A taxonomic review of *Penicillium* species producing conidiophores with solitary phialides, classified in section *Torulomyces*

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**Key words**

beta-tubulin  
calmodulin  
*Eupenicillium*  
internal transcribed spacer rDNA region  
low temperature Scanning Electron Microscopy (cryo-SEM)  
*Monocillium*  
RNA polymerase II second largest subunit  
*Trichocomaceae*  
Wollemi pine

**Abstract**  
The genus *Torulomyces* was characterised by species that typically have conidiophores consisting of solitary phialides that produce long chains of conidia connected by disjunctors. Based on the phylogenetic position of *P. lagena* (generic ex-neotype), the genus and its seven species were transferred to *Penicillium* and classified in sect. *Torulomyces* along with *P. cryptum* and *P. fassennii*. The aim of this study was to review the species currently classified in sect. *Torulomyces* using morphology and phylogenies of the ITS, *BenA*, *CaM* and *RPB2* regions. Based on our results, we accept 16 species in sect. *Torulomyces*, including 12 new species described as *P. aenio*, *P. austrochilicum*, *P. cantabricum*, *P. catalonicum*, *P. oregonense*, *P. mariae-christenseniae*, *P. riverlandense*, *P. tubakianum*, *P. variatorum*, *P. williamettense*, *P. wisconsinensis* and *P. wollemicola*. In addition, we reclassify *P. laeve* and *P. ovatum* in sect. *Elixicaulis* and correct the typification of *P. lagena*. We provide descriptions and notes on the identification of the species.

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**INTRODUCTION**

Delitsch (1943) introduced the hyphomycete genus *Torulomyces* with *T. lagena* as generic type. The genus included species producing conidiophores consisting of solitary, swollen phialides borne on short, unbranched stipes (Delitsch 1943, Barron 1967, Gams 1971, Stolk & Samson 1983, Ando et al. 1998, Seifert et al. 2011). Conidia are linked together by connectives, i.e. short intercalary cylinders of cell wall material, to form very long chains (Gams 1971, Stolk & Samson 1983, Ando et al. 1998). Colonies of *T. lagena* and related species are brownish and their slow growth on agar media means that they are overgrown easily by other fungi and are infrequently isolated.

Stolk & Samson (1983) linked *T. lagena* with the sexual morph *Eupenicillium limoneum*, suggesting an association with *Penicillium* and introduced *P. lagena* as a new combination. Later, they proposed a sectional classification for *Penicillium* asexual morphs, including sect. *Torulomyces* for *P. lagena* (Stolk & Samson 1985). Pitt & Hocking (1985) argued that *T. lagena* did not fit the generic concept of *Penicillium*, at that time defined by a conidiophore with branches forming a penicillus. The transfer also was not accepted in the list of Current Names in Use for the *Trichocomaceae* (Pitt & Samson 1993, Pitt et al. 2000).

The taxonomy of such morphologically undifferentiated hyphomycetes is confusing, and prior to the availability of DNA-based phylogenies it was difficult to evaluate the phylogenetic significance of characters such as colony colours, phialide shape and the nature of connectives between individual conidia in chains. Apart from *T. lagena*, 10 similar fungi were classified as monophalic species of *Paecilomyces* (Onions & Barron 1967), but following the conclusions of Gams (1971), the monograph of Samson (1974) excluded these from *Paecilomyces*. They were distributed by various authors into genera such as:

i. *Monocillium* S.B. Saksena (1955), sexual morphs in *Niesslia* (*Niessliaceae*, *Hypocreales*), including species with thick-walled, centrally swollen phialides, conidial chains lacking connectives, or often slimy rather than in chains, sometimes confused with *Torulomyces* (Hashmi et al. 1972);

ii. *Phialosimplex* Sigler et al. (2010), sexual morphs unknown, including species with thin-walled, scarcely swollen mono- or polyphialides, and conidial chains with connectives, proposed as a synonym of *Aspergillus* (*Aspergillaceae*, *Eurotiales*) by Houbraken & Samson (2011) and Samson et al. (2014), but perhaps phylogenetically distinct (Tanney, pers. comm.);

iii. *Sagenomella* W. Gams (1978), sexual morphs *Sagenoma* (*Trichocomaceae*, *Eurotiales*), including species with thin-walled, unswollen mono- or polyphialides, and conidial chains with connectives;

iv. *Taifanglania* Z.Q. Liang et al. (2009), sexual morphs unknown but *Chaetomiaceae*, *Sordariales*, including species with thin-walled, flask-shaped monophialides and conidial chains without connectives, now considered a synonym of *Acrophialophora* Edward (Zhang et al. 2015).

Thus, it is clear that the combination of solitary phialides with chains of ameroconidia is plesiomorphic, and its notable that this combination of characters appears in several unrelated monophyletic groups, nested among species with more complex conidiophores. In particular, *Phialosimplex* appears to represent a reduced form of the vesiculate *Aspergillus* conidiophores,
<table>
<thead>
<tr>
<th>Species name</th>
<th>Collection accession nr.</th>
<th>Substrate, Locality</th>
<th>GenBank accession nr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. austrocola</td>
<td>CBS 135897 = DTO 207D4</td>
<td>Unknown strain</td>
<td>KF303654</td>
</tr>
<tr>
<td>P. bennettii</td>
<td>CBS 135898 = CV 184</td>
<td>Soil, South Africa</td>
<td>KF303655</td>
</tr>
<tr>
<td>P. bennettii</td>
<td>CBS 135901 = CV 189</td>
<td>Soil, South Africa</td>
<td>KF303656</td>
</tr>
<tr>
<td>P. bretxi</td>
<td>CBS 135902 = CV 190</td>
<td>Soil, South Africa</td>
<td>KF303657</td>
</tr>
<tr>
<td>P. cantabricum</td>
<td>CBS 120415T = DTO 76I9</td>
<td>Soil, Cantabria, Spain</td>
<td>KF303655</td>
</tr>
<tr>
<td>P. catalonicum</td>
<td>CBS 110532T = DTO 78H5</td>
<td>Soil, Catalonia, Montseny Natural Park, Spain</td>
<td>KF303650</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 330.79 = IJFM 5147</td>
<td>Air sample, Barcelona, Spain</td>
<td>GU944557</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 271.89T = DTO 122C9</td>
<td>Soil from Quercus-Betula forest, New York, USA</td>
<td>KF303647</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 456.70T = NRRL 5207</td>
<td>Mangrove swamp soil, Tooraddin, Australia</td>
<td>AF081804</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 136665T = DTO 270G8</td>
<td>Soil, unknown, Thailand</td>
<td>KF667369</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 185.65T = DTO 77I8</td>
<td>Soil under Thuja plicata, Guelph, Canada</td>
<td>KF303665</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 277.70T = DTO 95D6</td>
<td>Soil under conifers, California, USA</td>
<td>KF303648</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 136664T = DTO 270G7</td>
<td>Soil under Pinus caribaea leaf litter, Kuala Lumpur, Malaysia</td>
<td>KF667370</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135884 = CV 1448</td>
<td>Bract from Protea repens infructescens, Malmesbury, South Africa</td>
<td>KF303664</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135885 = CV 1450</td>
<td>Bract from Protea repens infructescens, Malmesbury, South Africa</td>
<td>KF303665</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135886 = CV 1583</td>
<td>Bract from Protea repens infructescens, Malmesbury, South Africa</td>
<td>KF303666</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135887 = CV 2815</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303673</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135888 = CV 2819</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303674</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135889 = CV 2822</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303675</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135890 = CV 2831</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303676</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135891 = CV 2833</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303677</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135892 = CV 2839</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303678</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135893 = CV 2849</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303679</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135895 = CV 0959</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303680</td>
</tr>
<tr>
<td>P. corylophilum</td>
<td>CBS 135896T = CV 0979</td>
<td>Soil, Malmesbury, South Africa</td>
<td>KF303681</td>
</tr>
</tbody>
</table>

Table 1 Strains used for the phylogenetic analysis.
Fig. 1 Phylogenetic trees for ITS, BenA, CaM and RPB2 datasets of *Penicillium* sect. *Torulomyces*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (* = ex-type; ** = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). Species are indicated by coloured blocks.
of Matsushima (1987) (Ando based on the protologue Monocillium probably belongs in Torulomyces was not transferred because it Torulomyces macrosporus was available for the second original species of Aspergillus accepted determining the placement of the species (Stolk & Samson 1983, T. viscosus, and the original description is insufficient for de terming the placement of the species (Stolk & Samson 1983, Houbraken & Samson 2011).)

Following a four gene phylogenetic analysis of Penicillium, Aspergillus and related genera, Houbraken & Samson (2011) accepted Penicillium lagena as the correct classification for Delitsch’s T. lagena, and confirmed Torulomyces as a synonym of Penicillium. They transferred seven species from Torulomyces to Penicillium sect. Torulomyces, making the new combinations P. laeve, P. ovatum, P. parviverrucosum and P. porphyreum (for Monocillium humidica var. brunneum ≡ T. brun neus). Although they lack conidiophores with solitary phialides, P. cryptum and P. lasseni were included in the section. Torulomyces macrosporus was not transferred because it probably belongs in Monocillium based on the protologue of Matsushima (1987) (Ando et al. 1998). No type material was available for the second original species of Torulomyces, T. viscosus, and the original description is insufficient for determining the placement of the species (Stolk & Samson 1983, Houbraken & Samson 2011).

In this study, we re-evaluate the taxonomy of Penicillium sect. Torulomyces following the standardised methods and taxonomic approach advocated by Visagie et al. (2014b). We delineate species using phylogenies based on the nuc rDNA internal transcribed spacer region (ITS), and partial BenA (β-tubulin), CaM (calmodulin) and RPB2 (RNA polymerase II second largest subunit) gene sequences, as well as morphological characters. We describe 12 new species and provide descriptions for the six other accepted species, while also correcting the typification of P. lagena. Based on phylogenetic results, we classify P. laeve and P. ovatum in Penicillium sect. Exilicaulis. The most important morphological characters needed for species identification are summarised in table format.

MATERIALS AND METHODS

Strains

Strains used for this study (summarised in Table 1) were obtained from the CBS-KNAW Fungal Biodiversity Centre, the Netherlands (CBS) and the working collection of the Applied and Industrial Mycology department (DTO) at the same institute. Ex-type and representative cultures were also deposited in the Canadian Collection of Fungal Cultures, Ottawa (DAOMC). Reference material for P. laeve and P. ovatum was received from the Biological Resource Centre at the National Institute of Technology and Evaluation, Chiba, Japan (NBRC).

Table 2  Length of datasets and models used for phylogenetic analysis.

<table>
<thead>
<tr>
<th></th>
<th>ITS</th>
<th>BenA</th>
<th>CaM</th>
<th>RPB2</th>
<th>Concatenated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>513 bp</td>
<td>368 bp</td>
<td>551 bp</td>
<td>753 bp</td>
<td>2185 bp</td>
</tr>
<tr>
<td>Model</td>
<td>T92+G</td>
<td>K2+G</td>
<td>T92+G+I</td>
<td>TN93+G+I</td>
<td>TN93+G+I</td>
</tr>
<tr>
<td>Bayes</td>
<td>GTR+G</td>
<td>HKY+G+I</td>
<td>HKY+G+I</td>
<td>GTR+G+I</td>
<td>GTR+G+I</td>
</tr>
</tbody>
</table>

Abbreviations used in this table: T92 = Tamura 3-parameter; K2 = Kimura 2-parameter; TN93 = Tamura-Nei; GTR = General Time Reversible; HKY = Hasegawa-Kishino-Yano; +G = Gamma distribution; +I = Invariant sites.
Table 3: Summary of the most important morphological characters for species identification.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth rate (in mm)</th>
<th>Conidia&lt;br&gt;soluble&lt;br&gt;pigments</th>
<th>Conidia&lt;br&gt;branching</th>
<th>Ornamentation</th>
<th>Shape</th>
<th>Tubercle size (µm)</th>
<th>Connectives</th>
<th>Connectives&lt;br&gt;present&lt;br&gt;absent</th>
<th>Connectives&lt;br&gt;long, lack visible rings</th>
<th>Connectives&lt;br&gt;short, lack visible rings</th>
<th>Connectives&lt;br&gt;present, lack visible rings&lt;br&gt;absent</th>
<th>Connectives&lt;br&gt;n.a.</th>
<th>Connectives&lt;br&gt;n.a.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeris</td>
<td>4–5</td>
<td>9–10</td>
<td>no growth</td>
<td>brown</td>
<td>globose</td>
<td>0.21–0.4</td>
<td>absent</td>
<td>absent</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>long, lack visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. austricola</td>
<td>no growth</td>
<td>10–11</td>
<td>8–10</td>
<td>rough</td>
<td>globose</td>
<td>0.19–0.33</td>
<td>absent</td>
<td>present</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>long, lack visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. cantabricum</td>
<td>no growth</td>
<td>no growth</td>
<td>2–3</td>
<td>rough</td>
<td>subglobose</td>
<td>0.12–0.18</td>
<td>absent</td>
<td>present</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>long, visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. catalonicum</td>
<td>no growth</td>
<td>no growth</td>
<td>no growth</td>
<td>rough</td>
<td>subglobose</td>
<td>0.12–0.18</td>
<td>absent</td>
<td>present</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>long, visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. cryptum</td>
<td>no growth</td>
<td>6–9</td>
<td>no growth</td>
<td>rough</td>
<td>broadly ellipsoid</td>
<td>n.a.</td>
<td>n.a.</td>
<td>present</td>
<td>monoverticillate</td>
<td>smooth</td>
<td>smooth</td>
<td>present, lack visible rings</td>
<td>n.a.</td>
</tr>
<tr>
<td>P. laeve</td>
<td>4–5</td>
<td>10–13</td>
<td>6–7</td>
<td>rough</td>
<td>globose</td>
<td>0.19–0.33</td>
<td>absent</td>
<td>absent</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>long, lack visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. lagena</td>
<td>4–5</td>
<td>10–13</td>
<td>6–7</td>
<td>smooth</td>
<td>broadly ellipsoid</td>
<td>0.18–0.41</td>
<td>present</td>
<td>absent</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>present, lack visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. oregonense</td>
<td>no growth</td>
<td>8–10</td>
<td>no growth</td>
<td>smooth</td>
<td>broadly ellipsoid</td>
<td>0.24–0.38</td>
<td>absent</td>
<td>present</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>present, lack visible rings</td>
<td>absent</td>
</tr>
<tr>
<td>P. variratense</td>
<td>4–7</td>
<td>9–14</td>
<td>8–9</td>
<td>smooth</td>
<td>broadly ellipsoid</td>
<td>0.22–0.41</td>
<td>absent</td>
<td>present</td>
<td>solitary phialide</td>
<td>rough</td>
<td>smooth</td>
<td>present, lack visible rings</td>
<td>absent</td>
</tr>
</tbody>
</table>

DNA extraction, sequencing, and phylogenetic analysis

DNA extractions were made from colonies grown on OA for 8–10 d using the Ultraclean™ Microbial DNA isolation Kit (MoBio, Solana Beach, USA). Subsequent PCR amplification and sequencing of the ITS, BenA, CaM and RPB2 gene regions, were done using methods described by Visagie et al. (2014b). Sequence contigs were assembled in DNAStar® SeqMan v. 9.0.4. Newly generated sequences were deposited in GenBank (Table 1). Sequences were aligned in MAFFT v. 7.164 (Katoh & Standley 2013) with the L-INS-I option. For the multigene phylogeny, the datasets were concatenated in SeaView v. 4.4.1 (Gouy et al. 2010). Datasets were analysed using both Maximum Likelihood (ML) and Bayesian tree inference (BI). Trees and alignments were uploaded to TreeBASE (www.treebase.org) with submission ID 16300.

ML analyses were performed using MEGA v. 6.06 (Tamura et al. 2013). The most suitable substitution model for each dataset was selected using the model-test within MEGA, based on the lowest Bayesian information criterion (BIC) value. An initial tree was calculated with the Bio-Neighbour-Joining (BioNJ) option, with the subsequent Heuristic search done with Nearest-Neighbour-Interchange (NNI). A bootstrap analysis with 1 000 replications was used for calculating node support.

For BI analyses, the most suitable substitution model for each dataset, according to the lowest Bayesian information criterion (AIC) value, was selected using MrModeltest v. 2.3 (Nylander et al. 2004). Each analysis was run in MrBayes v. 3.2.1 (Ronquist & Huelsenbeck 2003), with two sets of four chains until the standard deviation of split frequencies reached 0.01. Sample frequency was set at 100 and 25 % of trees removed as burn in. ML phylograms were used for representing phylogenetic results, with both the bootstrap (bs) and posterior probability (pp) values, higher than 80 % bs and/or 0.95 pp given above thickened branch nodes.

Morphology

Standardised techniques for culturing conditions were used as described by Visagie et al. (2014b). Colony characters were recorded from strains grown on CYA (Czapek yeast autolysate agar), MEA (malt extract agar, Oxoid), YES (yeast extract sucrose agar), DG18 (dichloran 18 % glycerol agar), CYAS (CYA supplemented with 5 % NaCl), OA (oatmeal agar), SNA (Spezieller Nährstoffarmer agar) and CREA (creatine sucrose agar). All plates were incubated at 25 °C, with additional CYA plates incubated at 30 and 37 °C. Colour names and codes used for descriptions refer to the Methuen Handbook of Colour (Kornerup & Wanscher 1967).

Microscopic characters were recorded using both light-microscopy and low-temperature scanning electron microscopy (SEM). Microscopic preparations were made from 7–14-d-old cultures grown on OA, with 60 % lactic acid used as mounting fluid. Examinations were done using a Zeiss Axioskop 2 Plus light-microscope fitted with a Nikon microscope camera and pictures captured and analysed using Nikon NIS-elements D v. 4.0 software. In species descriptions, the average measurements and standard deviations are provided in brackets. Two-week-old colonies grown on OA were used for SEM observations. The technique used for SEM is similar to that described in Visagie et al. (2013). Photo plates were prepared using Adobe® Photoshop® Creative Suite v. 6. Photomicrographs were modified for aesthetic purposes using the healing brush tool, without altering areas of scientific significance.
RESULTS

Phylogeny
Phylogenies of all strains were prepared for the ITS region, and the partial BenA, CaM and RPB2 gene regions, and compared using the Genealogical Concordance Phylogenetic Species Recognition (GCP/SSR) concept (Taylor et al. 2000; Fig. 1). A multigene phylogeny of concatenated ITS, BenA, CaM and RPB2 sequences (Fig. 2) was prepared to evaluate the overall phylogenetic structure and the strength of support for nodes in sect. *Torulomyces*. The lengths of aligned datasets and substitution models selected for the analyses are provided in Table 2. Tree topologies did not differ between ML and BI analyses and ML trees are used here for presenting results, with BI pp values marked on relevant branches.

Phylogenies of BenA, CaM and RPB2 were congruent and variable enough to distinguish all species (Fig. 1b–d). The multigene phylogeny (Fig. 2) indicated the presence of 14 species in the main clade of sect. *Torulomyces*, with *P. cryptum* and *P. porphyreum* distinctly related. In the main clade, GCP/SSR confirmed the distinct nature of the previously described species *P. lagena* and *P. caulis*. A further 12 clades were recognized, and were congruent across the different phylogenies. These could be distinguished morphologically and are described as new species in the Taxonomy section below.

From the ITS phylogeny (Fig. 1a) it is apparent that the formal barcode for fungi (Schoch et al. 2012) does not work well for species identification in the section, with *P. austricola*, *P. wisconsinense*, *P. wollemincola* and some strains of *P. riverlandense* sharing identical ITS sequences. The three alternative genes used here can distinguish between all species in the section, but we follow Visagie et al. (2014b) in proposing the use of BenA as an identification marker.

Some degree of variation in gene sequences occurred within *P. austricola* and *P. riverlandense*, the two species recovered from ecological surveys of the fynbos and *Protea repens* infuctescences in South Africa. In the ITS tree, *P. riverlandense* strains had five different genotypes resolved into three main clades (red blocks in Fig. 1a). This conflicts with the other gene trees, where the same clades also occur, but are united in a monophyletic clade that is supported in the *CaM* (Fig. 1c) and RPB2 (Fig. 1d) phylogenies; all three clades are well-supported in the multigene phylogeny (Fig. 2). In *P. austricola*, there is one clade in the ITS tree, two in RPB2 and three in the CaM and BenA trees. These clades do not conflict, although one of them includes only a single strain (CBS 135903). Although it would be feasible to apply GCP/SSR and designate these clades within *P. austricola* and *P. riverlandense* as phylogenetic species, the lack of diagnostic phenotypic characters and their geographical and ecological coherence argues against this. Increased sampling of strains and genes should indicate whether our conservative approach is correct.

ITS sequences of *P. lassennii* and *P. cryptum* show that these species might not belong to sect. *Torulomyces* as defined here. They were basal to the section in the other phylogenies. Similarly, *P. laeve* and *P. ovatum* are shown to belong to sect. *Exilicaulis* with *P. demorphosphorum* as their closest relative.

Morphology
Strains used for phylogenetic analyses were grown for morphological analysis under standardised conditions, and the clades corresponded well with morphological differences. Colony growth rates on different media incubated at different temperatures were the most useful characters for identification. Conidiophores were essentially identical among the species of the section. The exceptions were *P. lassennii*, which produced mono- to biverticillate conidiophores, and *P. cryptum*, which produced mostly monoverticillate conidiophores, with a small proportion consisting of solitary phialides. Conidial shape varies from globose to ellipsoidal and is taxonomically informative. Conidial ornamentation was informative for species identification, but SEM observation is required for this. *Penicillium cryptum*, *P. lassennii*, *P. laeve* and *P. ovatum*, the species excluded from the section based on molecular results, produces smooth-walled conidia, not observed for other species in sect. *Torulomyces* s.str.

Morphological differences between the species described below are summarised in Table 3.

TAXONOMY

The following species are accepted in *Penicillium* sect. *Torulomyces*. *Penicillium ovatum* and *P. laeve*, previously classified in the section, are classified in *Penicillium* sect. *Exilicaulis*. Art. 9.3 of the Code (McNeill et al. 2012) explicitly includes published illustrations among ‘original material’, which precludes neotypification (Art. 9.7). Therefore the erroneous neotypification of *Torulomyces lagena* by Stolk & Samson (1983) is corrected by the designation of one of Delitsch’s (1943) original figures as lectotype and designation of the ‘neotype’ strain CBS 185.65 as the epitope. *Penicillium parviverrucosum* is considered a dubious species.

*Penicillium aeras* Visagie & Samson, sp. nov. — MycoBank MB808262; Fig. 3a–g, 4a–c

**ITS Barcode.** KF303654 (alternative markers: BenA = KF303614; CaM = KF303627; RPB2 = KF303681).

**Type** — Culture ex-type CBS 135907 = DUT 103456 (ectotroph). 

**Description** — Colony diameter, 7 d, in mm: CYA 11–12, CYO 30°C 9–10, CYO 37°C 4–5, MEA 6–7, YES 10–11, OA 9–10, DG18 11–12, CYAS 6–7, SNA 8–9, CRE 6–7, CREA 2–5 μm diam. Colony growth at 25°C, 7 d: CYA obverse pale orange (5A3), reverse pale orange (5A3); MEA obverse orange white (5A2), reverse yellowish brown (5D6); YES obverse greenish white (5A2), reverse brownish orange (5C5); OA obverse greyish green (26E5); DG18 obverse yellowish white (3A2), reverse yellowish white (3A2); CYAS obverse white, reverse white; SNA obverse greenish grey (26B2), reverse white to light greyish; CREA acid not produced.

**Conidiophores** as solitary phialides; stipes smooth-walled, 4–13.5 × 1.5–2 μm; phialides ampulliform, 3.5–6.5 × 2–3 μm (4.9 ± 0.5 × 2.4 ± 0.2); conidia rough to spiny, globose, 2–2.5 × 2–2.5 μm (2.1 ± 0.2 × 2.1 ± 0.2, n = 34), average width/length = 0.99; *sclerotia* not produced.

**SEM observations** — Conidia rough-walled with tubercles present, connects long without visible rings, tubercles 0.21–0.4 μm diam (0.31 ± 0.05).

*Penicillium australicum* Visagie & K. Jacobs, sp. nov. — MycoBank MB805184; Fig. 3h–n, 4d–f

**ITS Barcode.** JX091466 (alternative markers: BenA = JX091579; CaM = JX141600; RPB2 = KF303705).

**Etymology** — Latin, meaning resident from the south, in reference to the type strain isolated from an air sample.

**Type specimen** — Germany, indoor air, 2012, coll. I. Ilyashenko (CBS-H-21608 holotype, culture ex-type CBS 135697 = DUT 20704).

**Description** — Growth present on CYA at 37°C, no growth on CRE, conidia rough-walled, globose, 2–2.5 μm diam.

**Conidiophores** as solitary phialides; stipes smooth-walled, 4–13.5 × 1.5–2 μm; phialides ampulliform, 3.5–6.5 × 2–3 μm (4.9 ± 0.5 × 2.4 ± 0.2); conidia rough to spiny, globose, 2–2.5 × 2–2.5 μm (2.1 ± 0.2 × 2.1 ± 0.2, n = 34), average width/length = 0.99; *sclerotia* not produced.

**SEM observations** — Conidia rough-walled with tubercles present, connects long without visible rings, tubercles 0.21–0.4 μm diam (0.31 ± 0.05).

*Penicillium australicum* Visagie & K. Jacobs, sp. nov. — MycoBank MB805184; Fig. 3h–n, 4d–f

**ITS Barcode.** JX091466 (alternative markers: BenA = JX091579; CaM = JX141600; RPB2 = KF303705).

**Etymology** — Latin, meaning resident from the south, in reference to the type strain isolated close to the most southern tip of Africa at Struisbaai.

Fig. 3  Morphology of species characterised in this study. a, h, o. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–g, i–n, p–v. conidiophores and conidia produced on OA. — Scale bar: v = 10 µm, applies to all microscope pictures.
Diagnosis — No growth on CYA at 37 °C, growth on CYA at 30 °C and CREA, colonies typically produces brown soluble pigments, conidia rough-walled, globose, 1.5–2.5 μm diam.

Description — Colony diam, 7 d, in mm: CYA 10–13; CYA 30 °C 10–11; CYA 37 °C no growth; MEA 4–8; YES 10–11; OA 9–10; DG18 9–11; CYAS 8–10; SNA 9–11; CREA 3–4.

Colonies at 25 °C, 7 d: CYA obverse greyish to dull green (25B3–26D3), reverse olive to dark brown (4E3–6F8), brown soluble pigment produced in some isolates; MEA obverse greyish green (25B3–26B3), reverse light to dark brown (6D6–F8), brown soluble pigment produced in some isolates; YES obverse orange white (5A2) when not sporulating, greyish green (25C4) when sporulating, reverse greyish yellow (4B4); OA...
Conidiophores as solitary phialides; conidia smooth-walled, 4.1–1.5 × 2–2.5 μm; phialides ampulliform, 5–8 × 2–3 μm (6.1 ± 0.6 × 2.7 ± 0.2); conidia rough-walled, globose, 1.5–2.5 × 1.5–2.5 μm (2.1 ± 0.1 × 2.1 ± 0.1, n = 25), average width/length = 0.98; sclerotia not produced.

**Penicillium cantabricum** Visage & Samson, sp. nov. — MycoBank MB808263; Fig. 3o–v, 4g–i

*ITS Barcode.* KF303655 (alternative markers: BenA = KF303615; CaM = KF303646; RPB2 = KF303682).

Etymology. Latin, named after Cantabria, from where the type was isolated.

Type specimen. **Spain.** Cantabria, Reinosa, from soil, June 1996, isol. A.M. Stchigel (CBS H-21612 holotype, culture ex-type CBS 120415 = DTO 7619 = FMR 9121).

Diagnosis — Growth present on CYA at 37 °C, very weak acid produced within colony periphery on CREA, conidia finely rough-walled, globose, 2–2.5 μm.

Description — Colony diam, 7 d, in mm: CYA 13–14; CYA 30 °C 12–13; CYA 37 °C 3–5; MEA 6–7; YES 9–10; OA 10–11; DG18 6–8; CREA 3–4; SNA 7–8; CREA 3–4.

Colonies at 25 °C, 7 d: CYA obverse pale orange (6A3), reverse white to light greyish; CREA acid not produced.

Conidiophores as solitary phialides; conidia smooth-walled, 4.5–11 × 1.5–2 μm; phialides ampulliform, 5–7 × 2–3 μm (5.9 ± 0.6 × 2.4 ± 0.2); conidia finely rough, subglobose to broadly ellipsoidal, 2–2.5 × 2–2.5 μm (2.16 ± 0.1 × 2.11 ± 0.1, n = 41), average width/length = 0.98; sclerotia not produced.

**Penicillium cryptum** Goch., Mycotaxon 26: 349. 1986 — MycoBank MB103648; Fig. 4m–o, 5h–p


*ITS Barcode.* KF303647 (alternative markers: BenA = KF303608; CaM = KF303626; RPB2 = JN121478).

Type specimen. **USA.** New York, Long Island, from soil in Quercus-Betula forest, Oct. 1983, isol. S.E. Gochenaur (NY 769 holotype, culture ex-type CBS 271.89 = DTO 1222 = ATCC 60138 = IMI 296794 = NRRL 13460).

Diagnosis — No growth on CYA at 37 °C or CREA, growth on CYA at 30 °C, conidiophores typically monophasial to monoverticillate, conidia smooth-walled, broadly ellipsoidal, 2–2.5 × 1.5–2 μm.

Description — Colony diam, 7 d, in mm: CYA 6–7; CYA 30 °C 6–9; CYA 37 °C no growth; MEA 6–7; YES 5–6; OA 5–6; DG18 1–2; CREA no growth; SNA 5–6; CREA no growth.

Conidiophores as solitary phialides; stipes smooth-walled, 4.5–24 × 1.5–2 μm; phialides ampulliform, 3.5–5.5 × 2.5–3 μm (4.5 ± 0.5 × 2.6 ± 0.2); conidia finely rough-walled, globose, 2–2.5 × 2–2.5 μm (2.2 ± 0.1 × 2.2 ± 0.1, n = 41), average width/length = 0.99; sclerotia not produced.

**Penicillium catalonicum** Visage & Samson, sp. nov. — MycoBank MB808265; Fig. 4j–l, 5a–g

*ITS Barcode.* KF303650 (alternative markers: BenA = KF303609; CaM = KF303644; RPB2 = KF303683).

Etymology. Latin, named after Catalonia, from where the type was isolated.

Type specimen. **Spain.** Catalonia, Montseny Natural Park, from soil, unknown date, isol. A.M. Stchigel & M. Calduch (CBS H-21610 holotype, culture ex-type CBS 110532 = DTO 78H5).

Diagnosis — No growth on CYA at 30 °C, growth on CYAS, conidia finely rough-walled, with tubercles forming rings around conidia under SEM, subglobose to broadly ellipsoidal, 2–2.5 × 2–2.5 μm.

Description — Colony diam, 7 d, in mm: CYA 6–8; CYA 30 °C no growth; CYA 37 °C no growth; MEA 4–5; YES 5–6; OA 6–7; DG18 7–8; CREA 2–3; SNA 6–7; CREA no growth.

Conidiophores as solitary phialides; stipes smooth-walled, 4.5–11 × 1.5–2 μm; phialides ampulliform, 5–7 × 2–3 μm (5.9 ± 0.6 × 2.4 ± 0.2); conidia finely rough, subglobose to broadly ellipsoidal, 2–2.5 × 2–2.5 μm (2.16 ± 0.1 × 2.11 ± 0.1, n = 41), average width/length = 0.98; sclerotia not produced.

**Penicillium laeve** (K. Ando & Manoch) Houbraeken & Samson, Stud. Mycol. 70: 47. 2011 — MycoBank MB561960; Fig. 5q–w, 6a–c


*ITS Barcode.* KF667369 (alternative markers: BenA = KF667365; CaM = KF667367; RPB2 = KF667371).
Fig. 5  Morphology of species characterised in this study. a, h, q. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–g, i–p, r–w. conidiophores, conidia, asci and ascospores (in w) produced on OA. — Scale bar: w = 10 µm, applies to all microscope pictures.

Diagnosis — No growth on CYA at 37 °C or CREA, growth on CYA at 30 °C, conidia smooth-walled, globose, 2.5–3 μm diam. Ascomata inconspicuous, asci 3–8 μm diam, ascospores smooth-walled, globose, 1.5–2 μm diam.

Description — Colony diam, 7 d, in mm: CYA 8–9; CYA 30 °C 4–5; CYA 37 °C no growth; MEA microcolonies; YES 8–9; OA 7–8; DG18 5–7; CYAS no growth; SNA 7–8; CREA no growth. Colonies at 25 °C, 7 d: CYA obverse white, reverse greyish yellow (3C3); MEA obverse white, reverse yellowish brown (5D6); YES obverse greenish grey (25B2), reverse brownish orange (5C4); OA obverse greyish white (1B1); DG18 obverse greyish white (1B1), reverse greyish yellow (3B3); SNA obverse white to light greyish, reverse white. CREA acid not produced. Conidiophores as solitary phialides; stipes smooth-walled, 2–6 × 1–1.5 μm; phialides ampulliform, 4–6 × 2–3 μm (5.2 ± 0.6 μm).

Fig. 6 SEM micrographs showing characters of monophialidic *Penicillium* species. a, d, g, j, m. Overview of conidiogenous areas; b, e, h, k, n. phialides; c, f, i, l, o. conidia. — Scale bars: a, d, g, j, m = 10 μm; b, e, h, k, n = 5 μm; c, f, i, l, o = 2 μm.
Penicillium lassenii  

Paden, Mycopathol. Mycol. Appl. 43: 266. 1971. — MycoBank MB319281; Fig. 7a–h  


ITS Barcode. KF303648 (alternative markers: BenA = KF303607; CaM = KF303629; RPB2 = JN121481).  

Type specimen. USA, California, from soil under conifers, May 1969, isol. J.W. Paden (JWP 69-26 (UVIC) holotype, culture ex-type CBS 277.70 = DTO 950D = NRRL 5272 = ATCC 22054 = FRR 858 = IMI 148395).  

Diagnosis — No growth on CYA at 37 °C, growth on CYA at 30 °C and CREA, conidiophores typically mono- to biverticillate, conidia smooth-walled, broadly ellipsoidal, 2.5–3 × 2–3 μm, produces pale to brown sclerotia.  

Penicillium lagena  

Delitsch & Samson, Stud. Mycol. 23: 100. 1983 — MycoBank MB109162; Fig. 6d–f, 7a–h  


ITS Barcode. KF303665 (alternative markers: BenA = KF303619; CaM = KF303634; RPB2 = JN121450).  

Type specimen. USA, Wisconsin, unknown source and date, isol. M. Christensen (CBS H-21613 holotype, culture ex-type CBS 129213 = DTO 20185).  

Diagnosis — No growth on CYA at 37 °C, CREA or CYAS, growth on CYA at 30 °C, colonies on CYA 7–8 mm and SNA 11–12 mm, conidia rough-walled, with large well-defined tubercles (average 0.26 ± 0.03 μm) under SEM, subglobose, 2–2.5 × 2–2.5 μm.  

Penicillium marthae-christenseniae Visagie & Samson, sp. nov. — MycoBank MB808267; Fig. 4g–i, 7p–w  

ITS Barcode. KF303651 (alternative markers: BenA = KF303613; CaM = KF303645; RPB2 = KF303711).  

Etymology. Latin, named after Martha Christensen, who isolated and deposited the ex-type strain into the CBS collection.  

Type specimen. USA, Wisconsin, unknown source and date, isol. M. Christensen (CBS H-21613 holotype, culture ex-type CBS 129213 = DTO 20185).  

Diagnosis — No growth on CYA at 37 °C, CREA or CYAS, growth on CYA at 30 °C, colonies on CYA 7–8 mm and SNA 11–12 mm, conidia rough-walled, with large well-defined tubercles (average 0.26 ± 0.03 μm) under SEM, subglobose, 2–2.5 × 2–2.5 μm.  

Penicillium oregonense Visagie, M. Chr. & Samson, sp. nov. — MycoBank MB808268; Fig. 6j–l, 7a–g  

ITS Barcode. KF303668 (alternative markers: BenA = KF303623; CaM = KF303640; RPB2 = KF303710).  

Etymology. Latin, named after Oregon State, from where the type was isolated.  

Type specimen. USA, Oregon, Williamette National Forest near Blue River, unknown source and date, isol. M. Christensen (CBS H-21607 holotype, culture ex-type CBS 129775 = DTO 208A5).  

Diagnosis — No growth on CYA at 30 °C or CYAS, colonies on CYA at 25 °C 4–5 mm, conidia rough-walled with tubercles in rings under SEM, subglobose, 2–2.5 × 2–2.5 μm.
Fig. 7 Morphology of species characterised in this study. a, i, p. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–h, j–o, q–w. conidiophores, conidia and sclerotia (q) produced on OA. — Scale bar: w = 10 µm, applies to all microscope pictures.
Fig. 8 Morphology of species characterised in this study. a, h, o. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–g, i–n, p–u. conidiophores and conidia produced on OA. — Scale bar: u = 10 µm, applies to all microscope pictures.
Description — Colony diam, 7 d, in mm: CYA 4–5; CYA 30 °C no growth; CYA 37 °C no growth; MEA 4–5; YES 5–7; OA 9–10; DG18 8–9; CYAS no growth; SNA 7–8; CREA no growth. Colonies at 25 °C, 7 d: CYA obverse white, reverse pale yellow (3B3); MEA obverse orange white (5A2), reverse yellowish brown (5D6); YES obverse yellowish white (3A2), reverse yellowish white (3A2); OA obverse dull green (28E4); DG18 obverse yellowish white (3A2), reverse yellowish white (3A2); SNA obverse white to light greyish, reverse white to light greyish; CREA acid not produced.

Conidiophores as solitary phialides; stipes smooth-walled, 2.5–14 × 1.5–2 μm; phialides ampulliform, 4.5–6 × 2.5–3.5 μm (5.5 ± 0.4 × 2.8 ± 0.2); conidia rough-walled, globose, 2–2.5 μm (2.15 ± 0.1 × 2.15 ± 0.1, n = 32), average width/length = 0.99; sclerotia not produced.

Fig. 9 SEM micrographs showing characters of monophialidic *Penicillium* species. a, d, g, j, m. Overview of conidiogenous areas; b, e, h, k, n. phialides; c, f, i, l, o. conidia. — Scale bars: a, d, g, j, m = 10 μm; b, e, h, k, n = 5 μm; c, f, i, l, o = 2 μm.
SEM observations: Conidia rough-walled, with tubercles forming conspicuous parallel rings, connectives moderately long and well defined without visible rings, tubercles 0.18–0.24 μm diam (0.22 ± 0.04).

**Penicillium ovatum** (K. Ando & Nawawi) Houbraken & Samson, Stud. Mycol. 70: 48. 2011 — MycoBank MB561961; Fig. 6m–o, 6n–n


*ITS Barcode.* _KF667370_ (alternative markers: _BenA_ = _KF667366_; _CalM_ = _KF667368_; _RPB2_ = _KF667372_).

_Type specimen._ _Malaysia,_ Kuala Lumpur, from soil under _Pinus caribaea_, Nov. 1989, isol. K. _Ando_ (TNS-F-238518 holotype, culture ex-type CBS 136664 = KY 12726 = DTO 27007 = NBRC 109725).

Diagnosis — No growth on CYA at 37 °C or CREA, growth on CYA at 30 °C, conidia smooth-walled, ellipsoidal, 2–3 × 1–2 μm.

Description — Colony diam, 7 d, in mm: CYA 10–11; CYA 30 °C 12–14; CYA 37 °C no growth; MEA 7–8; YES 13–14; OA 10–11; DG18 9–11; CYAS no growth; SNA 8–10; CREA no growth. Colonies at 25 °C, 7 d: CYA obverse greyish white (1B1), reverse brownish orange (5C4); colonics producing orange colours after prolonged incubation; MEA obverse greyish white (1B1), reverse yellowish brown (SD6); YES obverse greenish grey (29C2), reverse greyish yellow (4C4); OA obverse greyish white (1B1); DG18 obverse greyish green (29C2), reverse greyish yellow (3C4); SNA obverse white to light greyish, reverse white; _CREA_ acid not produced.

_Conidiophores_ as solitary phialides; _stipes_ smooth-walled, 3.5–15 × 1–2 μm; _phialides_ ampulliform, 4.5–7 × 2–3 μm (5.7 ± 0.5 × 2.5 ± 0.25); _conidia_ smooth-walled, ellipsoidal, 2–3 × 1.5–2 μm (2.4 ± 0.2 × 1.8 ± 0.1, n = 27), average width/length = 0.76; _sclerotia_ not produced.

SEM observations: Conidia ellipsoidal, smooth-walled, connectives present without visible rings.

**Penicillium porphyreum** Houbraken & Samson, Stud. Mycol. 70: 48. 2011 — MycoBank MB561959; Fig. 8o–u, 9a–c


*ITS Barcode.* _KF303666_ (alternative markers: _BenA_ = _KF303621_; _CalM_ = _KF303636_; _RPB2_ = _KF303677_).

_Type specimen._ USA, Wisconsin, from soil in community of _Pinus strobus_, July 1955, isol. M. _Christensen_ (NY barcode 00985491 holotype, culture ex-type CBS 382.64 = ATCC 15571 = DBO 7867 = KY 12723 = WSF 9-C).

Diagnosis — No growth on CYA at 37 °C or CREA, growth on CYA at 30 °C, colonies on CYA 10–11 mm, conidia rough-walled with small tubercles (average 0.19 ± 0.02 μm), connectives have two clearly visible rings.

Description — Colony diam, 7 d, in mm: CYA 10–11; CYA 30 °C 11–12; CYA 37 °C no growth; MEA 9–10; YES 10–12; OA 10–11; DG18 12–13; CYAS 2–3; SNA 11–12; CREA no growth. Colonies at 25 °C, 7 d: CYA obverse yellowish to orange white (4A2–5A2), reverse orange white (5A2), brown soluble pigment typical; _MEA_ obverse yellowish to orange white (4A2–5A2), reverse light brown (6D7), brown soluble pigment typical; YES obverse turquoise white to greenish white (24A2–25A2), reverse brownish orange (5C4); OA obverse greyish green (25C3–26C3), brownish olive soluble pigment produced; DG18 obverse yellowish grey (3B2), reverse greyish orange (5C4); CYAS obverse yellowish white (3A2), reverse yellowish white (3A2); SNA obverse white to light greyish, reverse white to light greyish; _CREA_ acid not produced.

_Conidiophores_ as solitary phialides; _stipes_ smooth-walled, 5–17 × 1–1.5 μm; _phialides_ ampulliform, 4.5–7.5 × 2–2.5 μm (6 ± 0.8 × 2.3 ± 0.2); _conidia_ rough-walled, globose, 2–2.5 × 2–2.5 μm (2.16 ± 0.1 × 2.16 ± 0.1, n = 43), average width/length = 0.99; _sclerotia_ not produced.

SEM observations: Conidia rough-walled with pronounced large to moderately sized tubercles present, connectives long, consistently having two visible rings, tubercles 0.16–0.23 μm diam (0.19 ± 0.02).

**Penicillium riverlandense** Visagie & K. Jacobs, sp. nov. — MycoBank MB808269; Fig. 9d–f, 10a–g

*ITS Barcode.* _JX091457_ (alternative markers: _BenA_ = _JX091580_; _CalM_ = _JX141153_; _RPB2_ = _KF303686_).

_Etymology._ Latin, named after Riverlands Nature Reserve, in reference to the location of the type origin.


Diagnosis — Growth observed on CYA at 37 °C, colonies producing brown soluble pigment, conidia rough-walled, globose, mostly smaller than 2 μm diam.

Description — Colony diam, 7 d, in mm: CYA 10–14; CYA 30 °C 9–14; CYA 37 °C 4–7; MEA 6–8; YES 10–15; OA 10–14; DG18 10–12; CYAS 8–9; SNA 10–12; CREA 3–4. Colonies at 25 °C, 7 d: CYA obverse greyish green grey (25B2), reverse olive to dark brown (5E5–6F8), brown soluble pigment produced in all isolates; MEA obverse light to greyish green (25A5–C5), reverse dark brown (6F8), brown soluble pigment produced in all isolates; YES obverse greyish green (25B4–26B4), sporulation sometimes absent, then pale to white colonies, brown soluble pigment produced, reverse brownish orange to brown (5C5–5F8); OA obverse dull green (25D4), brownish olive soluble pigment produced; DG18 obverse white to pale yellow (3A3), reverse yellowish white to dull yellow (3A2–B4); CYAS obverse greyish green (25B2), reverse brown (6E6), SNA obverse dull green (29D4), reverse greyish green (29B2); _CREA_ acid not produced.

_Conidiophores_ as solitary phialides; _stipes_ smooth-walled, 3.5–10 × 1–2 μm; _phialides_ ampulliform, 4–8 × 2–3 μm (5.5 ± 0.9 × 2.6 ± 0.2); _conidia_ smooth-walled, ellipsoidal, 2–2.5 × 1–1.5 μm (3.1 ± 0.8 × 1.3 ± 0.3, n = 35), average width/length = 0.78; _sclerotia_ not produced.

SEM observations: Conidia rough-walled with tubercles present, connectives long without visible rings, tubercles 0.19–0.35 μm diam (0.27 ± 0.04).

Fig. 10  Morphology of species characterised in this study. a, h, o. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–g, i–n, p–u. conidiophores and conidia produced on OA. — Scale bar: u = 10 µm, applies to all microscope pictures.
**Penicillium tubakianum** Visagie & Samson, sp. nov. — MycoBank MB808271; Fig. 9i–l, 10h–n

*ITS Barcode*. KF303652 (alternative markers: BenA = KF303611; CaM = KF303637; RPB2 = KF303712).

**Etymology.** Latin, name after K. Tubaki, who isolated the ex-type strain of the species.

**Type specimen.** NEW ZEALAND, from dead bark of Cyathea, Dec. 1963, isol. K. Tubaki (CBS H-21504 holotype, culture ex-type CBS 287.66 = DTO 138D9 = MUCL 8519 = IPO 8315).

**Diagnosis** — No growth on CYA at 37 °C, CREA, growth on CYA at 30 °C, colonies on CYA 8–9 mm, CYAS 2–4 mm and SNA 5–7 mm, conidia rough-walled, globose, 2–2.5 μm diam, sclerotia not produced.

**Description** — Colony diam, 7 d, in mm: CYA 8–9; CYA 30 °C 5–6; CYAS 37 °C no growth; CREA 4–5; YES 7–8; OA 8–9; DG18 9–10; CYAS 2–4; SNA 5–7; CREA no growth.

Colonies at 25 °C, 7 d: CYA obverse yellowish to orange white (4A2–5A2), reverse orange white (5A2), reverse yellowish brown (5D6); YES obverse pale orange brown (5A3), reverse pale orange (5A4); OA obverse greyish green (26C4); DG18 obverse yellowish white (3A2), reverse yellowish brown (3A2); CYAS obverse yellowish white (3A2), reverse yellowish white (3A2); SNA obverse white to light greyish, reverse white to light greyish; CREA acid not produced.

**Conidiophores** as solitary phialides; *stipes* smooth-walled, 2.5–10 × 1–1.5 μm; *phialides* ampulliform, 3.5–6.5 × 2–3 μm (4.6 ± 0.2 × 2.5 ± 0.2); *conidia* rough-walled, globose, 2–2.5 × 2–2.5 μm (2.1 ± 0.1 × 2.1 ± 0.1, n = 34), average width/length = 0.99; *sclerotia* not produced.

SEM observations: Conidia rough-walled with tubercles present, connects present without visible rings, tubercles 0.17–0.28 μm diam (0.23 ± 0.03).

**Penicillium variaritense** Visagie & Samson, sp. nov. — MycoBank MB808271; Fig. 9i–l, 10h–n

*ITS Barcode*. KF303649 (alternative markers: BenA = KF303610; CaM = KF303630; RPB2 = KF303675).

**Etymology.** Latin, named after Varirata National Park in Papua New Guinea, from where the type was isolated.

**Type specimen.** PAPUA NEW GUINEA, Varirata National Park near Port Moresby, from humus soil, Dec. 1996, isol. A. Aptroot & A. van Iperen (CBS H-21611 holotype, culture ex-type CBS 337.97 = DTO H-21611 holotype, culture ex-type CBS 337.97 = DTO 137C8).

**Diagnosis** — No growth on CYA at 37 °C, growth on CYA at 30 °C and CREA, conidia smooth-walled by light microscope, finely roughened under SEM, broadly ellipsoidal, 2–3 × 2.5 μm.

**Description** — Colony diam, 7 d, in mm: CYA 9–10; CYA 30 °C 10–12; CYA 37 °C no growth; MEA 4–5; YES 7–8; OA 8–9; DG18 7–8; CYAS 8–9; SNA 9–10; CREA 2–3.

Colonies at 25 °C, 7 d: CYA obverse greyish green (25B4), reverse greyish yellow to greyish orange (4B4–5B4); MEA obverse orange white (5A2), reverse yellowish brown (5D6); YES obverse greenish white (27A2), reverse brownish orange (5C5); OA obverse dark green (27F6); DG18 obverse pale yellow (1A3), reverse light yellow (3A4); CYAS obverse greyish green (25B4), reverse greyish yellow (3B5); SNA obverse white to light greyish, reverse white to light greyish; CREA acid not produced.

**Conidiophores** as solitary phialides; *stipes* smooth-walled, 3.5–11.5 × 1–2 μm; *phialides* ampulliform, 4.5–7 × 2–3.5 μm (5.7 ± 0.6 × 2.6 ± 0.2); *conidia* smooth-walled, broadly ellipsoidal, 2–3 × 2–2.5 μm (2.5 ± 0.2 × 2.3 ± 0.1, n = 30), average width/length = 0.91; *sclerotia* not produced.

**Penicillium williamettense** Visagie, M. Chr. & Samson, sp. nov. — MycoBank MB808272; Fig. 9m, n, 11a–h

*ITS Barcode*. KF303667 (alternative markers: BenA = KF303622; CaM = KF303639; RPB2 = KF303709).

**Etymology.** Latin, named after Williamette National Forest in Oregon, from where the type was isolated.

**Type specimen.** USA, Oregon, Williamette National Forest near Blue River, unknown source and date, isol. M. Christensen (CBS H-21609 holotype, culture ex-type CBS 129774 = DTO 208A4).

**Diagnosis** — No growth on CYA at 30 °C or CYAS, colonies on CYA at 25 °C 7–8 mm, conidia rough-walled with big tubercles, visible with SEM, globose, 2–2.5 μm diam.

**Description** — Colony diam, 7 d, in mm: CYA 7–8; CYA 30 °C no growth; CYAS 37 °C no growth; MEA 4–5; YES 7–8; OA 5–6; DG18 7–8; CYAS no growth; SNA 6–7; CREA no growth.

Colonies at 25 °C, 7 d: CYA obverse white, reverse pale yellow (3B3); MEA obverse orange white (5A2), reverse yellowish brown (5D6); YES obverse yellowish white (3A2), reverse yellowish white (3A2); OA obverse dull green (28E4); DG18 obverse yellowish white (3A2), reverse yellowish white (3A2); SNA obverse white to light greyish, reverse white to light greyish; CREA acid not produced.

**Conidiophores** as solitary phialides; *stipes* smooth-walled, 5–16 × 1–1.5 μm; *phialides* ampulliform, 4–6.5 × 2–3 μm (5.4 ± 0.5 × 2.6 ± 0.2); *conidia* rough-walled, globose, 2–2.5 × 2–2.5 μm (2.2 ± 0.2 × 2.2 ± 0.2, n = 25), average width/length = 0.99; *sclerotia* not produced.

SEM observations: Conidia rough-walled with very big tubercles present, connects long with rings barely visible, tubercles 0.23–0.42 μm diam (0.33 ± 0.04).

**Penicillium wisconsinense** Visagie, M. Chr. & Samson, sp. nov. — MycoBank MB808273; Fig. 11i–o, 12a–c

*ITS Barcode*. KF303670 (alternative markers: BenA = KF303624; CaM = KF303641; RPB2 = KF303706).

**Etymology.** Latin, named after Wisconsin, from where the type was isolated.

**Type specimen.** USA, Wisconsin, unknown source and date, isol. M. Christensen (CBS H-21614 holotype, culture ex-type CBS 128279 = DTO 198H7 = WSF 3132).

**Diagnosis** — No growth on CYA at 37 °C or CREA, growth on CYA at 30 °C, colonies on CYA 7–10 mm, CYAS 0–2 mm, SNA 9–10 mm, conidia rough-walled with very big tubercles under SEM, globose, 2–2.5 × 2–2.5 μm.

**Description** — Colony diam, 7 d, in mm: CYA 7–10; CYA 30 °C 3–5; CYA 37 °C no growth; MEA 3–6; YES 7–11; OA 9–11; DG18 8–10; CYAS no growth, sometimes 2; SNA 9–10; CREA no growth.

Colonies at 25 °C, 7 d: CYA obverse yellowish to orange white (4A2–5A2), reverse orange white (5A2), sometimes greyish brown (5D3); MEA obverse orange white (5A2), reverse yellowish brown (5D6); YES obverse greyish turquoise to greyish green (24B3–25B3), reverse greyish green (28C3), some yellowish grey (4C4); CYAS obverse pale when growth, reverse pale when growth; SNA obverse dull green (29D4), reverse greenish grey (29B2); CREA acid not produced.

**Conidiophores** as solitary phialides; *stipes* smooth-walled, 5–45 × 1–2 μm; *phialides* ampulliform, 4.5–7.5 × 2.5–3 μm (5.7 ± 0.7 × 0.8 ± 0.7 μm).
Fig. 11  Morphology of species characterised in this study. a, i, p. Colony morphology from left to right, top row: CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS, OA; from left to right, bottom row: reverse colonies on CYA, CYA 30 °C, CYA 37 °C, MEA, YES, DG18, CYAS and obverse on CREA; b–h, j–o, q–w. conidiophores, conidia, ascocarps (in v) and asci (in w) produced on OA. — Scale bar: w = 10 µm, applies to all microscope pictures, except v = 20 µm.
Penicillium species producing conidiophores with solitary phialides

- Conidiophores as solitary phialides; stipes smooth-walled, 4–13 × 1–1.5 μm; phialides ampulliform, 4.5–6.5 × 2.5–3 μm (5.6 ± 0.4 × 2.8 ± 0.3); conidia distinctly rough to spiny, globose, 2–2.5–2.5 μm (2.3 ± 0.1 × 2.3 ± 0.1, n = 25), average width/length = 0.99; sclerotia abundant, colourless, 30–94 × 24–90 μm; cleistothecia observed on old dry plate, similarly sized to sclerotia, wall pseudoparenchymatous, single layer, asci not observed, ascospores lenticular with two widely separated ridges, smooth-walled, 2.5–3.5 × 2.5–3 μm.

SEM observations: Conidia rough-walled with tubercles present, connectives relatively long without visible rings, tubercles 0.22–0.41 μm diam, average 0.31 ± 0.05.

DUBIOUS SPECIES


Notes — Because of commercial restrictions placed on the use of the ex-type strain, material was unavailable for examination. Morphologically, the species was distinguished from close relatives by its verrucose conidia that have small tubercles under SEM (Ando et al. 1998). Because of the overlapping characters of conidia observed during SEM examinations with other species in this section, it is clear that DNA sequence data is necessary to determine the precise taxonomic placement of P. parviverrucosum. Presently, we consider it a dubious species and should be reconsidered when material is made available.

DISCUSSION

Penicillium sect. Torulomyces was introduced by Houbraken & Samson (2011) for P. lagena (CBS 185.65) and the phylogenetically related P. lasseni and P. cryptum, with the consequence that the genus Torulomyces was confirmed as a synonym of Penicillium based on multigene phylogenetic evidence. This group of species all display restricted growth on media and the majority produce conidiophores consisting of solitary phialides. The aim of our study was to re-evaluate the taxonomy of species currently classified in the section in the context of the discovery of 12 new species, described above in the taxonomy.
section. This considerably extends the species sampling for this section, allowing a stringent evaluation of species concepts and their significance. Inconspicuous morphological characters, and some consideration of ecological and biogeographic data in the resulting taxonomic structure. Our multigene phylogenies combined with morphological data recognize 16 species in the section. As noted above, identical ITS sequences are shared by several species; *BenA* was proposed as a secondary marker for *Penicillium* by Visagie *et al.* (2014b) and using *BenA* works well for identification of species in this section.

Species of sect. *Torulomyces* are morphologically challenging to identify. Despite this, their ability or inability to grow on certain media or at higher temperatures makes identification into smaller phenotypic groups possible. Further, within small groups of phylogenetically related species, phenotypic or morphological characters were discerned to distinguish most species pairs. For example, *P. aemis* and *P. cantabrum* are consistently resolved as close relatives in our phylogenies, but *P. aemis* has rougher conidia than *P. cantabrum* (Fig. 4c, i) and does not grow on CREA. *Penicillium tubakianum* and *P. wollemicola* are close relatives, but differ in growth rates and conidia tubercle sizes (Fig. 9i, 12f). Our multigene phylogeny places *P. marthae-christenseniae*, which grows on CYA 30 °C, closest to *P. catalonica*, which does not. Conidial shape also differs between the two species. *Penicillium williamstetten* and *P. oregonense* are close relatives, but they have different growth rates and *P. oregonense* has conidia that are roughened in parallel rings (Fig. 6i, 9e). When one considers *P. aemis* / *P. austricola* and *P. riverlandense*, clearly distinct species under GCPSR, the only morphological difference is that *P. riverlandense* produces smaller colonies at 37 °C. As the most frequently sampled species, the phylogenetic distance and coherence of each species in the single gene phylogenies support the idea that the observed ecological differences between them, discussed below, indicate they are different species rather than different populations of one species.

SEM examination of conidial ornamentations and connectives also reveals differences that distinguish species. Similar observations were made by Ando *et al.* (1998), who reported that *P. laeve* and *P. ovatum* have smooth conidia, but they were able to distinguish between *P. lagena* and *P. porphyreum* based on the latter’s smaller conidial tubercles. In addition to size differences in tubercles, various aspects of conidial ornamentation are informative for some species. For example, *P. variatense* had very finely roughened conidia compared to the rather large ornamentations in *P. williamstetten*. In *P. catalonica* and *P. oregonense* conidial ornamentations formed parallel rings. Connectives were also sometimes useful, with those of *P. porphyreum* having two distinct, clearly visible rings. Although the combination of SEM and growth characters under different conditions allows phenotype-based identification, routine species identification by these means is unrealistic, because having two distinct, clearly visible rings, *P. porphyreum* is less accessible and more time-consuming than routine molecular methods. Although we incorporated these observations for species delineation in this study, we suggest that DNA sequences should be used for reliable identification.

Nine of the new species are based on single isolates. This is not ideal and reflects the difficulty inherent in isolating such slow-growing fungi. Although the two species from the heavily sampled fynbos soils, *P. austricola* and *P. riverlandense*, are represented by many strains, only one culture of *P. wollemicola* was isolated from Wollemi pine litter among more than 700 strains isolated via dilution to extinction, a method designed to recover slow-growing fungi. Future isolations should result in the recovery of more strains that can be used to better document species of sect. *Torulomyces*. In most cases, the genetic distances between our species, including those represented by single strains, corresponds with that observed among phylogenetic species in other groups of *Penicillium*. Although strict application of a phylogenetic species concept requires more than one representative of each taxon, our newly proposed species otherwise conform with GCPSR. Unfortunately, the number of singletons in the phylogenies make bootstrap values and posterior probabilities uninformative to some degree.

Determining the distribution of this group of species from the literature is hampered because until recently, most slow-growing, brown, monophialidic strains were considered to represent one species, *P. lagena*, which was considered widespread but rarely isolated (Domsch et al. 1980). Many of the species newly described in this paper were deposited into culture collections as that species. The literature mostly reports *P. lagena*, and the three other previously known species, from soil surveys (Christensen & Backus 1962, Barron 1967, Gochnau 1978, Domsch *et al.*, 1980, Ando *et al.*, 1998, Renker *et al.*, 2005), although *P. lagena* has also been reported from other sources like dead bark and conmeal (Domsch *et al.*, 1980). In this study, *P. riverlandense* and *P. austricola* were frequently isolated from *Protea repens* infructescences and the mites living in them. Our single strain of *P. wollemicola* was isolated from needle debris of *Wollemia nobilis*, the so-called ‘dinosaur tree’ or ‘living fossil’ that was discovered in the Blue Mountains of Australia in 1994 (Jones *et al.*, 1995). *Penicillium austricola* and *P. riverlandense* were isolated from the fynbos biome situated in the Western Cape of South Africa. One of the fynbos sampling sites in the Riverlands Nature Reserve near Malmesbury was surveyed over a period of four years and *P. riverlandense* was consistently recovered from these samples. In addition, *P. austricola* was only found at a fynbos sampling site at Struisbaai. Our success at isolating these two species makes us optimistic that the development of appropriate sampling or selective isolation techniques will eventually lead to increased sampling of other species, and better understanding of their ecologies.

Culture independent, environmental DNA sequencing might allow enhanced detection of the distribution of this group. Because ITS, the most frequently used marker in such studies, is uninformative at the species level in this group, presently we can only consider data at the sectional level. A BLAST search with the ITS sequence of *T. lagena* (CBS 185.65; KF303665) on GenBank results in only 14 hits that can be assigned to the section. These hits originate from soil (Czech Republic, one unknown location), plant material (Lithuania, Ecuador), marine sediments (China), plant root tissue (Australia, South Africa), a lichen (*Letharia vulpina*, USA) and house dust (Finland). Most of these hits are from cultured isolates, with only two hits (dust from Finland; soil from unreported country) from culture-independent detection techniques. This seems to support that these species are widespread but occur in very low numbers as reported in Domsch *et al.* (1980). The diversity of identified habitats from these studies suggests an even greater diversity of trophic modes for these species than we have so far explored.

Whether the use of a gene with higher species resolution, such as the *BenA*, *CaM* or *RPB2* used here, will allow more sensitive detection remains to be seen.

In our study, only two of the species, *P. laeve* and *P. wollemicola* produced ascospores in some culture transfers. The ascospores of *P. laeve* are smooth-walled and globose, while those of *P. wollemicola* are lenticular and have two broadly spaced ridges. The latter are similar to those described for *P. lagena* (Stolk & Samson 1983, as *Eupen. limoneum*), but we did not observe ascospores in that species. *Penicillium lasseni* was originally described in the sexual genus *Eupenicillium*, but we observed only sclerotia in our strains. The paucity of ascomata observed in our study suggests that either the ability to produce the sexual morph is easily lost in culture if the species are homothallic, or that the species are heterothallic. Thus, it is difficult to confidently interpret sexual characters for identi-
fying species, although the lack of ridges on ascospores of *P. laeve*, and the difference in shape from the other two species, presently seems diagnostic.

Our phylogenies reveal that *P. laeve* and *P. ovatum*, previously included in sect. *Torulomyces* by Houbraken & Samson (2011), should be classified in sect. *Eutychiales* with *P. dimorphosporum* as their closest relative. The placement of *P. lasseni* and *P. cryptum* in sect. *Torulomyces* remains questionable, because our phylogenies resolve them as distant relatives to the well-supported clade that contains the majority of species of sect. *Torulomyces* (Fig. 1, 2). A BLAST search of the *P. lasseni* ITS sequence results in the closest match of 93 % to *P. sublatentarium* and *P. cyanenum*, classified in sect. *Ragimena*. For *P. cryptum*, a BLAST search results in a 93 % match to *P. donkii* and *P. boreae*, classified in sect. *Stoklia*. The closest GenBank match for *RPB2* of *P. lasseni* and *P. cryptum*, respectively results in a 84 % similar match to *P. ornatum* and 83 % similar to *P. lagena*. Houbraken & Samson (2011) used *RPB1*, *RPB2*, *Tsr1* and *Cdt8* for their sectional reclassification of *Penicillium*, which placed both species in a clade with *P. lagena*. However, the branches for these three species in the clade were very long and perhaps provided deceptive support for recognising them as separate and distinct sections. In a similar situation, Visagie et al. (2014a) recently described *P. alfredii*, but were unable to classify it in any of the 25 sections proposed by Houbraken & Samson (2011). Although it seemed that *P. alfredii* represented a new section, they opted to leave it unclassified because of the uncertain phylogenetic position of *P. cryptum* and *P. lasseni*. In their *RPB2* phylogeny, *P. cryptum* made sect. *Torulomyces* polyphyletic with sect. *Fracta*. The fact that these two species are inconsistently placed with seemingly every gene analysed, hints that they may represent two distinct sections that are presently undersampled and thus unrecognizable by cladistic approaches. Conidiophore branching patterns further support the idea that they do not belong in sect. *Torulomyces*, but without isolating additional strains, closely related species and/or analysing more alternative gene sequences, the placement of *P. cryptum*, *P. lasseni* and *P. alfredii* will remain problematic. As a result, for the time being, we believe it best to leave *P. cryptum* and *P. lasseni* in sect. *Torulomyces* as proposed by Houbraken & Samson (2011).

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