Penicillium poederi and P. tirolense, two new species of section Torulomyces

M. Kirchmair1,*, J. Embacher1, D. Heimdörfer2, G. Walch3, S. Neuhauser4

1Institute of Microbiology, University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria
2Division of Genomics and RNomics, Biocenter, Medical University of Innsbruck, 6020 Innsbruck, Austria

*Corresponding author: martin.kirchmair@uibk.ac.at

Abstract: Here we describe two new species of the genus Penicillium section Torulomyces with solitary phialides. Penicillium poederi sp. nov. was isolated from volcanic soils in Iceland. Penicillium tirolense sp. nov. was isolated from a sporocarp of Serpula lacrymans. Both species are characterised by slow growth rates and the production of a brown soluble pigment on CYA, conidiophores with solitary ampulliform phialides with smooth-walled stipes and warty, globose conidia and with connectives without visible rings. The spores of P. poederi are 2.5 µm diam, while the spores of P. tirolense are 2.0 µm diam. In a multigene phylogeny based on the ITS, BenA, CaM and RPB2 gene regions P. tubakianum and P. wollemiicola are the closest relatives of P. poederi. This species differs from P. tubakianum and P. wollemiicola by its growth rates and by its pigmentation. The holotype of P. poederi is IB2017/0007, while SF014017 (CBS 147622) is a culture derived from the holotype. The closest relatives of P. tirolense are P. australicola and P. riverlandense. It differs from P. australicola by lower growth rates on all tested media and temperatures and by its larger spores. It differs from P. riverlandense by lower growth rates and the absence of growth at 37 °C. The holotype of P. tirolense is IBF2019/0162, while SF015108 (CBS 147625) is a culture derived from the holotype.

INTRODUCTION

Newly created environments are found after the retreat of glaciers, after volcanic eruptions or on a newly created island like Surtsey. Studying such sites allows insight in the development of soils and organic matter. A well-studied volcanic succession site is Mt. Hekla, Iceland, where larger eruptions created a series of soils and organic matter. A well-studied volcanic succession site Surtsey. Studying such sites allows insight in the development of glaciers, after volcanic eruptions or on a newly created island like New...
0.05 % Tetracycline, and 0.01 % Dicloran. Agar plates were incubated at 25 °C for 14 d and screened daily for fungal growth. Colonies of interest were transferred onto PDA.

Parts of a sporocarp of *S. lacrymans* (0.1–1 g) were suspended in 20 mL 0.9 % sodium chloride solution supplemented with 0.05 % Tween 80 and mixed together with 10 sterilised glass beads (Ø 4 mm) for 10 min with an overhead shaker (Embacher et al. 2021). The suspension was plated onto malt extract agar (MEA; according to Pitt 1988) supplemented with 1 % Streptomycin, 0.05 % Tetracycline. Agar plates were incubated at 25 °C for 7 d and screened daily for fungal growth. Colonies of interest were transferred onto MEA.

A dilution to extinction approach was applied to find a maximum number of different fungal strains (Unterseher & Schnittler 2009). Ninety-six-well plates were prepared with PDA supplemented with antibiotics (see above). Each well contained 0.5 mL of PDA. Aliquots of 10 µL of the soil and *S. lacrymans* sporocarp suspension were pipetted into each well. The 96-well plates were incubated at 25 °C and screened daily for fungal growth. Agar plugs with a single visible fungal colony were transferred onto fresh MEA agar plates.

Cultures were deposited at the Jena Microbial Resource Collection (JMRC), at the collection culture (CBS) of the Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands, and at the Institute of Microbiology, University Innsbruck, Austria. Dried specimens were deposited at the National Science Collection of the Tiroler Landesmuseum (IBF).

**Molecular characterisation**

Fungal colonies were grown on PDA for 8–10 d, and DNA extraction performed using a CTAB-based extraction protocol (Neuhauser et al. 2009). The methods of Visagie et al. (2014) were followed for PCR amplification of the ITS, *BenA*, and *CaM* gene regions. For *RPB2* a touch down PCR protocol was used (98 °C for 2 min, 4 cycles of 96 °C for 20 s, 60 °C for 45 s, 72 °C for 60 s, 4 cycles of 96 °C for 20 s, 58 °C for 45 s, 72 °C for 60 s, 28 cycles of 96 °C for 20 s, 55 °C for 45 s, 72 °C for 60 s and a final incubation step at 72 °C for 7 min.

The PCR products were purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and sequenced by GATC Biotech. Sequences were quality checked and aligned with sequences of known *Torulomyces* species (Table 1) using MAFFT v. 7.308 (Katoh & Stanley 2013) implemented in Geneious v. 9.1.7. To concatenate the multigene alignment the individual alignments were combined manually leaving a gap between the individual genes in the following order: ITS, *BenA*, *CaM*, *RPB2*. The datasets were analysed using Maximum Likelihood in MEGA X v. 10.1 (Kumar et al. 2018). The most suitable substitution model for each dataset (ITS, *BenA*, *CaM*, *RPB2*, and the concatenated alignment of all four genes) was selected using the model-test within MEGA, based on the lowest Bayesian information criterion (BIC) value (Table 2). An initial tree was calculated with the Bio-Neighbour-Joining (BioNJ) option, with the subsequent Heuristic search done with Nearest-Neighbour-Interchange (NNI). For calculating node support a bootstrap analysis with 1 000 replicates was performed. Alignments and raw trees have been deposited at Figshare (https://doi.org/10.6084/m9.figshare.20631792.v1).

**Morphology**

Isolates were grown as described by Pitt (1988) and Visagie et al. (2014). Colony characteristics were recorded from strains grown on CREA (creatinine sucrose agar), CYA (Czapek yeast autolysate agar), CYAS (CYA supplemented with 5 % NaCl), DG18 (dichloran 18 % glycerol agar), G2SN (25 % glycerol nitrate agar), MEA, YES (yeast extract sucrose agar), OA (oatmeal agar), and SNA (synthetic nutrient-poor agar). All four isolates were grown on all media at 25 °C. Additionally all isolates were incubated at 10 °C, 30 °C, and 37 °C on CYA and MEA. Growth measurements are given in the form (minimum)–median–(maximum) after 7 and 14 d incubation. Colour names and codes used for descriptions refer to the Methuen Handbook of Colour (Kornerup & Wanscher 1967).

Microscopic characters were examined using a Nikon Optiphot compound microscope with Nomarski interference contrast, fitted with a Nikon DS-Fi3 microscope camera and pictures captured and analysed using Nikon Nis-elements D v. 3.0 software. Microscopic specimens were prepared from 7–14-d-old cultures grown on MEA, with 3 % KOH as mounting medium.

For scanning electron microscopy (SEM) culture discs were fixed with Roti®Histofix 4 % (Carl Roth) for 30 min, washed in PBS (phosphate buffered saline buffer: 0.02 mol/L sodium phosphate buffer with 0.15 mol/L sodium chloride, pH adjusted to 7.4), dehydrated in an ascending methanol series and critical point dried. The specimens were mounted on aluminium stubs and sputtered with gold. Micrographs were taken using a Zeiss DSM 950 scanning electron microscope (SEM).

Photo plates were prepared using Adobe® Photoshop® Creative Suite v. 5. Photomicrographs were modified for aesthetic purposes, without altering areas of scientific significance. All measurements are given in the form (minimum) mean ± standard deviation (maximum).

**RESULTS**

**Taxonomy**


**Etymology:** Dedicated to our teacher, mentor, and friend, the Austrian mycologist Reinhold “Bodo” Pöder, who died on Aug. 20th 2015 at the age of 66.

**Type**: Iceland, from soil on the lava flow from 1158 (19°52’W, 63°59’N) at Mt Hekla, 24 Nov. 2016, D. Heimdörfer & M. Kirchmair (*holotype* IFF2017/0007, preserved as dried specimen, ex-type culture SFO14017 = CBS 147622 = Bq214).

**Soil characteristics:** pH = 5.3–6.6; SOM = 10.8 ± 0.9 %, Mean grain size: coarse sand 24.7 % ± 1.2 %, fine sand 70.8 % ± 1.2 %, silt 4.5 % ± 0.2 %, clay 0.0 %, total N 0.1 ± 0.01, total P<sub>M</sub> (mg/L) 13.4 ± 0.3, total P<sub>o</sub> (mg/L) 7.6± 1.5, total K<sub>s</sub> (mg/L) 1.1 ± 0.1, total K<sub>x</sub> (mg/L) 0.4 ± 0.1. (Cutler et al. 2014).

**ITS Barcode:** MF611757 (alternative markers: *BenA* = MF611760; *CaM* = MF611763; *RPB2* = MF611766).
Table 1. Sequence data of *Penicillum* spp. used for phylogenetic analyses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>ITS</th>
<th>BenA</th>
<th>CaM</th>
<th>RPB2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. poederi</em> sp. nov.</td>
<td>Bq214 = CBS 147622 = IBF2017/0007 = SF014017</td>
<td>MF611757</td>
<td>MF611760</td>
<td>MF611763</td>
<td>MF611766</td>
</tr>
<tr>
<td></td>
<td>Ad249 = IBF2017/0006 = SF014018 = CBS 147623</td>
<td>MF611758</td>
<td>MF611761</td>
<td>MF611764</td>
<td>MF611767</td>
</tr>
<tr>
<td></td>
<td>Ar233 = IBF2017/0008 = SF014019 = CBS 147624</td>
<td>MF611759</td>
<td>MF611762</td>
<td>MF611765</td>
<td>MF611768</td>
</tr>
<tr>
<td><em>P. tirolense</em> sp. nov.</td>
<td>IBF2019/0162 = SF015108 = CBS 147625</td>
<td>MW145398</td>
<td>MW143069</td>
<td>MW143068</td>
<td>MW143067</td>
</tr>
<tr>
<td><em>P. aeris</em></td>
<td>DTO207D4</td>
<td>KF303654</td>
<td>KF303614</td>
<td>KF303627</td>
<td>KF303681</td>
</tr>
<tr>
<td><em>P. austrocila</em></td>
<td>CBS 135900</td>
<td>JX091466</td>
<td>JX091579</td>
<td>JX141600</td>
<td>KF303705</td>
</tr>
<tr>
<td><em>P. cantabricum</em></td>
<td>CBS 135903</td>
<td>JX091469</td>
<td>JX091588</td>
<td>JX141604</td>
<td>KF303699</td>
</tr>
<tr>
<td><em>P. corylophilum</em></td>
<td>CBS 330.79</td>
<td>GU944557</td>
<td>GU944519</td>
<td>GU944607</td>
<td>JN046569</td>
</tr>
<tr>
<td><em>P. cryptum</em></td>
<td>CBS 271.89</td>
<td>KF303647</td>
<td>KF303608</td>
<td>KF303628</td>
<td>JN121478</td>
</tr>
<tr>
<td><em>P. dimorphosporum</em></td>
<td>CBS 456.70</td>
<td>AF081804</td>
<td>KJ834448</td>
<td>KPO16783</td>
<td>JN121517</td>
</tr>
<tr>
<td><em>P. leavis</em></td>
<td>DTO270G8</td>
<td>KF667369</td>
<td>KF667365</td>
<td>KF667367</td>
<td>KF667371</td>
</tr>
<tr>
<td><em>P. lagena</em></td>
<td>CBS 185.65</td>
<td>KF303665</td>
<td>KF303619</td>
<td>KF303634</td>
<td>JN121450</td>
</tr>
<tr>
<td><em>P. lassenei</em></td>
<td>CBS 277.70</td>
<td>KF303648</td>
<td>KF303607</td>
<td>KF303629</td>
<td>JN121481</td>
</tr>
<tr>
<td><em>P. martiae-christenseniae</em></td>
<td>CBS 129213</td>
<td>KF303651</td>
<td>KF303613</td>
<td>KF303645</td>
<td>KF303711</td>
</tr>
<tr>
<td><em>P. oregonense</em></td>
<td>CBS 129775</td>
<td>KF303668</td>
<td>KF303623</td>
<td>KF303640</td>
<td>KF303710</td>
</tr>
<tr>
<td><em>P. ovatum</em></td>
<td>DTO270G7</td>
<td>KF667370</td>
<td>KF667366</td>
<td>KF667368</td>
<td>KF667372</td>
</tr>
<tr>
<td><em>P. porphyreum</em></td>
<td>CBS 382.64</td>
<td>KF303666</td>
<td>KF303621</td>
<td>KF303636</td>
<td>KF303677</td>
</tr>
<tr>
<td><em>P. restrictum</em></td>
<td>CBS 367.48</td>
<td>AF033457</td>
<td>KJ834486</td>
<td>KPO16803</td>
<td>JN121506</td>
</tr>
<tr>
<td><em>P. riverlandense</em></td>
<td>CBS 135896</td>
<td>JX091457</td>
<td>JX091580</td>
<td>JX141593</td>
<td>KF303685</td>
</tr>
<tr>
<td></td>
<td>CBS 135892</td>
<td>KF303659</td>
<td>KF301959</td>
<td>JX141615</td>
<td>KF303697</td>
</tr>
<tr>
<td></td>
<td>CBS 135894</td>
<td>JX091463</td>
<td>JX091592</td>
<td>JX141608</td>
<td>KF303694</td>
</tr>
<tr>
<td></td>
<td>CBS 135887</td>
<td>KF303873</td>
<td>JX091590</td>
<td>JX141606</td>
<td>KF303691</td>
</tr>
<tr>
<td></td>
<td>CBS 135886</td>
<td>KF303657</td>
<td>KF301977</td>
<td>JX141597</td>
<td>KF303689</td>
</tr>
<tr>
<td></td>
<td>CBS 135884</td>
<td>JX091459</td>
<td>JX091582</td>
<td>JX141595</td>
<td>KF303684</td>
</tr>
<tr>
<td><em>P. toxicarium</em></td>
<td>NRRL 6172</td>
<td>EF198650</td>
<td>EF198620</td>
<td>EF198631</td>
<td>EF198499</td>
</tr>
<tr>
<td><em>P. tubakianum</em></td>
<td>DTO138D9</td>
<td>KF303652</td>
<td>KF303611</td>
<td>KF303637</td>
<td>KF303712</td>
</tr>
<tr>
<td><em>P. variratense</em></td>
<td>CBS 337.97</td>
<td>KF303649</td>
<td>KF303610</td>
<td>KF303630</td>
<td>KF303675</td>
</tr>
<tr>
<td><em>P. williamettense</em></td>
<td>CBS 129774</td>
<td>KF303667</td>
<td>KF303622</td>
<td>KF303639</td>
<td>KF303709</td>
</tr>
<tr>
<td><em>P. wisconsinense</em></td>
<td>CBS 128279</td>
<td>KF303670</td>
<td>KF303624</td>
<td>KF303641</td>
<td>KF303706</td>
</tr>
<tr>
<td><em>P. wolleiicola</em></td>
<td>CBS 137177</td>
<td>KJ174314</td>
<td>KJ174315</td>
<td>KJ174313</td>
<td>KJ174313</td>
</tr>
</tbody>
</table>

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

<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>587 bp</td>
<td>532 bp</td>
<td>597 bp</td>
<td>887 bp</td>
<td>2 621 bp</td>
</tr>
<tr>
<td>Model ML</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>
</tbody>
</table>

Abbreviations: T92 = Tamura 3-parameter; K2 = Kimura 2-parameter; TN93 = Tamura-Nei; +G = Gamma distribution; +I = Invariant sites.
Additional cultures examined: Iceland, from soil at Mt Hekla, D. Heimdörfer & M. Kirchmair, culture IB2017/0006 = SF014018 = CBS 147623 = Ad249; ibid., culture IB2017/0008 = SF014019 = CBS 147624 = Ar233.

Colony diam, 7 d (in mm): CYA 25 °C (1–)2(–3); CYA 10 °C microcolonies (< 1); CYA 30 °C microcolonies (< 1); CYA 37 °C no germination; MEA 25 °C (9–)10(–12); MEA 10 °C 1(–3); MEA 30 °C microcolonies (< 1); MEA 37 °C no germination; G25N 25 °C (2–)3(–3); YES 25 °C (3–)4(–6); OA 25°C (6–)7(–9); DG18 25°C (4–)6(–7); CYAS 25 °C no germination; SNA 25 °C (6–)7(–10); CREA 25 °C: 1.

Colony characters, 7 d (Fig. 1): CYA 25 °C: crateriform to irregularly sulcate, mycelia white, sporulation sparse, soluble pigments absent, obverse and reverse yellowish white (4A2); CYA 10 °C: floccose, no sporulation, soluble pigment absent, colours not determinable (colonies too small); CYA 30 °C: floccose, no sporulation, soluble pigment absent, colours not determinable (colonies too small); MEA 25 °C: velvety, sulcation only indicated, mycelia white, sporulation moderately dense, conidia en masse greenish grey (28E2), soluble pigments absent, reverse light brown (5C3); MEA 10 °C: floccose, mycelia white, no sporulation, soluble pigment absent, colours not determinable (colonies too small); MEA 30 °C: floccose, mycelia white, no sporulation, soluble pigment absent, colours not determinable (colonies too small); YES 25 °C: crateriform to slightly sulcate, fasciculate at the centre, mycelia white, sporulation sparse, soluble pigment light brown, reverse yellowish white (4A3); DG18 25 °C: slightly sulcate, mycelia white, sporulation sparse, soluble pigments absent, obverse and reverse white; G25N 25 °C: velvety, mycelia white, sporulation sparse, soluble pigments absent, obverse and reverse white; SNA 25 °C: low, mycelia white, sporulation sparse, soluble
pigments absent, obverse and reverse white (4A1); OA 25 °C: velvety, mycelia white sporulation moderately dense, conidia en masse greenish grey (28E2), soluble pigments absent; CREA 25 °C: acid not produced.

Colony diam, 14 d (in mm): CYA 25 °C: (4–)4(–5); CYA 10 °C 1; CYA 30 °C: (microcolonies) 1; CYA 37 °C: no germination; MEA 25 °C: (17–)20(–23); MEA 10 °C: (3–)4(–6); MEA 30 °C: (1–)1(–2); MEA 37 °C: no germination; YES 25 °C: (5–)7(–10); DG18 25 °C: (13–)14(–16); G25N 25 °C: (5–)7(–9); CYAS 25 °C: no germination; SNA 25 °C: (10–)16(–20); OA 25 °C: (15–)17(–20); CREA 25 °C: 1.

Colony characters, 14 d (supplementary Fig. S1): CYA 25 °C: irregularly sulcate, mycelia white, sporulation sparse, soluble pigment brown, obverse yellowish white (4A3), reverse olive brown (4E5); CYA 10 °C: dense with a floccose overlay, mycelia white, sporulation sparse, soluble pigment absent, obverse and reverse white; CYA 30 °C: dense, waxy, mycelia white, no sporulation, soluble pigment absent, obverse and reverse white; CYA 37 °C: no germination; MEA 25 °C: radially sulcate, low with concentrical rings near the margin, mycelia white, sporulation moderately to dense, soluble pigment absent, obverse greenish grey (28E2) when sporulating, in non or sparsely sporulating cultures white, reverse brown to dark brown (5F4–5F5); MEA 10 °C: velvety to slightly fasciculate; mycelia white, sporulation sparse, soluble pigment absent, obverse and revers white; MEA 30 °C: velvety, mycelia white, sporulation sparse, soluble pigment absent, obverse and revers white; MEA 37 °C: no germination; YES 25 °C: dense, irregularly sulcate, occasionally crateriform, velvety to slightly fasciculate at the centre; mycelia white, sporulation sparse, soluble pigment brown, obverse white, reverse yellowish brown (5E5); DG18 25 °C: plane irregularly to radially sulcate, velvety, mycelia white, sporulation sparse, soluble pigment absent, obverse olive brown (4D3) when sporulating, in non or sparsely sporulating cultures yellowish grey (4B2), reverse olive brown (4F5) when sporulating, in non or sparsely sporulating cultures greyish yellow (4B4); G25N 25 °C: dense, irregularly sulcate, occasionally crateriform, velvety to slightly fasciculate, mycelia white, sporulation sparse,
soluble pigment absent, obverse greenish grey (28E2), revers greyish green (28E5); CYAS 25 °C: no germination; SNA 25 °C: plane with irregular margins, mycelia white, sporulation sparse, soluble pigment absent, obverse greenish grey (26B2), reverse white to light greyish. OA 25 °C: plane, velutinous, mycelia white, sporulation moderate, soluble pigment absent, conidia \textit{en masse} greenish grey (28E2); CREA 25 °C: acid not produced.

**Micromorphology** (Fig. 2): Conidiophores as solitary phialides; stipes smooth-walled, \( (4.0–)6.5 \pm 1.4(–9.3) \times (1.3–)1.7 \pm 0.2(–2.1) \) \( \mu m \) \( (n = 25) \); phialides ampulliform, \( (4.9–)6.1 \pm 0.9(–9.0) \times (2.1–)2.6 \pm 0.3(–3.2) \) \( \mu m \) \( (n = 37) \); conidioyta warty, globose, \( (2.1–)2.5 \pm 0.2(–2.9) \) \( \mu m \) \( (n = 38) \), average width/length quotient = 1; sclerotia not produced.

**SEM observations** (Fig. 3): Conidia warty, tubercles \( (0.26–)0.36 \pm 0.04(–0.43) \) \( \mu m \) diam, \( (0.14–)0.19 \pm 0.03(–0.21) \) high, connectives long without visible rings.

**Notes**: In ML phylogenies of the \textit{RBP2}, \textit{CaM}, \textit{BenA} as well as in the combined dataset (Fig. 7); Supplementary Figs S3–S6, \textit{P. poederi} forms one clade together with \textit{P. tubakianum} and \textit{P. wollemiicola}, and branches consequently as a separate and distinct clade.

\textit{Penicillium poederi} differs from \textit{P. tubakianum} and \textit{P. wollemiicola} by its lower growth rates on CYA when incubated on 25 °C and 30 °C, lower growth rates on YES and DG18 incubated at 25 °C, and higher growth rates on MEA at 25 °C. The obverse on MEA 25 °C is \textit{P. poederi} is greenish grey when sporulating or white in non-sporulating cultures, while in \textit{P. tubakianum} the obverse is orange-white. In contrast to \textit{P. tubakianum}, a brown soluble pigment is produced on CYA and YES at 25 °C by \textit{P. poederi} (Table 3).


**Etymology**: Named after Tyrol, a province in Austria from where the new species was isolated.

**Typus**: Austria, Tyrol, Matrei am Brenner (11°7'W, 47°27'N), from a sporocarp of \textit{Serpula lacrymans}, 14 Oct. 2019, J. Embacher & M. Kirchmair (holotype IBF2019/0162, preserved as dried specimen, culture ex-type SF014017 = CBS 147625).

**ITS Barcode**: MW145398 (alternative markers: \textit{BenA} = MW143069; \textit{CaM} = MW143068; \textit{RBP2} = MW143067).

**Colony diam, 7 d (in mm)**: CYA 25 °C: \( (3–)4(–5) \); CYA 10 °C: microcolonies < 1; CYA 30 °C \( (2–)3(–5) \); CYA 37 °C: no germination; MEA 25 °C: \( (4–)5(–6) \); MEA 10 °C: microcolonies < 1; MEA 30 °C: \( (2–)3(–5) \); MEA 37 °C: no germination; G25N 25 °C: growth rates (mm)

**Table 3. Summary of the most important morphological characters. Conspicuous differences between the new species (bold) and their closest relatives are printed in bold.**

<table>
<thead>
<tr>
<th></th>
<th>\textit{P. austricola}</th>
<th>\textit{P. tirolense}</th>
<th>\textit{P. poederi}</th>
<th>\textit{P. tubakianum}</th>
<th>\textit{P. wollemiicola}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rates</td>
<td>CYA 37: 2–4</td>
<td>2–4</td>
<td>2–4</td>
<td>2–4</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>CYA 30: 10–12</td>
<td>10–12</td>
<td>10–12</td>
<td>10–12</td>
<td>10–12</td>
</tr>
<tr>
<td></td>
<td>CYAS 1–3</td>
<td>1–3</td>
<td>1–3</td>
<td>1–3</td>
<td>1–3</td>
</tr>
<tr>
<td></td>
<td>CREA 2–3</td>
<td>2–3</td>
<td>2–3</td>
<td>2–3</td>
<td>2–3</td>
</tr>
<tr>
<td></td>
<td>OA 2–4</td>
<td>2–4</td>
<td>2–4</td>
<td>2–4</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>MEA 4–6</td>
<td>4–6</td>
<td>4–6</td>
<td>4–6</td>
<td>4–6</td>
</tr>
<tr>
<td></td>
<td>YES 3–6</td>
<td>3–6</td>
<td>3–6</td>
<td>3–6</td>
<td>3–6</td>
</tr>
<tr>
<td></td>
<td>SNA 2–6</td>
<td>2–6</td>
<td>2–6</td>
<td>2–6</td>
<td>2–6</td>
</tr>
<tr>
<td></td>
<td>DG18 4–7</td>
<td>4–7</td>
<td>4–7</td>
<td>4–7</td>
<td>4–7</td>
</tr>
<tr>
<td>Conidia</td>
<td>size [µm]</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>shape</td>
<td>globose</td>
<td>globose</td>
<td>globose</td>
<td>globose</td>
</tr>
<tr>
<td></td>
<td>tubercle size [µm]</td>
<td>0.23 ± 0.34</td>
<td>0.23 ± 0.34</td>
<td>0.23 ± 0.34</td>
<td>0.23 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>connectives</td>
<td>present</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td></td>
<td>acid on CREA</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
</tbody>
</table>

*Data from Visagie et al. (2016).*
Penicillium poederi and *P. tirolense* spp. nov.

**Fig. 4.** Culture characteristics of *Penicillium tirolense* sp. nov. after 1 wk incubation. From top to bottom: obverse, reverse, single colony. 

(1–)2(–3); YES 25 °C: (3–)5(–6); OA (2–)4(–5); DG18 25 °C: (4–)5(–7); CYAS: (1–)2(–3); SNA 25 °C: (2–)3(–6); CREA 25 °C: 1(–2).

**Colony characters, 7 d** (Fig. 4): CYA 25 °C: dense, somewhat fasciculate, mycelia white, sporulation sparse, soluble pigment brown, obverse white (4A1), reverse brown (5F4); CYA 10 °C: dense, mycelia white, no sporulation, soluble pigment absent, obverse and reverse white (4A1); CYA 30 °C: dense, muriform, irregularly sulcate, mycelium white, sporulation sparse, soluble pigment absent, obverse white (4A1), revers greyish beige to golden grey (4C2); CYA 37 °C: no germination. MEA 25 °C: crateriform, irregularly sulcate, velvety to slightly fasciculate, mycelia white, sporulation moderate, soluble pigment brown, obverse greenish grey (26C2–26D2, 25C2–25D2, 27D2), reverse brown to reddish brown (7B8 and 8B8). MEA 10 °C: waxy; mycelia white, sporulation sparse, soluble pigment absent, obverse and reverse white (4A2); MEA 30 °C: dense, crateriform, mycelia white, sporulation sparse, soluble pigment absent, obverse white (4A1), revers yellowish brown to brown (5E5 and 6E4). MEA 37 °C: no germination. YES 25 °C: dense, crateriform, irregularly sulcate; mycelia white, sporulation sparse, soluble pigment brown, obverse white (4A1), reverse yellowish brown (5E5). DG18 25 °C: plane to crateriform, velvety, mycelia white, sporulation sparse, soluble pigment brown (very weak), obverse yellowish grey (4B2), reverse greyish yellow (4B4), G25N 25 °C: dense, crateriform, velvety to slightly fasciculate, mycelia white, sporulation sparse, soluble pigment brown (very weak), obverse white (4A1), revers yellowish white (4A2); SNA 25 °C: plane with inconspicuous concentric rings, mycelia white, sporulation moderate, soluble pigment absent, obverse white to olive green (4A1, 2E3), reverse white (4A1). OA 25 °C: plane, velutinous, mycelia white, sporulation moderate, soluble pigment brown (very weak), obverse greenish grey (27D3), reverse white.

Colony diam, 14 d (in mm): CYA 25 °C: (8–)9(–10); CYA 10 °C: (1–)2(–3); CYA 30 °C: (7–)8(–9); CYA 37 °C: no germination; MEA 25 °C: (15–)16(–17); MEA 10 °C: (2–)3(–4); MEA 30 °C: (9–)10(–11); MEA 37 °C: no germination. YES 25 °C: (12–)14(–15); DG18 25 °C: (9–)12(–13); G25N 25 °C: (5–)6(–7); SNA 25 °C: (11–)14(–16); OA 25 °C: (7–)8(–10); CYAS 25 °C: (3–)4(–6); CREA 25 °C: (3–)4(–5).

Colony characters, 14 d (supplementary Fig. S2): CYA 25 °C: dense, crateriform, irregularly sulcate, mycelia white, sporulation sparse, soluble pigment brown, obverse grey (28C1 and 29B1–29C1), reverse dark brown (7F7–7F8 and 8F8); CYA 10 °C: dense, mycelia white, no sporulation, obverse and reverse white (4A1), soluble pigment absent. CYA 30 °C: dense, moriform, irregularly sulcate, mycelium white, sporulation sparse, soluble pigment absent, obverse white (4A1), revers greyish beige to golden grey (4C2). CYA 37 °C: no germination. MEA 25 °C: irregularly to radially sulcate, velvety to slightly fasciculate, mycelia white, sporulation moderate, soluble pigment brown, obverse greenish grey (26C2–26D2, 25C2–25D2, 27D2), reverse brown to reddish brown (7B8 and 8B8). MEA 10 °C: velvety to slightly fasciculate; mycelia white, sporulation sparse, soluble pigment absent, obverse greyish yellow (4B3), revers white to greenish grey (4C2). MEA 30 °C: dense, crateriform, irregularly sulcate, mycelia white, sporulation sparse, soluble pigment absent, obverse greenish grey (28C2 to 28B2), revers greyish brown to brown (7E3 and 6E4). MEA 37 °C: no germination. YES 25 °C: dense, crateriform, irregularly sulcate; mycelia white, sporulation moderate, soluble pigment brown, obverse greyish orange to dull yellow (5B3 and 4A2), reverse greyish brown (5B3 and 8D3). DG18 25 °C: plane radially sulcate, velvety, mycelia white, sporulation sparse to moderate, soluble pigment brown present, obverse olive brown (4D3) when sporulating, in non or sparsely sporulating cultures yellowish grey (4B2), reverse olive brown (4F5) when sporulating; in non or sparsely sporulating cultures greyish yellow (4B4), G25N 25 °C: dense, irregularly sulcate, velvety to slightly fasciculate, mycelia white, sporulation present, obverse brown (4D3) when sporulating, in non or sparsely sporulating cultures yellowish grey (4B2), reverse olive brown (4F5) when sporulating; in non or sparsely sporulating cultures greyish yellow (4B4), G25N 25 °C: dense, irregularly sulcate, velvety to slightly fasciculate, mycelia white, sporulation present, obverse brown (4D3) when sporulating, in non or sparsely sporulating cultures yellowish grey (4B2), reverse olive brown (4F5) when sporulating; in non or sparsely sporulating cultures greyish yellow (4B4),
Fig. 7. Multigene phylogeny (maximum likelihood) for a combined ITS, BenA, CaM & RPB2 dataset of *Penicillium* sect. *Torulomyces*. Bootstrap values higher than 80% are indicated above branches. The new species are highlighted in bold font.
sparse, soluble pigment brown, obverse white (3A1), revers yellowish white to pale yellow (4A2 to 4A3). SNA 25 °C: plane with inconspicuous concentric rings, mycelia white, sporulation moderate, soluble pigment absent, obverse olive green to dull green (2E3, 2D2, 2E63), reverse white to greenish grey (2A1 to 2B2). OA 25 °C: plane, velutinous, mycelia white, sporulation moderate, soluble pigment brown (very weak), obverse greenish grey to dull green (2D73, 2C72), reverse olive to dull green (3D04, 3D3). CYAS 25 °C: dense, slightly succulate, mycelia white, sporulation sparse, soluble pigment absent, obverse white to grey (8A1 to 8B1, SC1), revers brownish grey (SC2 to 5D2). CREA 25 °C: acid not produced.

**Micromorphology** (Fig. 5): Conidiophores as solitary phialides; stipes smooth-walled; phialides ampulliform, (4.6–)6.2 ± 1.0 (–8.5) × (1.9–)2.6 ± 0.3(–3.2) μm (n = 32); conidia smooth to slightly rough, globose, (1.8–)2.0 ± 0.1(–2.2) μm (n = 41), average width/length quotient = 1; sclerotia not produced.

**SEM observations** (Fig. 6): Conidia warty, tubercles (0.23–)0.29 ± 0.04(–0.34) μm diam, (0.11–)0.13 ± 0.02(–0.16) high, connects short without visible rings.

**Notes:** In ML phylogenies of the RPB2, CaM, BenA as well as in the combined datasets (Fig. 7; Supplementary Figs S3–S6), *P. tiroliense* forms one branch together with *P. australicola* and *P. riverlandense*. From those species, *P. tiroliense* differs in its lower growth rates on all tested media and temperatures. The obverse and reverse on DG18 are olive brown in sporulating cultures while in *P. australicola* the obverse is greenish white to pale green and the revers dull green. In *P. riverlandense* the obverse is white to pale yellow and the reverse yellowish white to dull yellow.

**DISCUSSION**

The genus *Torulomyces* (type species: *T. lagena*) was morphologically characterised by solitary phialides and dry, basipetal conidial chains (Delitsch 1943). *Torulomyces viscous* was described at the same time, but the description is patchy and type material is lacking. Therefore, the species is considered as doubtful (Stolk & Samson 1983). *Torulomyces lagena* was recognised as asexual morph of *Eupenicillium limoneum* and the asexual morph was combined to *Penicillium lagena* (Stolk & Samson 1983). *Torulomyces lagena* was originally isolated from bark from Cyathea in Iceland. We would like to thank Bettina Schneidhofer for assistance in the laboratory. We are indebted to Nick Cutler for providing us with soil samples from Iceland. We would like to thank Bettina Schneidhofer for assistance in the laboratory.

**ACKNOWLEDGEMENTS**

SN was funded by the Austrian Science Fund (FWF) project Y801-B16. We are indebted to Nick Cutler for providing us with soil samples from Iceland. We would like to thank Bettina Schneidhofer for assistance in the laboratory.

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

**REFERENCES**


Supplementary Material: http://fuse-journal.org/
Fig. S3

*Penicillium austrolicola* CBS 135903 (JX091469)
*Penicillium austrolicola* CBS 135904 (JX091465)
*Penicillium austrolicola* CBS 135900 (JX091466)
*Penicillium riverlandense* CBS 135887 (KF303673)
*Penicillium wolleiica* CBS 137177 (KJ174314)
*Penicillium wisconsinense* CBS 128279 (KF303670)
*Penicillium williamettense* CBS 129774 (KF303667)

**Penicillium tirolense** CBS 147625 (MW145398)
*Penicillium oregonense* CBS 129775 (KF303668)
*Penicillium riverlandense* CBS 135886 (KF303657)
*Penicillium riverlandense* CBS 135892 (KF303659)
*Penicillium catalonicum* CBS 110532 (KF303650)
*Penicillium tubakianum* DTO138 D9 (KF303652)

**Penicillium poederi** CBS 147622 (MF611757)

**Penicillium poederi** CBS 147623 (MF611758)
**Penicillium poederi** CBS 147624 (MF611759)

*Penicillium riverlandense* CBS 135884 (JX091462)
*Penicillium riverlandense* CBS 135889 (JX091463)
*Penicillium martha-christenseniae* CBS 129213 (KF303651)
*Penicillium lagena* CBS 185.65 (KF303665)
*Penicillium porphyreum* CBS 382.64 (KF303666)
*Penicillium cantabricum* CBS 120415 (KF303655)
*Penicillium aereis* DTO207 D4 (KF303654)
*Penicillium variatense* CBS 337.97 (KF303649)

**Penicillium cryptum** CBS 271.89 (KF303647)
**Penicillium lasseni** CBS 277.70 (KF303648)

**Penicillium dimorphosporum** NRRL 5207 (AF081804)

*Penicillium laeve* DTO270 G8 (KF667369)
*Penicillium ovatum* DTO270 G7 (KF667370)

**Penicillium toxicari** NRRL 6172 (EF198650)
**Penicillium corylophilum** CBS 330.79 (GU944557)
**Penicillium restrictum** NRRL 1748 (AF033457)

---

**Fig. S3.** ITS1-5.8S-ITS2 phylogeny (maximum likelihood) of *Penicillium* sect. *Torulomyces*. Bootstrap values higher than 80% are indicated above branches. The new species are highlighted in bold font.
Fig. S4: BenA phylogeny (maximum likelihood) of *Penicillium* sect. *Torulomyces*. Bootstrap values higher than 80% are indicated above branches. The new species are highlighted in bold font.
Fig. S5. CaM phylogeny (maximum likelihood) of *Penicillium* sect. *Torulomyces*. Bootstrap values higher than 80% are indicated above branches. The new species are highlighted in bold font.
Fig. S6. RBP2 phylogeny (maximum likelihood) of *Penicillium* sect. *Torulomyces*. Bootstrap values higher than 80% are indicated above branches. The new species are highlighted in bold font.