecilomyces tabacinus Jurjević *et al.*, *Persoonia* **36**: 409. 2016. [MB#816870]. — Type: BPI 910044. Ex-type: CBS 141098 = DTO 412-B7 = CCF 5290. Reproduction: asexual. DNA barcodes: ITS = LT548280; *BenA* = MN969434; *CaM* = LT548288; *RPB2* = MN969210. Reference genome: Not available.

Paecilomyces variotii Bainier, *Bull. Soc. Mycol. France* **23**: 27. 1907. [MB#248517]. — Type: Unknown. Ex-type: CBS 102.74 = DTO 032-I8 = DTO 280-D5 = CECT 2803 = NRRL 1116. Reproduction: heterothallic. DNA barcodes: ITS = EU037055; *BenA* = EU037073; *CaM* = EU037038; *RPB2* = MN969153. Reference genomes: MSJH02, JANCMO01, JANCMP01, JANCMQ01, JAROOK01, JAROOL01, JAROOM01, JAROON01, JAROOO01, JAROOP01, JAROOQ01, JAROOR01, JAROOS01, JAROOT01, JAROOU01, JAROOV01, JAROOW01, JAROOY01, JAROOZ01, JAROPA01, JAROPB01, JAROPC01, JAROPD01, JAROPE01, JAROPF01, JAROPG01, JAROPH01, RCHW01, RCNU01, RHLL01, SAMN40250923.

Note: Samson (1974) mentioned that CBS 102.74 was probably the ex-type culture, sent to Charles Thom in 1922 as strain no. 436 from France by O. da Fonseca (D. I. Fennell, pers. comm.). We were unable to confirm the existence of holotype material.

Paecilomyces zollerniae Stolk & Samson, *Persoonia* **6**: 356 (1971) [MB#319129]. — Type: CBS 374.70. Ex-type: CBS 374.70 = JCM 12808. Reproduction: homothallic. DNA barcodes: ITS = FJ389933; *BenA* = FJ390008; *CaM* = FJ389966; *RPB2* = n.a. Reference genome: Not available.

DISCUSSION

This study reports five *Paecilomyces* species isolated from wooden utility poles in South Africa, including *P. brunneolus*, *P. dactylethromorphus*, *P. lecythidis*, *P. paravariotii* and a new species *P. lignorum*. This finding is supported by morphological observations, phylogenetic (based on ITS, *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8* and *Tsr1*) and phylogenomic analyses (comparing a set of 77 genomes of which 31 were generated in this study).

Paecilomyces species have been isolated from wooden utility poles in various parts of the world (Zabel *et al.* 1985, Lopez *et al.* 1990, Wang & Worrall 1992, Laks 1994, Wong & Pearce 1997, Anagnost *et al.* 2006). Most of these reports listed only *P. variotii*, but their identifications were based only on morphological observations. The fact that we discovered five different species of *Paecilomyces* and none of which represent *P. variotii*, suggests that some of the previous reports of this fungus from wooden poles could represent species other than *P. variotii*. Our results suggest that many *Paecilomyces* species are present on wooden poles and that many new fungal taxa from this niche await discovery. Among the novel species obtained during the survey were three *Sporothrix* species (de Meyer *et al.* 2008), a *Phaeoconiella* species (de Meyer 2007), as well as the

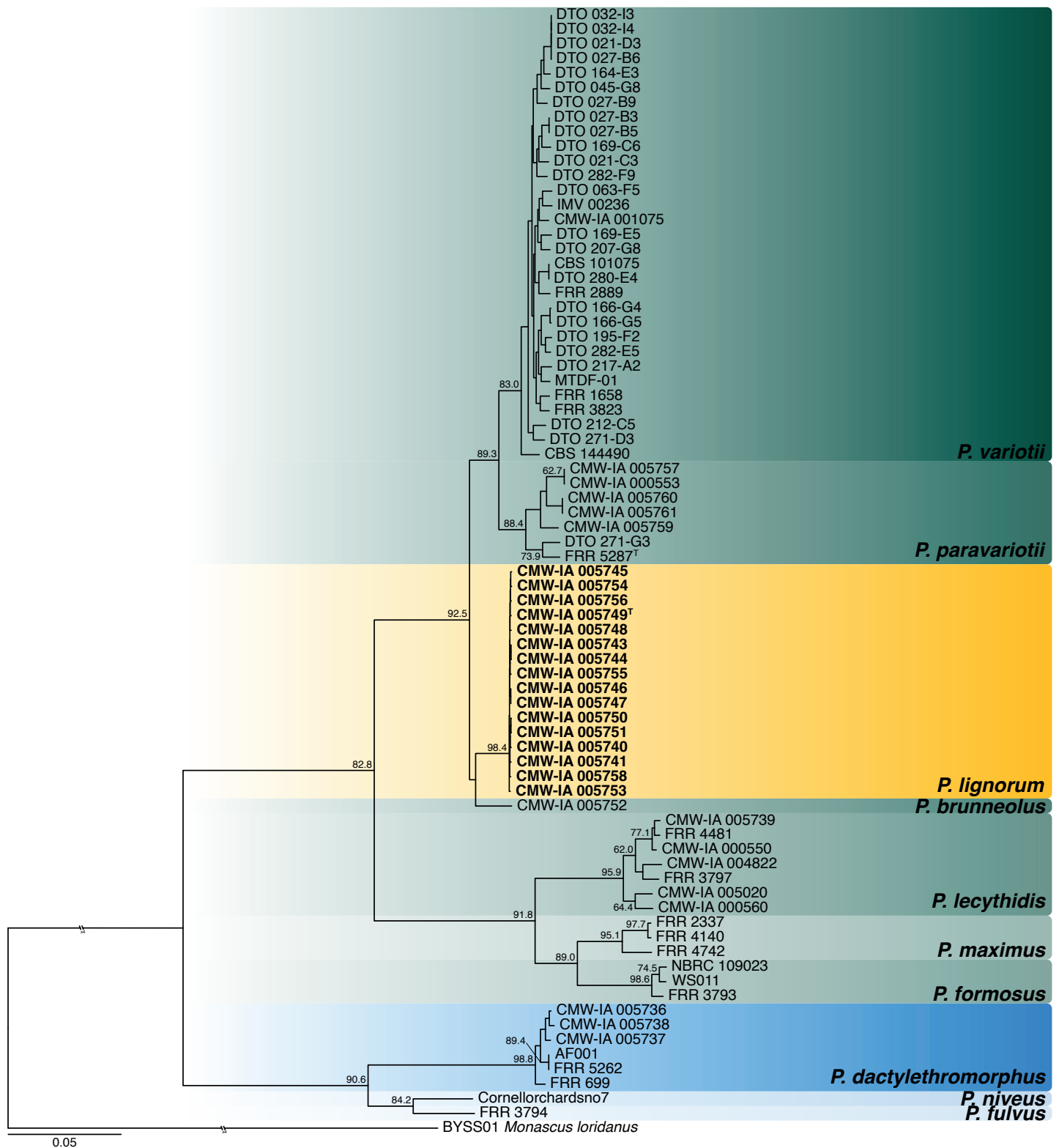


Fig. 2. Phylogenomic tree of *Paecilomyces* based on 3 132 single copy genes present in 77 genomes. Numbers above branches represents the gene concordance factors (gcf). Values for minor terminal clades were not indicated. The tree was rooted to *Monascus floridanus*.

Paecilomyces species described above.

The most common species found during our survey of wooden utility poles was *P. lecythidis*. This species was originally described from a wood sample of *Lecythis unsitatae* in Brazil where it caused brown discoloration to piles of wood (Ram 1968). Our survey isolated the species from the treated outer regions through to the untreated inner core of creosote treated pine and eucalypt poles from five different sites in four provinces (de Meyer 2007). It is clearly a widely distributed fungus that is common on this niche, irrespective of substrate and climatic

differences.

The three dominant *Paecilomyces* species, including *P. lecythidis*, *P. lignorum* and *P. dactylethromorphus*, obtained from utility poles during the survey were tested for their ability to cause soft rot, but only caused limited weight loss of the wood blocks (de Meyer 2007). Other researchers have, however, found that *P. variotii* can cause soft rot in wood (Leightley 1978). The latter species was one of the dominant fungi found on wooden utility poles in Australia and was able to grow on medium containing chromated copper arsenate, copper sulphate and creosote. It is

Table 2. Strains used for phylogenomic analysis of *Paecilomyces*.

Current name	Submitted genome name	Strain	Genome assembly date	WGS accession	BUSCO completeness (4 191 BUSCOs) ¹
<i>Monascus floridanus</i>	<i>Byssochlamys</i> sp. BYSS01	BYSS01	2017-08-07	NIXA01	C:90.6 % [S:90.2 %, D:0.4 %], F:0.9 %, M:8.5 %
<i>P. brunneolus</i>	<i>P. brunneolus</i>	CMW-IA 005752	2023-02-01	SAMN40250940	C:93.5 % [S:93.3 %, D:0.2 %], F:0.8 %, M:5.7 %
<i>P. dactylethromorphus</i>	<i>Byssochlamys</i> sp. AF001	AF001	2018-02-02	PNEM01	C:93.9 % [S:93.4 %, D:0.5 %], F:0.6 %, M:5.5 %
	<i>P. dactylethromorphus</i>	CMW-IA 005736	2023-02-01	SAMN40250926	C:93.8 % [S:93.3 %, D:0.5 %], F:0.8 %, M:5.4 %
	<i>P. dactylethromorphus</i>	CMW-IA 005737	2023-02-01	SAMN40250927	C:93.7 % [S:93.2 %, D:0.5 %], F:0.7 %, M:5.6 %
	<i>P. dactylethromorphus</i>	CMW-IA 005738	2023-02-01	SAMN40250928	C:94.0 % [S:93.5 %, D:0.5 %], F:0.7 %, M:5.3 %
	<i>P. dactylethromorphus</i>	FRR 5262	2023-04-06	JAPVCE01	C:93.9 % [S:93.4 %, D:0.5 %], F:0.6 %, M:5.5 %
	<i>P. dactylethromorphus</i>	FRR 699	2023-04-06	JAPVCF01	C:94.1 % [S:93.6 %, D:0.5 %], F:0.6 %, M:5.3 %
<i>P. formosus</i>	<i>P. maximus</i>	FRR 3793	2023-04-06	JAPVCB01	C:94.5 % [S:94.2 %, D:0.3 %], F:0.5 %, M:5.0 %
	<i>P. variotii</i>	NBRC109023	2013-11-12	BAUL01	C:94.3 % [S:94.0 %, D:0.3 %], F:0.9 %, M:4.8 %
	<i>P. variotii</i>	WS011	2020-11-06	JACXGS01	C:94.7 % [S:94.4 %, D:0.3 %], F:0.5 %, M:4.8 %
<i>P. fulvus</i>	<i>P. fulvus</i>	FRR 3794	2023-04-06	JAPVCG01	C:93.6 % [S:93.4 %, D:0.2 %], F:0.6 %, M:5.8 %
	<i>P. lecythidis</i>	CMW-IA 000550	2023-02-01	SAMN36999128	C:94.5 % [S:94.1 %, D:0.4 %], F:0.5 %, M:5.0 %
	<i>P. lecythidis</i>	CMW-IA 000560	2023-02-01	SAMN40250922	C:94.4 % [S:94.0 %, D:0.4 %], F:0.6 %, M:5.0 %
	<i>P. lecythidis</i>	CMW-IA 004822	2023-02-01	SAMN40250924	C:94.5 % [S:94.0 %, D:0.5 %], F:0.7 %, M:4.8 %
	<i>P. lecythidis</i>	CMW-IA 005020	2023-02-01	SAMN40250925	C:94.1 % [S:93.8 %, D:0.3 %], F:0.8 %, M:5.1 %
	<i>P. lecythidis</i>	CMW-IA 005739	2023-02-01	SAMN36999129	C:94.5 % [S:94.1 %, D:0.4 %], F:0.5 %, M:5.0 %
	<i>P. lecythidis</i>	FRR 3797	2023-04-06	JAPVCD01	C:94.5 % [S:94.1 %, D:0.4 %], F:0.6 %, M:4.9 %
	<i>P. lecythidis</i>	FRR 4481	2023-04-06	JAPVCC01	C:94.5 % [S:94.0 %, D:0.5 %], F:0.5 %, M:5.0 %
<i>P. lignorum</i>	<i>P. lignorum</i>	CMW-IA 005740	2023-02-01	SAMN40250929	C:94.0 % [S:93.8 %, D:0.2 %], F:0.5 %, M:5.5 %
	<i>P. lignorum</i>	CMW-IA 005741	2023-02-01	SAMN40250930	C:94.2 % [S:94.0 %, D:0.2 %], F:0.5 %, M:5.3 %
	<i>P. lignorum</i>	CMW-IA 005743	2023-02-01	SAMN40250931	C:94.1 % [S:93.9 %, D:0.2 %], F:0.4 %, M:5.5 %
	<i>P. lignorum</i>	CMW-IA 005744	2023-02-01	SAMN40250932	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005745	2023-02-01	SAMN40250933	C:94.3 % [S:94.0 %, D:0.3 %], F:0.5 %, M:5.2 %
	<i>P. lignorum</i>	CMW-IA 005746	2023-02-01	SAMN40250934	C:94.0 % [S:93.8 %, D:0.2 %], F:0.5 %, M:5.5 %
	<i>P. lignorum</i>	CMW-IA 005747	2023-02-01	SAMN40250935	C:93.9 % [S:93.7 %, D:0.2 %], F:0.5 %, M:5.6 %
	<i>P. lignorum</i>	CMW-IA 005748	2023-02-01	SAMN40250936	C:94.3 % [S:94.1 %, D:0.2 %], F:0.5 %, M:5.2 %
	<i>P. lignorum</i>	CMW-IA 005749	2023-02-01	SAMN40250937	C:94.1 % [S:92.8 %, D:1.3 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005750	2023-02-01	SAMN40250938	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005751	2023-02-01	SAMN40250939	C:94.2 % [S:94.0 %, D:0.2 %], F:0.5 %, M:5.3 %
	<i>P. lignorum</i>	CMW-IA 005753	2023-02-01	SAMN40250941	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005754	2023-02-01	SAMN40250942	C:94.2 % [S:94.0 %, D:0.2 %], F:0.5 %, M:5.3 %

Table 2. (Continued).

Current name	Submitted genome name	Strain	Genome assembly date	WGS accession	BUSCO completeness (4 191 BUSCOs) ¹
<i>P. maximus</i>	<i>P. lignorum</i>	CMW-IA 005755	2023-02-01	SAMN40250943	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005756	2023-02-01	SAMN40250944	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. lignorum</i>	CMW-IA 005758	2023-02-01	SAMN40250946	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
<i>P. niveus</i>	<i>P. maximus</i>	FRR 2337	2023-04-06	JAPVBZ01	C:94.2 % [S:93.9 %, D:0.3 %], F:0.6 %, M:5.2 %
	<i>P. maximus</i>	FRR 4140	2023-04-06	JAPVBY01	C:94.2 % [S:93.9 %, D:0.3 %], F:0.6 %, M:5.2 %
	<i>P. maximus</i>	FRR 4742	2023-04-06	JAPVCA01	C:94.3 % [S:94.0 %, D:0.3 %], F:0.5 %, M:5.2 %
<i>P. paravariotii</i>	<i>P. niveus</i>	Cornellorchardsno7	2018-05-11	QEIL01	C:94.1 % [S:93.8 %, D:0.3 %], F:0.5 %, M:5.4 %
	<i>P. paravariotii</i>	CMW-IA 000553	2023-02-01	SAMN40250921	C:94.2 % [S:93.8 %, D:0.4 %], F:0.5 %, M:5.3 %
	<i>P. paravariotii</i>	CMW-IA 005757	2023-02-01	SAMN40250945	C:93.9 % [S:93.7 %, D:0.2 %], F:0.5 %, M:5.6 %
	<i>P. paravariotii</i>	CMW-IA 005759	2023-02-01	SAMN40250947	C:94.0 % [S:93.8 %, D:0.2 %], F:0.6 %, M:5.4 %
	<i>P. paravariotii</i>	CMW-IA 005760	2023-02-01	SAMN40250948	C:94.4 % [S:94.2 %, D:0.2 %], F:0.5 %, M:5.1 %
	<i>P. paravariotii</i>	CMW-IA 005761	2023-02-01	SAMN40250949	C:94.4 % [S:94.1 %, D:0.3 %], F:0.5 %, M:5.1 %
	<i>P. variotii</i>	DTO 271-G3	2023-05-22	JAROOX01	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. variotii</i>	FRR 5287	2023-04-06	JANCMN01	C:94.0 % [S:93.7 %, D:0.3 %], F:0.7 %, M:5.3 %
	<i>P. variotii</i>	CBS 101075	2019-01-14	RCNU01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.5 %, M:5.6 %
	<i>P. variotii</i>	CBS 144490	2019-01-14	RHLL01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
<i>P. variotii</i>	<i>P. variotii</i>	CMW-IA 001075	2023-02-01	SAMN40250923	C:94.2 % [S:93.7 %, D:0.5 %], F:0.7 %, M:5.1 %
	<i>P. variotii</i>	DTO 021-C3	2023-05-22	JAROOU01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.6 %, M:5.4 %
	<i>P. variotii</i>	DTO 021-D3	2023-05-22	JAROOV01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
	<i>P. variotii</i>	DTO 027-B3	2023-05-22	JAROOY01	C:94.1 % [S:93.9 %, D:0.2 %], F:0.7 %, M:5.2 %
	<i>P. variotii</i>	DTO 027-B5	2023-05-22	JAROOZ01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.6 %, M:5.5 %
	<i>P. variotii</i>	DTO 027-B6	2023-05-22	JAROPA01	C:93.8 % [S:93.6 %, D:0.2 %], F:0.7 %, M:5.5 %
	<i>P. variotii</i>	DTO 027-B9	2023-05-22	JAROPB01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.7 %, M:5.3 %
	<i>P. variotii</i>	DTO 032-I3	2023-05-22	JAROOK01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
	<i>P. variotii</i>	DTO 032-I4	2023-05-22	JAROPF01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
	<i>P. variotii</i>	DTO 045-G8	2023-05-22	JAROPG01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.8 %, M:5.3 %
	<i>P. variotii</i>	DTO 063-F5	2023-05-22	JAROPH01	C:94.1 % [S:93.9 %, D:0.2 %], F:0.6 %, M:5.3 %
	<i>P. variotii</i>	DTO 164-E3	2023-05-22	JAROOI01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
	<i>P. variotii</i>	DTO 166-G4	2023-05-22	JAROOM01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.6 %, M:5.5 %
	<i>P. variotii</i>	DTO 166-G5	2023-05-22	JAROON01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.7 %, M:5.4 %
	<i>P. variotii</i>	DTO 169-C6	2023-05-22	JAROOO01	C:94.2 % [S:94.0 %, D:0.2 %], F:0.7 %, M:5.1 %
<i>P. variotii</i>	DTO 169-E5	2023-05-22	JAROOP01	C:94.2 % [S:94.0 %, D:0.2 %], F:0.7 %, M:5.1 %	

Table 2. (Continued).

Current name	Submitted genome name	Strain	Genome assembly date	WGS accession	BUSCO completeness (4 191 BUSCOs) ¹
	<i>P. variotii</i>	DTO 195-F2	2023-05-22	JAROOQ01	C:93.9 % [S:93.7 %, D:0.2 %], F:0.8 %, M:5.3 %
	<i>P. variotii</i>	DTO 207-G8	2023-05-22	JAROR01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.6 %, M:5.4 %
	<i>P. variotii</i>	DTO 212-C5	2023-05-22	JAROS01	C:94.2 % [S:94.0 %, D:0.2 %], F:0.7 %, M:5.1 %
	<i>P. variotii</i>	DTO 217-A2	2023-05-22	JAROOT01	C:94.2 % [S:93.9 %, D:0.3 %], F:0.7 %, M:5.1 %
	<i>P. variotii</i>	DTO 271-D3	2023-05-22	JAROW01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.7 %, M:5.3 %
	<i>P. variotii</i>	DTO 280-E4	2023-05-22	JAROPC01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.6 %, M:5.4 %
	<i>P. variotii</i>	DTO 282-E5	2023-05-22	JAROPD01	C:94.2 % [S:94.0 %, D:0.2 %], F:0.6 %, M:5.2 %
	<i>P. variotii</i>	DTO 282-F9	2023-05-22	JAROPE01	C:94.6 % [S:94.4 %, D:0.2 %], F:0.5 %, M:4.9 %
	<i>P. variotii</i>	FRR 1658	2023-04-06	JANCMQ01	C:94.0 % [S:93.8 %, D:0.2 %], F:0.6 %, M:5.4 %
	<i>P. variotii</i>	FRR 2889	2023-04-06	JANCMPO1	C:94.1 % [S:93.9 %, D:0.2 %], F:0.5 %, M:5.4 %
	<i>P. variotii</i>	FRR 3823	2023-04-06	JANCMO01	C:94.3 % [S:94.1 %, D:0.2 %], F:0.6 %, M:5.1 %
	<i>Byssochlamys</i> sp. IMV 00236	IMV 00236	2017-02-15	MSJH02	C:94.3 % [S:93.1 %, D:1.2 %], F:0.7 %, M:5.0 %
	<i>P. variotii</i>	MTDF-01	2019-01-04	RCHW01	C:93.7 % [S:93.5 %, D:0.2 %], F:0.8 %, M:5.5 %

¹C = Complete BUSCOs; S = Complete and single-copy BUSCOs; D = Complete and duplicated BUSCOs; F = Fragmented BUSCOs; M = Missing BUSCOs.

also capable of causing soft rot in *Eucalyptus maculata* and *Pinus eliottii* wood (Leightley 1978). The dominance of *Paecilomyces* species, together with *Hormoconis resinae* on treated wooden utility poles in South Africa supports previous data suggesting that these fungi may be tolerant to certain preservatives (Leightley 1978). They might even assist in the degradation of these chemicals, making the wood more susceptible to other agents of decay. A study showed that a *Paecilomyces* species was able to remove arsenic from aquatic systems (Acosta Rodriguez *et al.* 2013). García-Peña *et al.* (2008) also showed that *P. variotii* is capable of degrading benzene, toluene, ethyl benzene and xylenes. Another wood preservative, 2,4,6-tribromophenol (TBP), is converted to 2,4,6-tribromoanisole (TBA) by *P. variotii* (Whitfield *et al.* 1997). Clearly further research is necessary to determine the exact role of these fungi in the process of wood preservative degradation.

The availability of whole-genome sequence data, together with those newly generated in this study, has allowed us to construct an evolutionary backbone for the genus, providing insight into the evolutionary relationships between species of *Paecilomyces*. Our phylogenomic analysis has also enabled us to reidentify and correct mislabelled genomic sequences deposited in the NCBI genome database. The genome sequences generated for five different species in this study have contributed to the growing genomics resources, which will facilitate future comparative genomics studies aimed at understanding the biology and ecology of species within the genus.

ACKNOWLEDGEMENTS

We dedicate this paper to Prof M.J. Wingfield on the occasion of his 70th birthday. As a former director of FABI, colleague and friend, he continues to play a major role in the lives of the authors of this paper, for which we are grateful. We acknowledge the National Research Foundation (NRF), Tree Protection Co-operative Programme (TPCP), the THRIP initiative of the Department of Trade and Industry (DTI) and the European Union's Horizon 2020 research and innovation program (RISE) under the Marie Skłodowska-Curie grant agreement No. 101008129, project acronym "Mycobiomics", for financial assistance that made this study possible. We are grateful for the advice and support provided during this project by W. de Beer, J. Houbraken and R.A. Samson. We thank S. Marincowitz who revived cultures from the CMW culture collection. We also acknowledge J. Spraker and the team at Hexagon Bio (CA, USA) who sequenced the genomes used in this study. We would like to thank K. Bensch for her assistance with Latin nomenclature.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

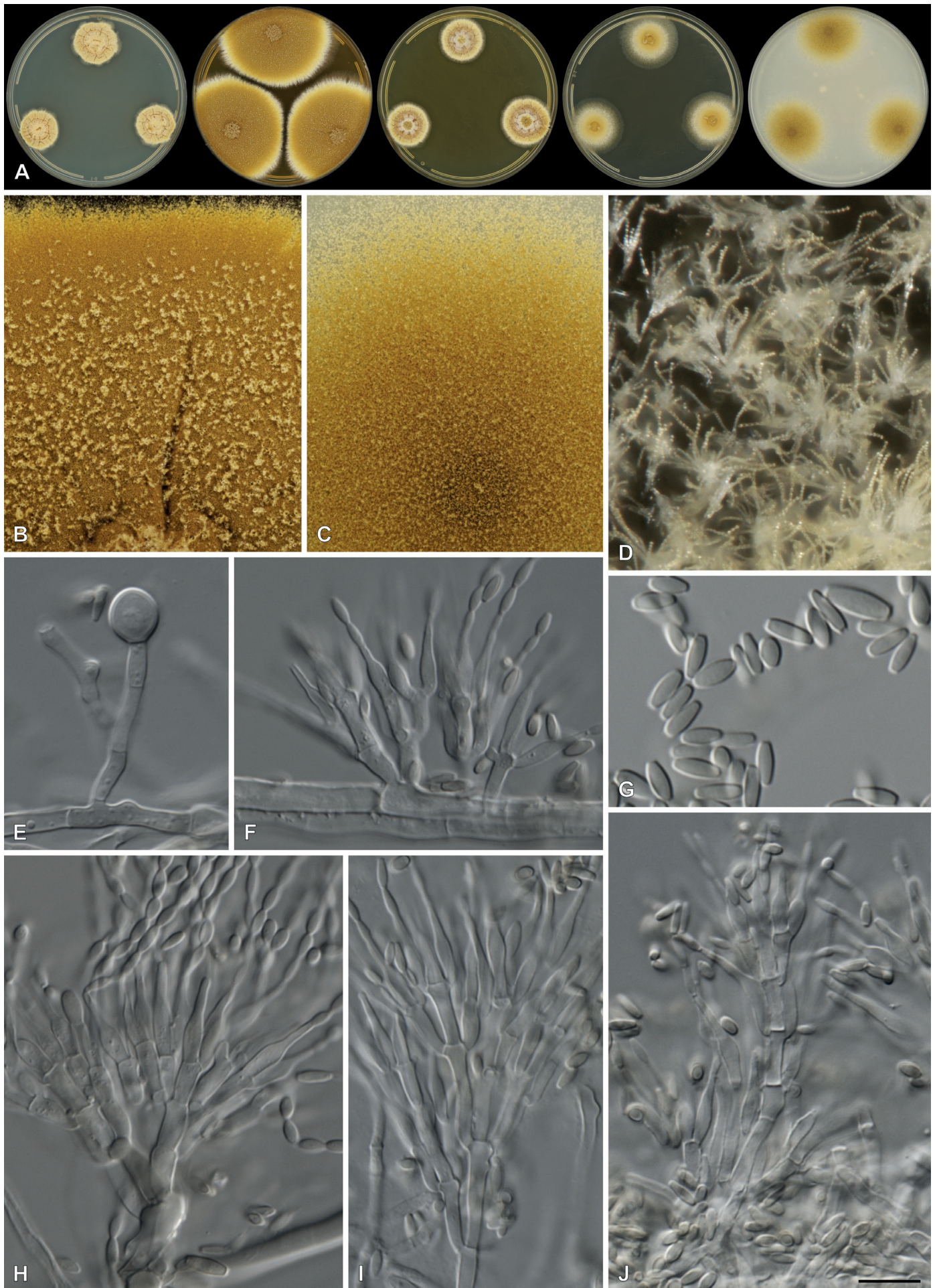


Fig. 3. *Paecilomyces lignorum*. **A.** Colonies from left to right, on CYA, MEA, YES, DG18 and OA. **B, D.** Colony close-up on MEA. **C.** Colony close-up on OA. **E.** Chlamydsopore. **F, H–J.** Conidiophores. **G.** Conidia. Scale bars: J = 10 μm, applies to E–I.

- Abadi S, Azouri D, Pupko T, *et al.* (2019). Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* **10**: 934.
- Acosta Rodriguez I, Martinez-Juarez VM, Cardenas-Gonzalez JF, *et al.* (2013). Biosorption of arsenic(III) from aqueous solutions by modified fungal biomass of *Paecilomyces* sp. *Bioinorganic Chemistry and Applications* **2013**: 376780.
- Anagnost SE, Zhou S, Yeo H, *et al.* (2006). Fungi inhabiting southern pine utility poles during manufacture. *Forest Products Journal* **56**: 53–59.
- Anderson S, Barrantes Murillo DF, Womble M, *et al.* (2022). Case Report: novel disseminated *Paecilomyces formosus* infection in a dog. *Frontiers in Veterinary Science* **9**: 878327.
- Bankevich A, Nurk S, Antipov D, *et al.* (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* **19**: 455–477.
- Bolger AM, Lohse M, Usadel B (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.
- Brown AHS, Smith G (1957). The genus *Paecilomyces* Bainier and its perfect stage *Byssoschlamys* Westling. *Transactions of the British Mycological Society* **40**: 17–89.
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009). trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**: 1972–1973.
- Crous PW, Wingfield MJ, Chooi YH, *et al.* (2020). Fungal Planet description sheets: 1042–1111. *Persoonia* **44**: 301–459.
- de Hoog GS, Gerrits van den Ende AH (1998). Molecular diagnostics of clinical strains of filamentous Basidiomycetes. *Mycoses* **41**: 183–189.
- de Meyer EM (2007). *Fungi from utility poles in South Africa: Taxonomy, phylogeny and effect on wood*. MSc thesis, University of Pretoria, Pretoria, South Africa.
- de Meyer EM, de Beer ZW, Summerbell RC, *et al.* (2008). Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* **100**: 647–661.
- García-Peña I, Ortiz I, Hernández S, *et al.* (2008). Biofiltration of BTEX by the fungus *Paecilomyces variotii*. *International Biodeterioration & Biodegradation* **62**: 442–447.
- Glass NL, Donaldson GC (1995). Development of premier sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. *Applied and Environmental Microbiology* **61**: 1323–1330.
- Heidarian R, Fotouhifar KB, Debets AJM, *et al.* (2018). Phylogeny of *Paecilomyces*, the causal agent of pistachio and some other trees dieback disease in Iran. *PLoS ONE* **13**: e0200794.
- Heshmatnia J, Marjani M, Mahdavian SA, *et al.* (2017). *Paecilomyces formosus* infection in an adult patient with undiagnosed chronic granulomatous disease. *Journal of Clinical Immunology* **37**: 342–346.
- Hoang DT, Chernomor O, von Haeseler A, *et al.* (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* **35**: 518–522.
- Houbraken J, Samson RA (2011). Phylogeny of *Penicillium* and the segregation of *Trichocomaceae* into three families. *Studies in Mycology* **70**: 1–51.
- Houbraken J, Kocsube S, Visagie CM, *et al.* (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.
- Kalyanamoorthy S, Minh BQ, Wong TKF, *et al.* (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.
- 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*. 2nd edn. Methuen & Co Ltd, London, England, UK.
- Kück P, Longo GC (2014). FASconCAT-G: extensive functions for multiple sequence alignment preparations concerning phylogenetic studies. *Frontiers in Zoology* **11**: 81.
- Laks P (1994). *Field evaluation of ammoniacal copper fatty acids as a utility pole preservative*. Electric Power Research Institute, Palo Alto, California, USA.
- Leightley L (1978). *Soft rot fungi found in copper/chrome/arsenic treated hardwood power transmission poles in Queensland*. The International Research Group on Wood Protection, document No. IRG/WP/185.
- Lopez S, Bertoni M, Cabral D (1990). Fungal decay in creosote-treated *Eucalyptus* power transmission poles I. Survey of the flora. *Material und Organismen* **25**: 287–293.
- Luangsa-Ard J, Houbraken J, van Doorn T, *et al.* (2011). *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. *FEMS Microbiology Letters* **321**: 141–149.
- Luangsa-ard JJ, Hywel-Jones NL, Manoch L, *et al.* (2005). On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycological Research* **109**: 581–589.
- Luangsa-Ard JJ, Hywel-Jones NL, Samson RA (2004). The polyphyletic nature of *Paecilomyces sensu lato* based on 18S-generated rDNA phylogeny. *Mycologia* **96**: 773–780.
- Manni M, Berkeley MR, Seppey M, *et al.* (2021). BUSCO: Assessing genomic data quality and beyond. *Current Protocols* **1**: e323.
- Masclaux F, Guého E, de Hoog GS, *et al.* (1995). Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. *Journal of Medical and Veterinary Mycology* **33**: 327–338.
- O'Donnell K, Cigelnik E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution* **7**: 103–116.
- Perdomo H, Garcia D, Gene J, *et al.* (2013). *Phialemoniopsis*, a new genus of *Sordariomycetes*, and new species of *Phialemonium* and *Lecythophora*. *Mycologia* **105**: 398–421.
- Pitt JI, Hocking AD (2009). *Fungi and Food Spoilage*. 3rd edition. Springer Dordrecht Heidelberg London New York Cambridge.
- Ram C (1968). Timber-attacking fungi from the state of Maranhao, Brazil. Some new species of *Paecilomyces* and its perfect state *Byssoschlamys* Westl. VIII. *Nova Hedwigia* **16**: 305–314.
- Reblova M, Miller AN, Rossman AY, *et al.* (2016). Recommendations for competing sexual-asexually typified generic names in *Sordariomycetes* (except *Diaporthales*, *Hypocreales*, and *Magnaporthales*). *IMA Fungus* **7**: 131–153.
- Rostami T, Jamali S (2023). Characterization, pathogenicity and host range studies of *Paecilomyces formosus* associated with dieback of Christ's thorn trees (*Paliurus spina-christi* Mill.) in Iran. *Forest Pathology* **53**: e12790.
- Sabernasab M, Jamali S, Marefat A, *et al.* (2019). Molecular and pathogenic characteristics of *Paecilomyces formosus*, a new causal agent of Oak tree dieback in Iran. *Forest Science* **65**: 743–750.
- Samson RA (1974). *Paecilomyces* and some allied Hyphomycetes. *Studies in Mycology* **6**: 1–76.
- Samson RA, Houbraken J, Thrane U, *et al.* (2019). *Food and indoor fungi*.

- 2nd edition. CBS Laboratory manual, the Netherlands.
- Samson RA, Houbraken J, Varga J, *et al.* (2009). Polyphasic taxonomy of the heat resistant ascomycete genus *Byssochlamys* and its *Paecilomyces* anamorphs. *Persoonia* **22**: 14-27.
- Samson RA, Visagie CM, Houbraken J, *et al.* (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology* **78**: 141–173.
- Spetik M, Berraf-Tebbal A, Gramaje D, *et al.* (2022). *Paecilomyces clematidis* (Eurotiales, Thermoascaceae): a new species from *Clematis* root. *Phytotaxa* **559**: 238–246.
- Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Stolk A, Samson RA (1971). Studies on *Talaromyces* and related genera I. *Hamigera* gen. nov. and *Byssochlamys*. *Persoonia* **6**: 341–357.
- Urquhart AS, Idnurm A (2023). A polyphasic approach including whole genome sequencing reveals *Paecilomyces paravariotii* sp. nov. as a cryptic sister species to *P. variotii*. *Journal of Fungi* **9**: 285.
- Urquhart AS, Vogan AA, Gardiner DM, *et al.* (2023). Starships are active eukaryotic transposable elements mobilized by a new family of tyrosine recombinases. *Proceedings of the National Academy of Sciences USA* **120**: e2214521120.
- Wang CJK, Worrall JJ (1992). *Soft rot decay capabilities and interactions of fungi and bacteria from fumigated utility poles*. Electric Power Research Institute, Palo Alto, California, USA.
- Whitfield F, Hill J, Shaw K (1997). 2, 4, 6-Tribromoanisole: a potential cause of mustiness in packaged food. *Journal of Agricultural and Food Chemistry* **45**: 889–893.
- Wong AHH, Pearce RB (1997). Soft rot decay and variable preservative retentions in copper-chrome-arsenic treated Malaysian hardwood utility poles. *Journal of Tropical Forest Products* **2**: 216–226.
- Zabel RA, Lombard FF, Wang CJK, *et al.* (1985). Fungi associated with decay in treated southern pine utility poles in the eastern United States. *Wood and Fiber Science* **17**: 75–91.

Supplementary Material: <http://fuse-journal.org/>

Fig. S1. Phylogenetic trees of *Paecilomyces* based on (from left to right) *BenA*, *CaM*, *RPB2*, *RPB1*, *Cct8*, *Tsr1* and ITS. Strains of the new species is shown in bold text. Bootstrap support in nodes higher than 80 % are indicated at relevant branches († = ex-type). The trees were rooted to *Monascus floricidus*.