



## Fifteen new species of *Penicillium*

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### Key words

beta-tubulin  
calmodulin  
internal transcribed spacer rDNA region  
*Trichocomaceae*

**Abstract** We introduce 15 new species of *Penicillium* isolated from a diverse range of locations, including Canada, Costa Rica, Germany, Italy, New Zealand, Tanzania, USA and the Dry Valleys of Antarctica, from a variety of habitats, including leaf surfaces in tropical rain forests, soil eaten by chimpanzees, infrabuccal pockets of carpenter ants, intestinal contents of caterpillars and soil. The new species are classified in sections *Aspergilloides* (1), *Canescentia* (2), *Charlesia* (1), *Exilicaulis* (3), *Lanata-Divaricata* (7) and *Stolkia* (1). Each is characterised and described using classical morphology, LC-MS based extrolite analyses and multigene phylogenies based on ITS, *BenA* and *CaM*. Significant extrolites detected include andrastin, pulvilloric acid, penitrem A and citrinin amongst many others.

**Article info** Received: 28 January 2016; Accepted: 12 March 2016; Published: 9 May 2016.

## INTRODUCTION

Because of the standardised approach to delineating species of *Penicillium* now available, with ex-type or representative strains and barcode sequences designated for almost all described species, including those considered synonyms (Visagie et al. 2014b), routine description of new species can now be approached with reasonable confidence. The exact set of genes used for multi-locus sequence typing (MLST) or Genealogical Concordance Phylogenetic Species Recognition (GCPSR) is not standardised among different laboratories, but most tend to use a similar core set of loci. Phenotypes such as irregular conidiophore branching, culture sectoring, and the relative abundance of conidia, sterile mycelium, soluble pigments or exudates can be interpreted as phylogenetically informative characters or artefacts of culture degeneration according to their correlation with robustly analysed DNA sequence data.

The historical focus of *Penicillium* taxonomy on strains isolated from food, soil and the indoor environment is understandable given the importance of these fungi as spoilage agents, producers of potent mycotoxins, and sources of valuable antibiotics. Much of the expansion of the number of species in *Penicillium* and its sister genus *Talaromyces* can be traced to the antibiotic revolution and the ease with which soil could be collected by travellers and used to isolate new strains upon return to the laboratory. This practise, however, obscured how *Penicillium*

species might actually function in nature. It was easy to assume that most species are generalists found wherever we care to make isolations.

In this paper, 15 species are described that originated from several studies in three Canadian laboratories over the past 20 years, which are now recognised as novel taxa because of the more complete reference data. Three species were isolated from soil of termite mounds selectively eaten by chimpanzees (*Pan troglodytes*) in the Mahale Mountains and Gombe areas of Tanzania (Ketch 1998). Thirty-one genera were identified from these termitarian soils and surrounding control soils. The most commonly isolated genus was *Penicillium* with over 42 species recovered, the most common of which was *Penicillium citrinum*. Clark (2002) isolated microfungi from the infrabuccal pockets of carpenter ants, especially *Camponotus pennsylvanicus*. These sac-like organs in the mouths of ants filter solids from their liquid foods to be expelled later, and thus capture fungal material collected during feeding and grooming. About 60 strains of *Penicillium* were isolated from extracted infrabuccal masses from ants collected in New Brunswick (Canada), Germany and the Netherlands, representing six morphospecies; three are described as new species here. A US National Science Foundation Microbial Observatory study of caterpillar intestines from Costa Rica previously yielded two undescribed species, *P. mallochii* and *P. guanacastense* that clearly were significant components of that niche, along with *P. citrinum* (Rivera et al. 2012). An additional new species, represented as a single strain among the 88 isolated, is described here. Other new species described in this paper were isolated from leaf surfaces of tropical plants, nuts in temperate hardwood forests, peat bogs and soil remote from agricultural regions.

Extrolite profiling brought a new dimension to *Penicillium* taxonomy about 25 years ago (Filtenborg et al. 1990), accompanied by a hypothesis that different isolates of a single species should produce a consistent profile of core extrolites. With the availability of a new generation of LC-MS-MS technology, we generated extrolite profiles for all the new species described in this paper. Although reports are unavailable for the metabolites produced by many species related to our new taxa, these observations will be useful for future comparative chemotaxonomic studies.

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## MATERIALS AND METHODS

### Strains

Cultures were isolated either by direct or dilution plating from various substrates or niches as indicated in the species descriptions, usually on 2 % malt extract agar with antibacterial antibiotics. Strains were recovered from the working collections of Keith Seifert (Agriculture and Agri-Food Canada, Ottawa) and David Malloch (formerly University of Toronto), the latter now maintained in the Seifert laboratory. Ex-type and other representative strains were deposited into the Canadian Collection of Fungal Cultures (DAOMC) and the CBS-KNAW Fungal Biodiversity Centre (CBS) in the Netherlands.

### Morphology

Colony characters were recorded from various media incubated for 7 d, including Czapek yeast autolysate agar (CYA), Blakeslee's (1915) malt extract agar (MEAb), yeast extract sucrose agar (YES), oatmeal agar (OA) and creatine sucrose agar (CREA). Bacto™ malt extract was used for MEAb preparation. Media preparations, inoculations, incubation conditions and microscopic preparations followed the recommendations by Visagie et al. (2014b). Colour names and codes used in descriptions are from Kornerup & Wanscher (1967). Microscopic observations were made on an Olympus SZX12 dissecting microscope and Olympus BX50 compound microscope equipped with Infinity3 and InfinityX cameras driven by Infinity Analyze v. 6.5.1 software (Lumenera Corp., Ottawa, Canada).

### DNA extraction, sequencing and phylogenetic analysis

Strains were grown on MEAb for 7 d and DNA extracted using the Ultraclean™ Microbial DNA isolation Kit (MoBio Laboratories Inc., Solana Beach, USA). ITS barcodes (Schoch et al. 2012), partial  $\beta$ -tubulin (*BenA*) and partial calmodulin (*CaM*) genes were amplified using the Illustra pureTaq Ready-To-Go™ PCR Beads (GE Healthcare Life Sciences) with conditions and primers as suggested in Visagie et al. (2014b). Sequencing reactions were set up with the same primer pairs and the Big Dye Terminator Cycle Premix Kit with products run on the ABI PRISM™ 310 DNA automated sequencer (Applied Biosystems, Waltham, USA). Contigs were assembled and edited in Geneious v. 8.1.5 (BioMatters Ltd., Auckland, New Zealand).

Newly generated sequences were submitted to GenBank under accession numbers KT887760–KT887876.

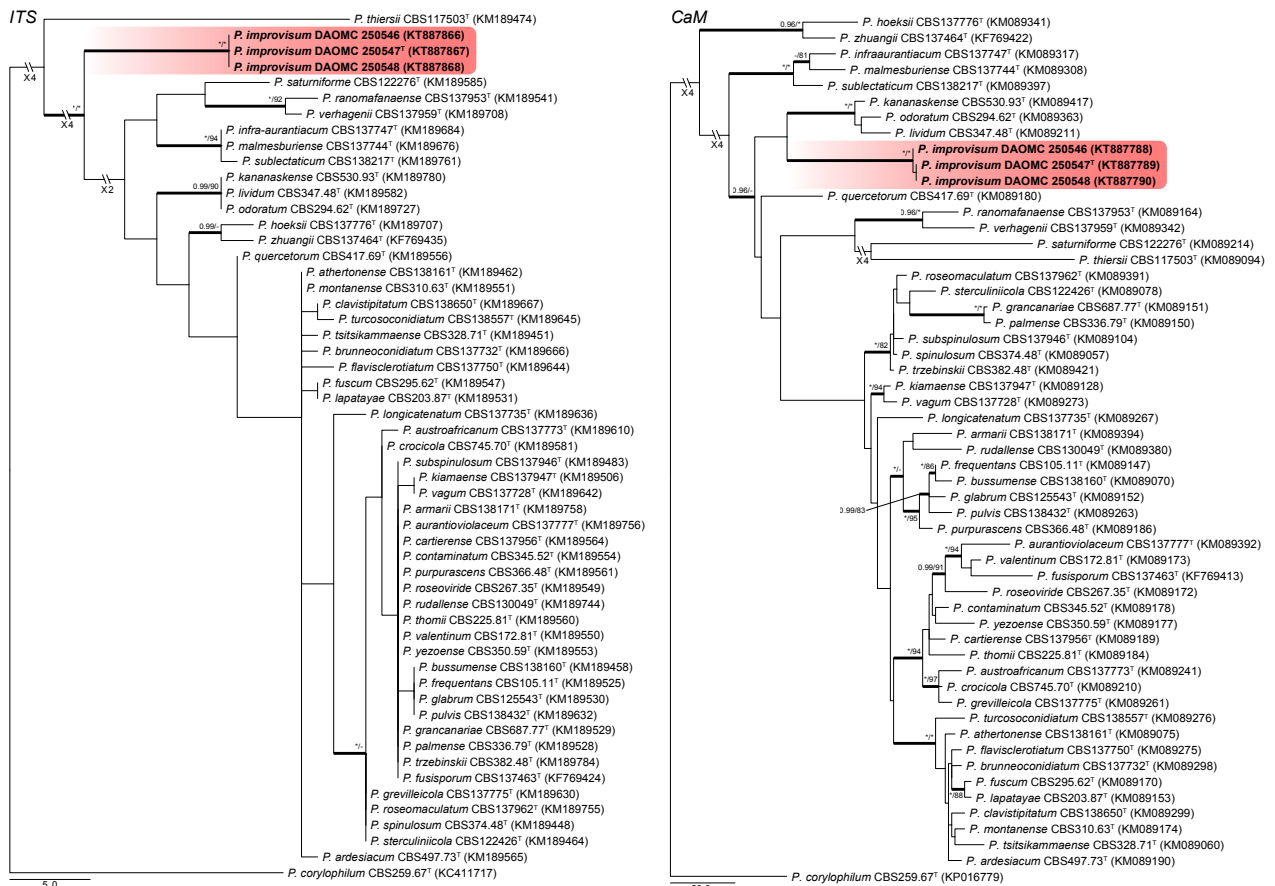
Gene sequences of the new species were compared with a reference sequence dataset (Visagie et al. 2014b), with phylogenies calculated for the relevant taxonomic sections in the system of Houbraken & Samson (2011). Datasets were aligned using MAFFT v. 7.221 (Kato & Standley 2013), with the L-INS-i algorithm selected for ITS and G-INS-i for *BenA* and *CaM*. Aligned datasets were adjusted and trimmed manually in Geneious. Datasets were subsequently analysed using Maximum Parsimony (MP) and Bayesian tree Inference (BI). MP heuristic searches were performed in PAUP v. 4.0b10 (Swofford 2003). BI analyses were run in MrBayes v. 3.2.5 (Ronquist & Huelsenbeck 2003). Model selections were made using MrModeltest v. 2.3 (Nylander et al. 2004) based on the lowest Akaike information criterion (AIC) value. Aligned datasets with the respective command blocks for MP and BI were uploaded onto TreeBASE (www.treebase.org) with accession no. S18638. Trees were prepared for publication in Adobe Illustrator CS6.

### Extrolites

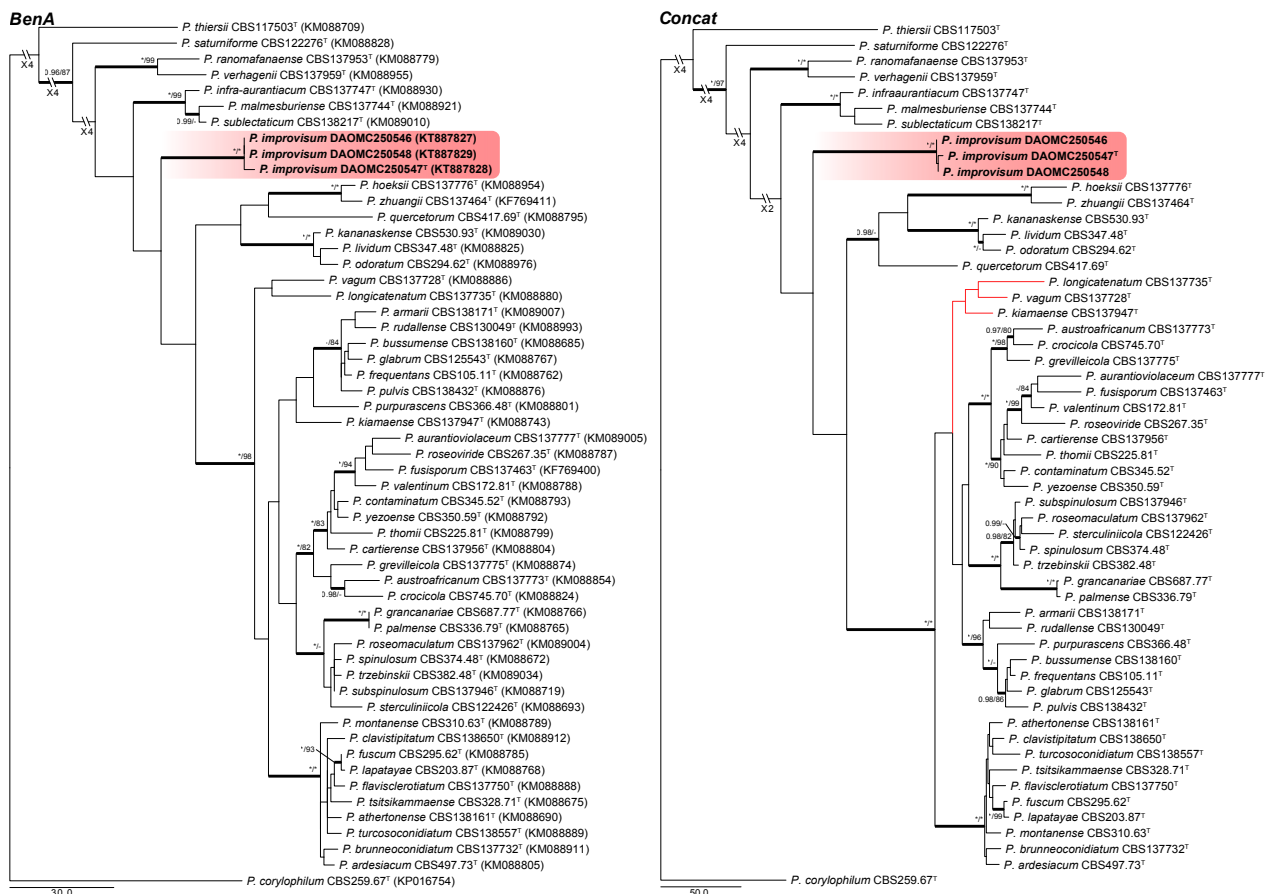
For extrolite analyses, all strains were grown in 9 cm polystyrene Petri dishes on YES (Frisvad 1981, Filtenborg et al. 1990) and CYA (Pitt 1980) incubated at 25 °C for 14 d. Strains were also grown in 200 mL of CYA broth incubated at 25 °C for 14 d. Extrolites were extracted from the agar plates using an established method (Bragulat et al. 2001) with some modifications. Three agar plugs from each fungal isolate were removed with a sterilised 7 mm cork borer and placed in a 7 mL glass scintillation vial containing 1.5 mL of 75 % methanol in water. The vials were vortexed for 30 s and sonicated at room temperature for 30 min. The extrolites in CYA broth media were extracted using 2× 150 mL ethyl acetate, combined, dried and reconstituted in 1 mL of methanol. The solutions were filtered into 1.5 mL amber HPLC glass vials using 0.45  $\mu$ m syringe filters. All extracts were analysed in both positive and negative ionization modes on a Thermo Q-Exactive Orbitrap coupled to an Agilent 1290 HPLC. Extrolites were identified by comparison with authentic standards when possible. When standards were unavailable, compounds were putatively identified by screening the high-resolution/accurate mass  $m/z$  signals with AntiBase2013 and comparing product ions observed with those published in the literature.

**Table 1** Overview and details used for phylogenetic analyses.

		Dataset				
		section <i>Aspergilloides</i>	section <i>Canescentia</i>	section <i>Charlesia &amp; Stolckiae</i>	section <i>Exilicaulis</i>	section <i>Lanata-Divaricata</i>
ITS dataset	Length (bp)	550	510	532	513	502
	Parsimony uninformative sites	34	31	22	51	41
	Parsimony informative sites	43	12	100	49	59
	Substitution model (BI)	GTR+G+I	HKY+I	GTR+G+I	GTR+G+I	GTR+G+I
<i>BenA</i> dataset	Length (bp)	450	403	465	489	535
	Parsimony uninformative sites	52	37	17	68	80
	Parsimony informative sites	168	110	197	197	215
	Substitution model (BI)	HKY+G+I	HKY+G	SYM+G+I	HKY+G	GTR+G+I
<i>CaM</i> dataset	Length (bp)	521	388	557	527	571
	Parsimony uninformative sites	55	41	25	47	65
	Parsimony informative sites	207	130	236	241	255
	Substitution model (BI)	GTR+G+I	K80+G	GTR+I+G	SYM+I+G	GTR+G+I
Concate- nated dataset	Length (bp)	1521	1301	1554	1513	1608
	Parsimony uninformative sites	141	109	64	164	188
	Parsimony informative sites	418	252	533	483	522



**Fig. 1** Phylogenetic trees for ITS & CaM datasets of *Penicillium* sect. *Aspergilloides*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches († = ex-type; \* = 100 % bs and/or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.



**Fig. 2** Phylogenetic trees for *BenA* & Concatenated (ITS, *BenA* & *CaM*) datasets of *Penicillium* sect. *Aspergilloides*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches († = ex-type; \* = 100 % bs and/or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.



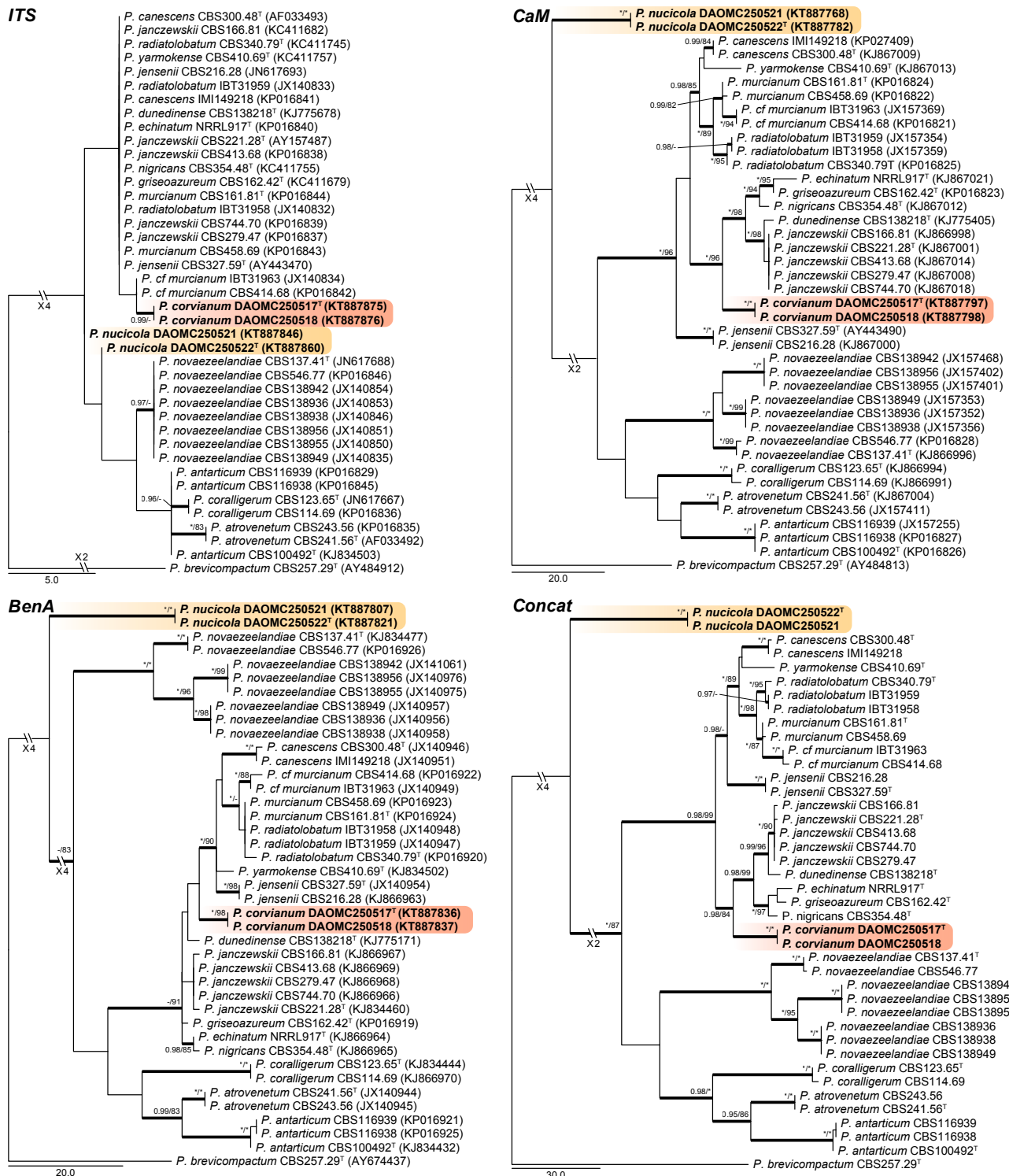
## RESULTS

## Morphology

We introduce 15 new species in the Taxonomy section below. These species belong to sections *Aspergilloides*, *Canescentia*, *Charlesia*, *Exilicaulis*, *Lanata-Divariata* and *Stolkia* based on the phylogenetic analyses. Strains conformed to the general morphological characters previously observed for species accepted in these sections. All of the new species were compared with respective close relatives, with notes provided on distinguishing characters after each species description in the taxonomy section.

## Phylogeny

A local BLAST search placed the new species into sections *Aspergilloides*, *Canescentia*, *Charlesia*, *Exilicaulis*, *Lanata-Divariata* and *Stolkia*. Phylogenies of each section were prepared for the ITS region, *BenA* and *CaM* genes, as well as a concatenation of the three. Phylogenies are presented in Fig. 1–8. The length, number of parsimony informative and uninformative sites, and substitution models (for BI) for each dataset are summarised in Table 1. Tree topologies did not differ between MP and BI analyses and MP trees were thus used for representing results, with BI posterior probability values



**Fig. 3** Phylogenetic trees for ITS, *BenA*, *CaM* & Concatenated (ITS, *BenA* & *CaM*) datasets of *Penicillium* sect. *Canescentia*. 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). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.

marked on relevant branches. The exception was the concatenated dataset for section *Aspergilloides*, where *P. longicatenatum* was resolved in an unsupported clade with ML (Fig 2, indicated by red branch), whilst in the BI tree the latter two were resolved in a well-supported clade with the *P. glabrum* and *P. thomii* clades, with *P. longicatenatum* a distant relative to this main clade.

#### Section *Aspergilloides* — Fig. 1, 2

Houbraken et al. (2014) reviewed section *Aspergilloides* and divided it into 12 clades. All of our phylogenies show that *P. improvisum* strains comprise a well-supported 13th clade. The new species can easily be identified using the ITS barcode, with *BenA* and *CaM* also distinguishing the species.

#### Section *Canescentia* — Fig. 3

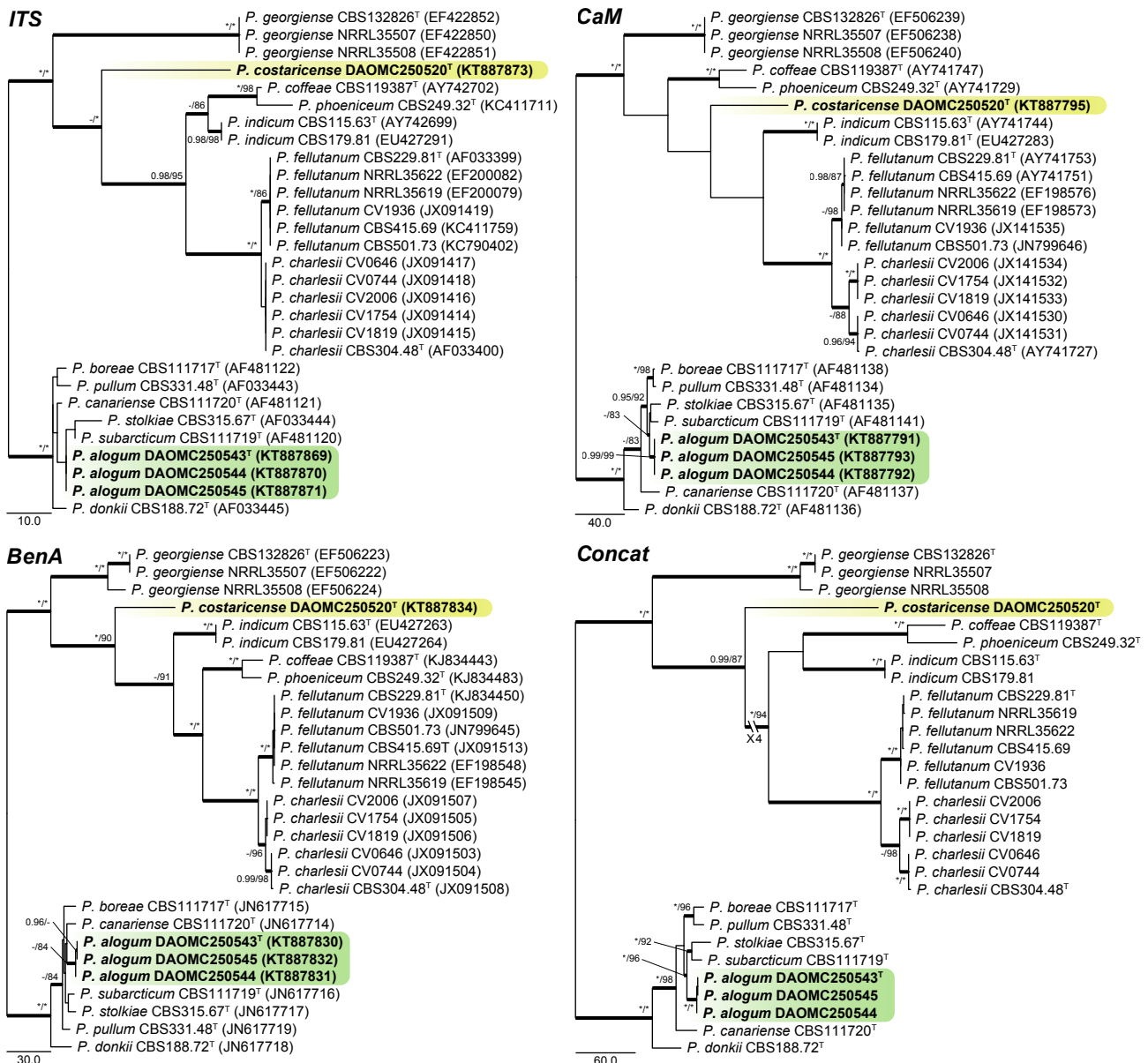
Two new species belong to section *Canescentia*. *Penicillium corvianum* is resolved in a distinct clade closely related to species of the *P. canescens* and *P. janczewskii* clades, while *P. nucicola* is distinct from all other species. The ITS barcode has poor discriminatory power in the section. *BenA* easily distinguishes the new species, but cannot reliably distinguish

among *P. radiatolobatum* and *P. murcianum* strains. In the strict sense, current data assessed with the Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept, suggests that these two represent synonyms and *P. radiatolobatum* (Publ. Soc. Nat. Rom. Pent. Stiinta Sol. 10B: 435, 1971) an older name than *P. murcianum* (Mycopathologia 74: 37, 1981).

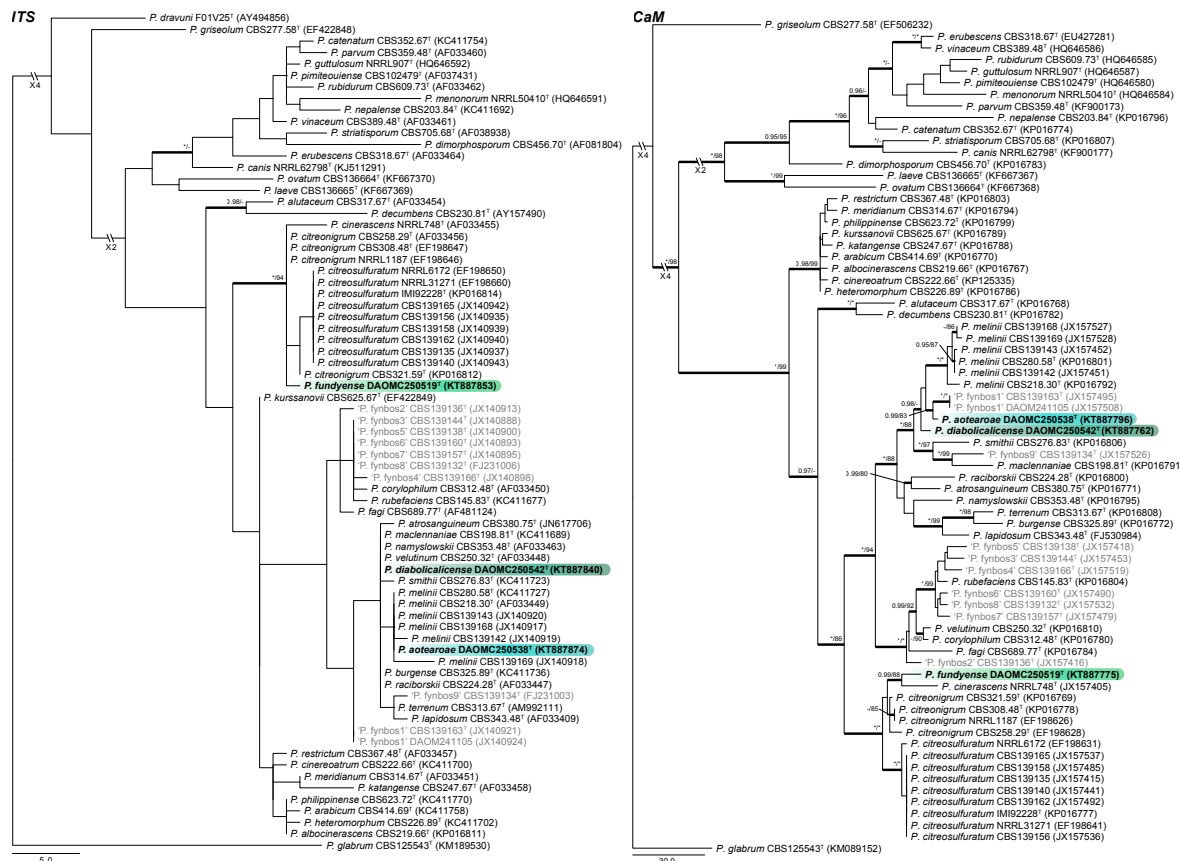
#### Sections *Charlesia* and *Stolkia* — Fig. 4

A new species is introduced in section *Charlesia*, where species are phylogenetically diverse enough for ITS to result in a positive identification. Phylogenies show the distinct identity of *P. charlesii* and *P. fellutanum*, with *P. multicolor* (CBS 501.73) and *P. ebenbitarianum* (CBS 415.69) confirmed as synonyms of the latter. The single strain of *P. costaricense* forms a branch distantly related to all other species of section *Charlesia*.

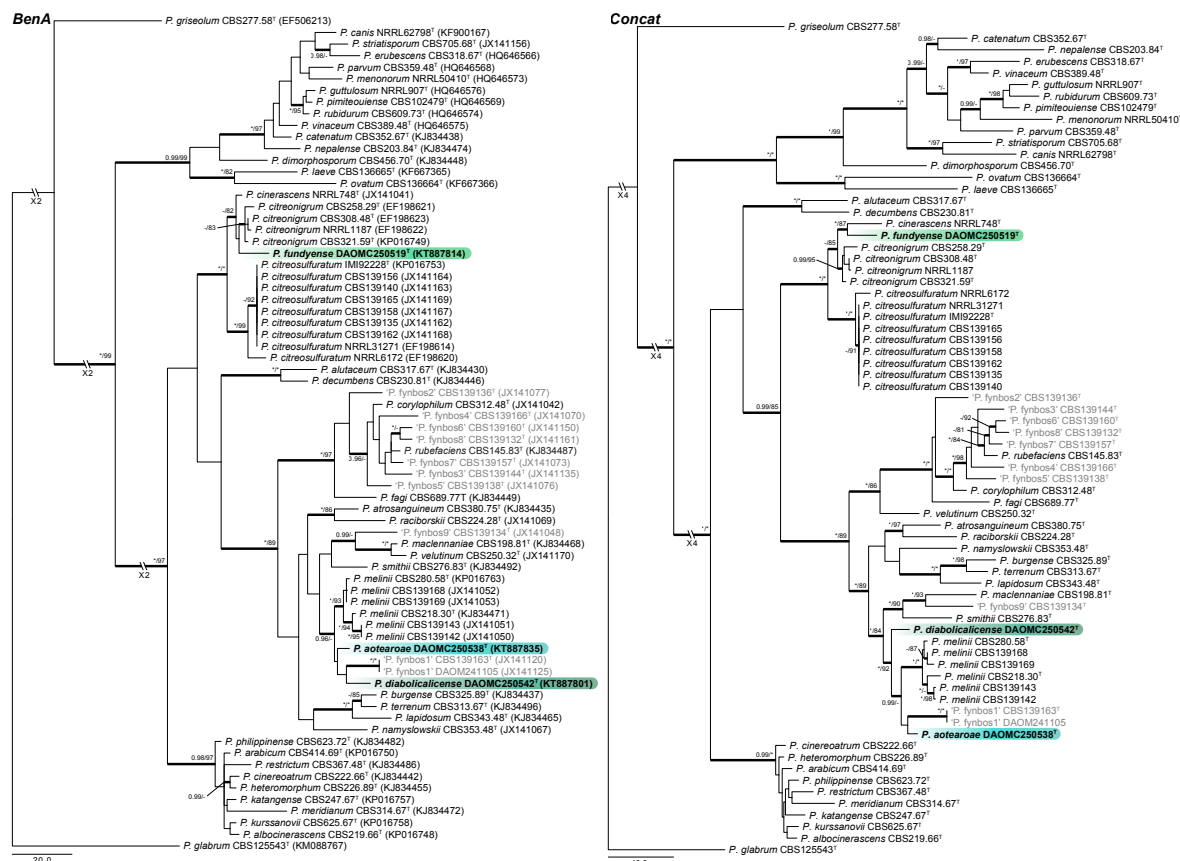
Section *Stolkia* is a small section of rather rare species. As with section *Charlesia*, the ITS barcode can distinguish between the six species and *P. alogum* described here. The concatenated phylogeny resolves *P. alogum* as a close relative of *P. stolkieae* and *P. subarcticum*.



**Fig. 4** Phylogenetic trees for ITS, *BenA*, *CaM* & Concatenated (ITS, *BenA* & *CaM*) datasets of *Penicillium* sections *Charlesia* and *Stolkia*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (<sup>T</sup> = ex-type; \* = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.

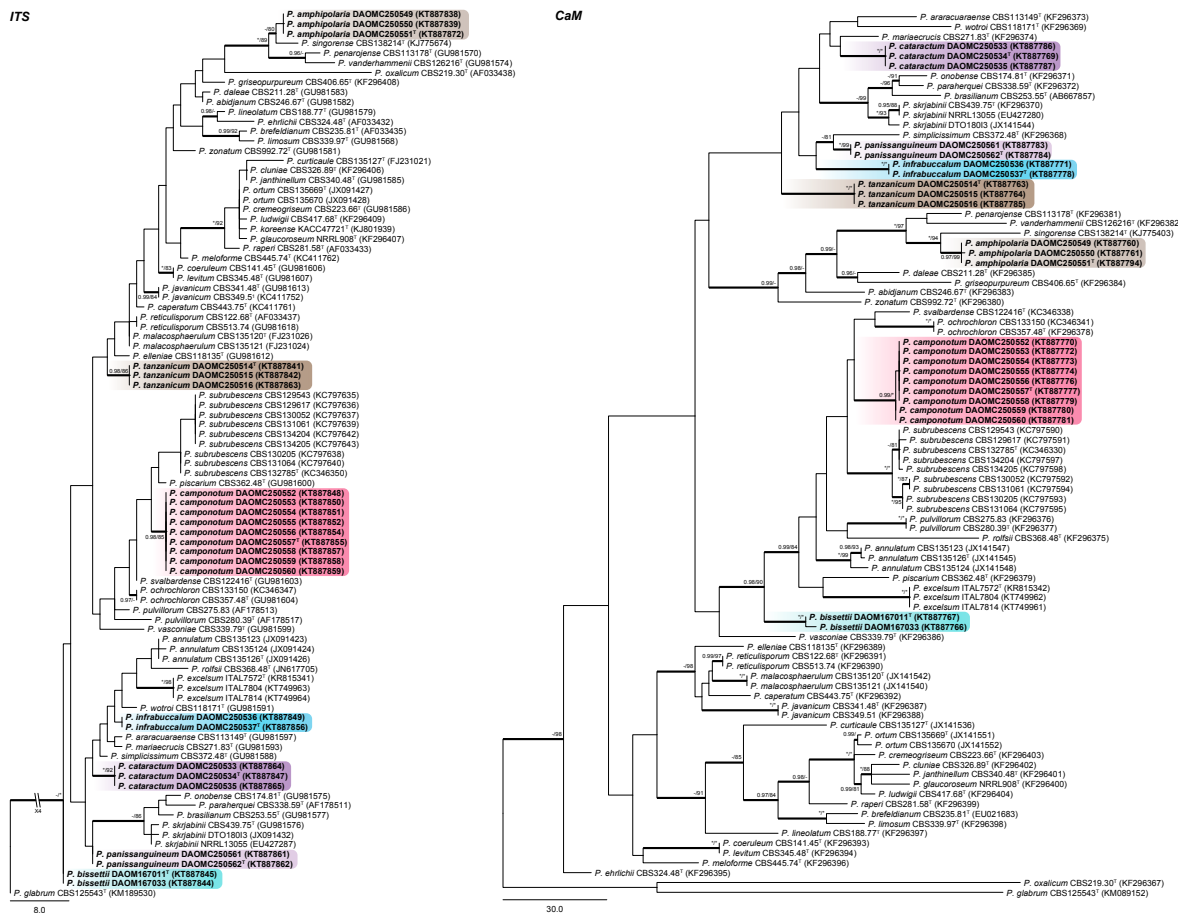


**Fig. 5** Phylogenetic trees for ITS & CaM datasets of *Penicillium* sect. *Exilicaulis*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (\* = ex-type; \* = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets. Taxon labels in grey, indicate unpublished new species from Visagie et al. (2016b).

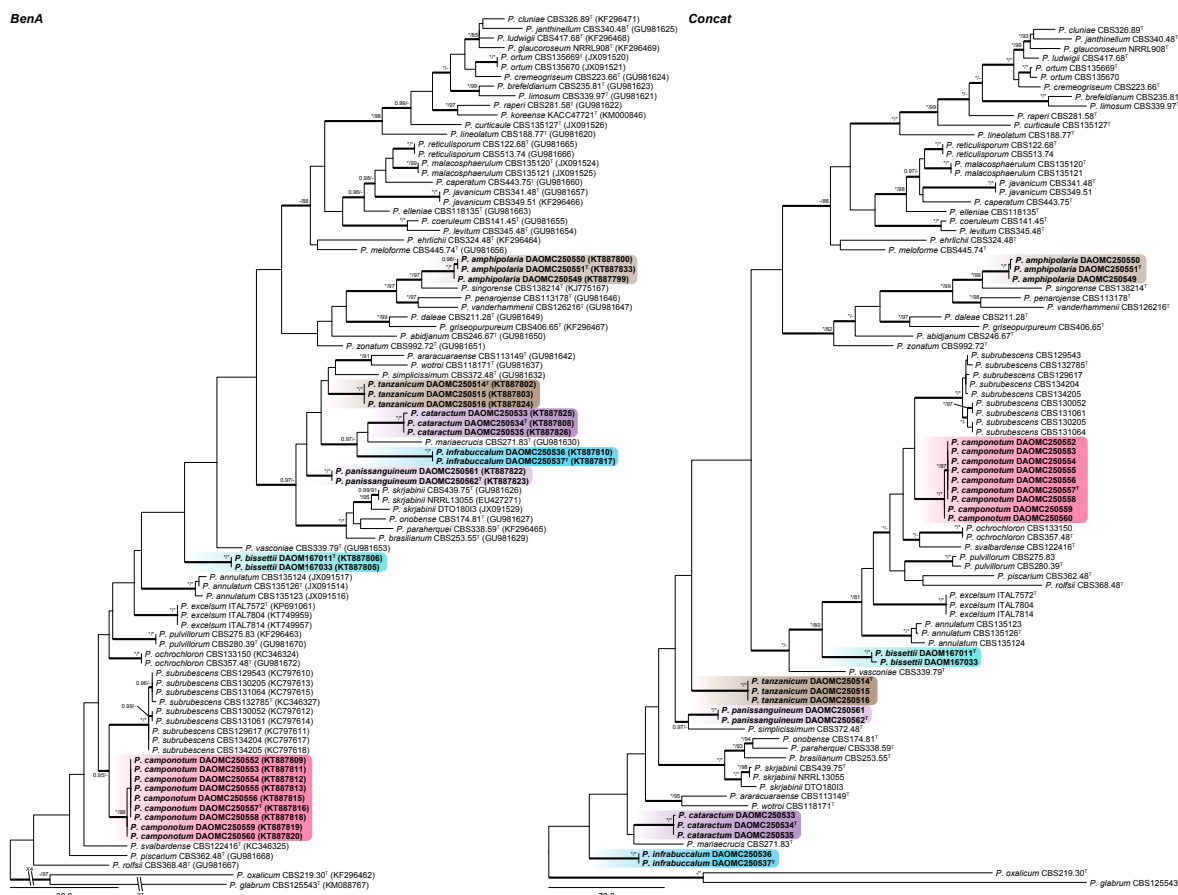


**Fig. 6** Phylogenetic trees for BenA & Concatenated (ITS, BenA & CaM) datasets of *Penicillium* sect. *Exilicaulis*. Branch support in nodes higher than 80 % bs and/or 0.95 pp are indicated above thickened branches (\* = ex-type; \* = 100 % bs or 1.00 pp; - = support lower than 80 % bs and/or 0.95 pp). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets. Taxon labels in grey, indicate unpublished new species from Visagie et al. (2016b).





**Fig. 7** Phylogenetic trees for ITS & CaM datasets of *Penicillium* sect. *Lanata-Divariata*. 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). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.



**Fig. 8** Phylogenetic trees for *BenA* & Concatenated (ITS, *BenA* & *CaM*) datasets of *Penicillium* sect. *Lanata-Divariata*. 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). New species are indicated by coloured blocks. GenBank accession numbers are provided between brackets.

### Section *Exilicaulis* — Fig. 5, 6

Three new species are introduced in section *Exilicaulis*. Sequence datasets contain some undescribed species from the fynbos, South Africa (in grey, Visagie et al. 2016b). ITS generally has poor resolution in this section. Of the new species, *P. diabolicalicense* and *P. aotearoae* share identical sequences with previously known species, whereas *P. fundyense* has a unique ITS sequence. The *BenA* and *CaM* genes distinguish the new species from its close relatives. *Penicillium fundyense* sits in a clade with *P. cinerascens*, *P. citreonigrum* and *P. citreosulfuratum* (= *P. toxicarium* fide Serra et al. 2008), recently reviewed in Visagie et al. (2016b). The single strain DAOMC 250519 was sufficiently distinct from other strains in the section to propose a new species. *Penicillium aotearoae* and *P. diabolicalicense* is resolved in the *P. melinii* clade closely related to *P. melinii* and *P. xanthomelinii*.

### Section *Lanata-Divaricata* — Fig. 7, 8

ITS performs remarkably well for identification of the seven new species we introduce in section *Lanata-Divaricata*, with all

having unique sequences. *BenA* and *CaM* also easily distinguish all species of the section. The concatenated phylogeny resolved *P. amphipolaria* as a close relative of *P. singorensis*; *P. camponotum* as closely related to *P. subrubescens*; while *P. tanzanicum*, *P. bissettii*, *P. cataractum*, *P. infrabuccalum* and *P. panissanguineum* resolved in several clades on a poorly supported back bone typically having species with rough walled conidiophores such as *P. simplicissimum*.

### Extrolites

An overview of the extrolites detected during this study and their product ions are summarised in Table 2, while Table 3 summarises the extrolites produced by each species analysed. We also list extrolites produced by each species with their respective descriptions in the taxonomy section.

Six of the *Penicillium* species studied here produced andrastin metabolites in varying amounts. *Penicillium cataractum* and *P. infrabuccalum* were both major producers of andrastin A–D. *Penicillium nucicola*, *P. panissanguineum* and two strains of

**Table 2** Overview of the major extrolites detected and product ions.

Code	m/z	Formula	Product ion m/z				
andrastin A	487.2687	C <sub>28</sub> H <sub>38</sub> O <sub>7</sub>	243.1743	395.2229	427.1995	349.2160	225.1637
andrastin B*	487.2702	C <sub>28</sub> H <sub>40</sub> O <sub>7</sub>	427.2486	455.2455	457.2603	419.0410	
andrastin C	473.2898	C <sub>28</sub> H <sub>40</sub> O <sub>6</sub>	203.1431	179.0702	441.2272	381.2057	335.2004
andrastin D	429.2636	C <sub>26</sub> H <sub>36</sub> O <sub>5</sub>	397.2374	369.2425	379.2267	245.1899	351.2318
aurantioclavine	227.1543	C <sub>15</sub> H <sub>18</sub> N <sub>2</sub>	210.1277	171.0916	58.0660	130.0651	154.0651
austin <sup>b</sup>	501.2481	C <sub>27</sub> H <sub>32</sub> O <sub>9</sub>	381.2056	363.1950	409.2004	391.1901	335.2000
bisdechlorogeodin <sup>b</sup>	331.0812	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	287.0911	299.0549	272.0677	255.0649	317.0653
citrinin	251.0915	C <sub>13</sub> H <sub>14</sub> O <sub>5</sub>	233.0808	205.0860			
dehydrohistidyltryptophanyldiketopiperazine	322.1299	C <sub>17</sub> H <sub>15</sub> N <sub>5</sub> O <sub>2</sub>	193.0720	130.0652	162.0295		
fiscalin A	474.2137	C <sub>26</sub> H <sub>27</sub> N <sub>5</sub> O <sub>4</sub>	258.1237	199.0865	171.0916		
fiscalin C	488.2290	C <sub>27</sub> H <sub>29</sub> N <sub>5</sub> O <sub>4</sub>	213.1021	258.1236	185.1072	201.1018	
fumitremorgin B	480.2523	C <sub>27</sub> H <sub>33</sub> N <sub>3</sub> O <sub>5</sub>	383.1967	355.2018	406.1764	424.1865	462.2385
fusaperazine E	361.1580	C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub> S	245.0920	217.0970	153.0367	293.0953	69.0707
fusaperazine related	406.1795	C <sub>20</sub> H <sub>27</sub> N <sub>3</sub> O <sub>4</sub> S	107.0495	216.1382	359.1836	290.1133	291.1210
glandicoline B	420.1667	C <sub>22</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>	289.0716	335.1018	261.0767	334.0935	307.1086
marcfortine A	478.2699	C <sub>28</sub> H <sub>35</sub> N <sub>3</sub> O <sub>4</sub>	450.2748	419.2314	348.1232		
marcfortine B	464.2544	C <sub>27</sub> H <sub>33</sub> N <sub>3</sub> O <sub>4</sub>	436.2593	419.2327	405.2178		
meleagrins	434.1820	C <sub>23</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub>	334.0931	403.1639	318.0741	289.0718	306.0985
mevastatin <sup>b</sup>	391.2477	C <sub>23</sub> H <sub>34</sub> O <sub>5</sub>	185.1324	159.1167	211.1480	229.1585	235.1482
N,N-dimethylrestrictin <sup>b</sup>	366.2639	C <sub>21</sub> H <sub>35</sub> NO <sub>4</sub>	115.0757	149.1326	231.1743	263.2004	83.0499
N6-formyl-roquefortine C	418.1874	C <sub>23</sub> H <sub>23</sub> O <sub>3</sub> N <sub>5</sub>	333.0977	349.1164	332.0900	350.1243	
neoxaline	436.1978	C <sub>23</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>	336.1087	405.1794	280.1192	263.1187	145.0760
oxalicine A	504.2379	C <sub>30</sub> H <sub>33</sub> NO <sub>6</sub>	148.0392	216.0291	122.0602	234.0396	363.1601
oxalicine B	520.2332	C <sub>30</sub> H <sub>33</sub> NO <sub>7</sub>	148.0394	216.0292	122.0603	234.0398	218.0449
oxaline	448.1981	C <sub>24</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>	348.1090	417.1794	332.0894	349.1168	386.1612
penicillic acid	171.0652	C <sub>8</sub> H <sub>10</sub> O <sub>4</sub>	125.0598	112.0522	97.0652	111.0444	83.0498
penitrem A	634.2930	C <sub>37</sub> H <sub>44</sub> ClNO <sub>6</sub>	Confirmed by RT and isotope pattern, CYA media only				
pulvilloric acid*	295.1186	C <sub>15</sub> H <sub>20</sub> O <sub>6</sub>	195.0293	277.1083	177.0185	151.0389	251.1286
restrictin <sup>b</sup>	338.2324	C <sub>19</sub> H <sub>31</sub> NO <sub>4</sub>	231.1742	170.0811	161.1324	263.2004	306.2063
isofumigaclavine A	299.1753	C <sub>18</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	239.1542	196.1122	197.1077	144.0808	168.0806
isofumigaclavine B	257.1647	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O	239.1542				
roquefortine C	390.1925	C <sub>22</sub> H <sub>23</sub> N <sub>5</sub> O <sub>2</sub>	322.1296	193.0719	198.1280	334.1296	358.1661
roquefortine M	422.1821	C <sub>22</sub> H <sub>23</sub> O <sub>4</sub> N <sub>5</sub>	192.0642	205.0722	353.1120		
roquefortine N	440.1928	C <sub>22</sub> H <sub>25</sub> O <sub>5</sub> N <sub>5</sub>	192.0642	154.0611	210.0747	422.1823	
trichodermamide A (penicillazine) <sup>b</sup>	433.1242	C <sub>20</sub> H <sub>20</sub> N <sub>2</sub> O <sub>9</sub>	248.0552	220.0603	192.0653	176.0343	
trichodermamide C <sup>b</sup>	447.1397	C <sub>21</sub> H <sub>22</sub> N <sub>2</sub> O <sub>9</sub>	262.0709	234.0761	206.0812	219.0764	176.0342

\* Analyzed as [M-H]-1 in negative mode. All other ions are [M+H]+.

<sup>b</sup> Putative assignments based interpretation of MS2 fragmentation pathways.



*P. camponotum* also produced andrastin, while only lower levels of andrastin A and C were produced by *P. costaricense*. There was a correlation between the production of andrastins and a pulvilloric acid related compound. As explained in the Discussion, this compound is either an unreported analogue of pulvilloric acid or the structure of pulvilloric acid has been mischaracterised in the literature. Of all the andrastin producers, only *P. camponotum*, *P. costaricense* and *P. nucicola* did

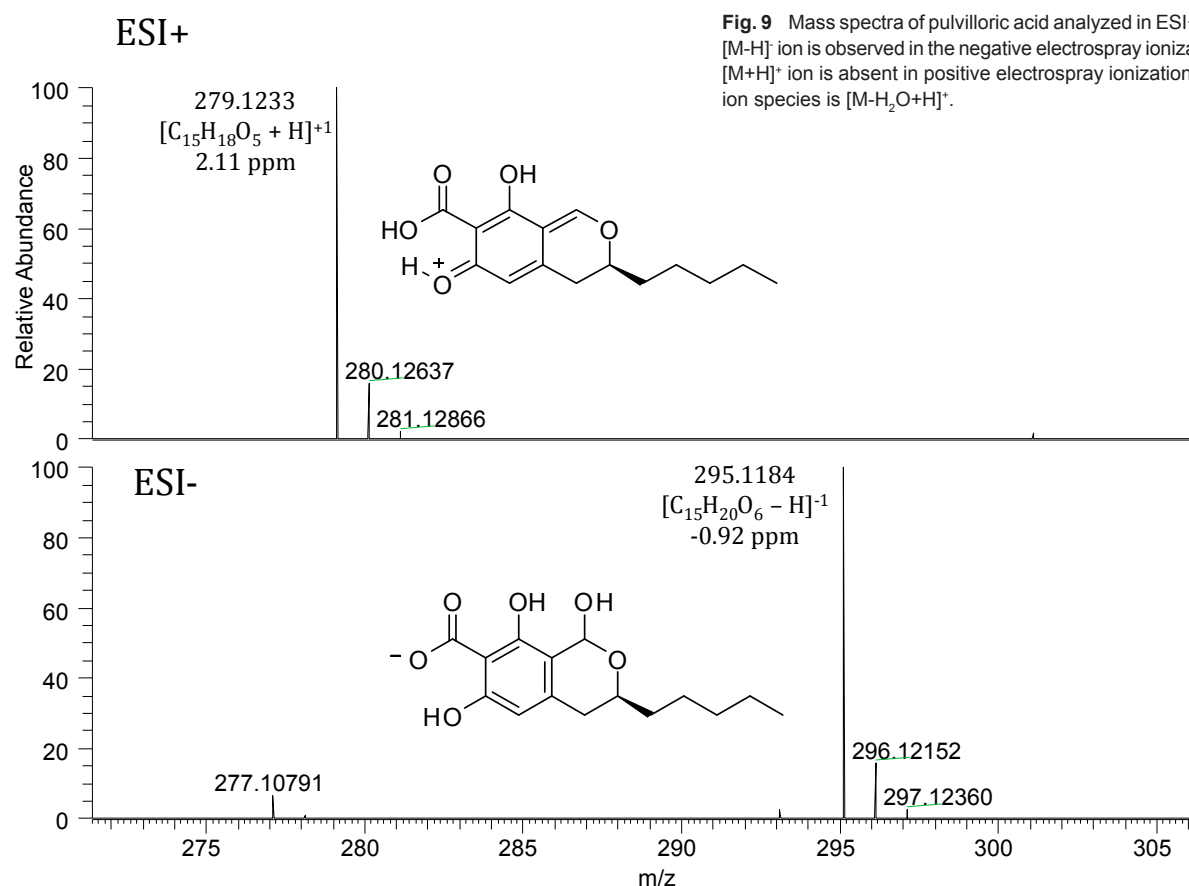
not produce this pulvilloric acid related compound. *Penicillium tanzanicum* also produced this compound.

Although all *P. camponotum* strains produced the indole alkaloid marcfortine A and B, their metabolite profiles could be separated into three groups that partly correlated with geographical origin. *Penicillium amphipolaria* was the only species that produced fumitremorgin compounds. The compounds produced on YES and CYA agar were fumitremorgin A, fumitremorgin C, dihydroxyfumitremorgin C and oxofumitremorgin B. When grown in

**Table 3** Overview of the extrolites produced by the strains of this study.

	<i>P. alogum</i>	<i>P. amphipolaria</i>	<i>P. aotearoae</i>	<i>P. bissettii</i>	<i>P. camponotum</i>	<i>P. cataractum</i>	<i>P. costaricense</i>	<i>P. corvianum</i>	<i>P. diabolicalcense</i>	<i>P. fundyense</i>	<i>P. improvisum</i>	<i>P. infrabuccalum</i>	<i>P. nucicola</i>	<i>P. parissanguineum</i>	<i>P. tanzanicum</i>
andrastin A	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-
andrastin B	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-
andrastin C	-	-	-	-	+	+	+	-	-	-	-	+	+	+	-
andrastin D	-	-	-	-	+	+	-	-	-	-	-	+	+	+	-
aurantioclavine	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
austin	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
bisdechlorogeodin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
citrinin <sup>c</sup>	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
dehydrohistidyl-tryptophanyl-diketopiperazine	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
fiscalin A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
fiscalin C	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
fumitremorgin B	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
fusaperazine E	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
fusaperazine related	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
glandicoline B	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
marcfortine A	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
marcfortine B	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
meleagrins <sup>c</sup>	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
mevastatin	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
N,N-dimethylrestricticin	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
N6-formyl-roquefortine C	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
neoxaline	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
oxalicine A	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
oxalicine B	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
oxaline	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
penicillic acid	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
penitrem A <sup>c</sup>	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
pulvilloric acid	-	-	-	-	-	+	-	-	-	-	-	+	-	+	+
restricticin	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
isofumigaclavine A <sup>c</sup>	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
isofumigaclavine B	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
roquefortine C <sup>c</sup>	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
roquefortine M	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
roquefortine N	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
trichodermamide C	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
trichodermamide A (penicillazine)	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
C <sub>11</sub> H <sub>15</sub> O <sub>3</sub> N	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
C <sub>18</sub> H <sub>22</sub> O <sub>5</sub>	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
C <sub>29</sub> H <sub>20</sub> O <sub>7</sub> S <sub>2</sub>	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
C <sub>20</sub> H <sub>30</sub> O <sub>6</sub> N <sub>2</sub>	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
C <sub>22</sub> H <sub>23</sub> O <sub>4</sub> N <sub>3</sub>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
C <sub>27</sub> H <sub>31</sub> O <sub>7</sub> N <sub>3</sub>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
C <sub>14</sub> H <sub>19</sub> O <sub>4</sub> N	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
C <sub>21</sub> H <sub>28</sub> O <sub>8</sub>	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C <sub>19</sub> H <sub>24</sub> O <sub>8</sub>	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
C <sub>20</sub> H <sub>20</sub> O <sub>6</sub>	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

<sup>c</sup> Confirmed by comparison to authentic standard.



**Fig. 9** Mass spectra of pulvilloric acid analyzed in ESI+ and ESI-. The intact [M-H]<sup>-</sup> ion is observed in the negative electrospray ionization mode. The intact [M+H]<sup>+</sup> ion is absent in positive electrospray ionization mode and the major ion species is [M-H<sub>2</sub>O+H]<sup>+</sup>.

CYA broth, other uncharacterised fumitremorgins were the extrolites produced. Very few extrolites were detected in cultures of *P. diabolicalicense*, but the fungal neurotoxin penitrem A was detected.

The two strains of *P. bissettii* produced significantly different extrolites. DAOMC 167033 produced the mycotoxin penicillic acid. DAOMC 167011 produced meleagrins, neoxaline and aurantioclavine along with other uncharacterised metabolites.

## TAXONOMY

### *Penicillium* section *Aspergilloides* — Fig. 1, 2

*Penicillium improvisum* Visagie, Malloch & Seifert, *sp. nov.* — MycoBank MB815769; Fig. 10

In: subgenus *Aspergilloides*, section *Aspergilloides*.

ITS barcode: KT887867. Alternative identification markers: *BenA* = KT887828, *CaM* = KT887789.

*Etymology.* Latin, *improvisum*, meaning unexpected/unforeseen, named for the conidiophores, which have randomly arranged branches.

*Type specimen.* CANADA, Ontario, Niagara falls, soil from oak/maple/ash forest, 28 Sept. 1996, *D. Malloch* (holotype DAOM 695768, culture ex-type DAOMC 250547 = CBS 140994 = KAS 2386 = W 156).

Colony morphology — Colony diam, 7 d, in mm: CYA 21–24; CYA 37 °C no growth; MEAb 24–30; YES 28–31; OA 18–22; CREA 14–15.

CYA 25 °C, 7 d: Colonies moderately deep, raised at centre, radially sulcate; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise (24D5–E5); soluble pigments absent; exudates absent; reverse greyish yellow (3B4), pale to yellowish grey (2A2–B2). MEA 25 °C, 7 d: Colonies low, plane; margins low, wide, irregular; mycelia white; texture velutinous; sporula-

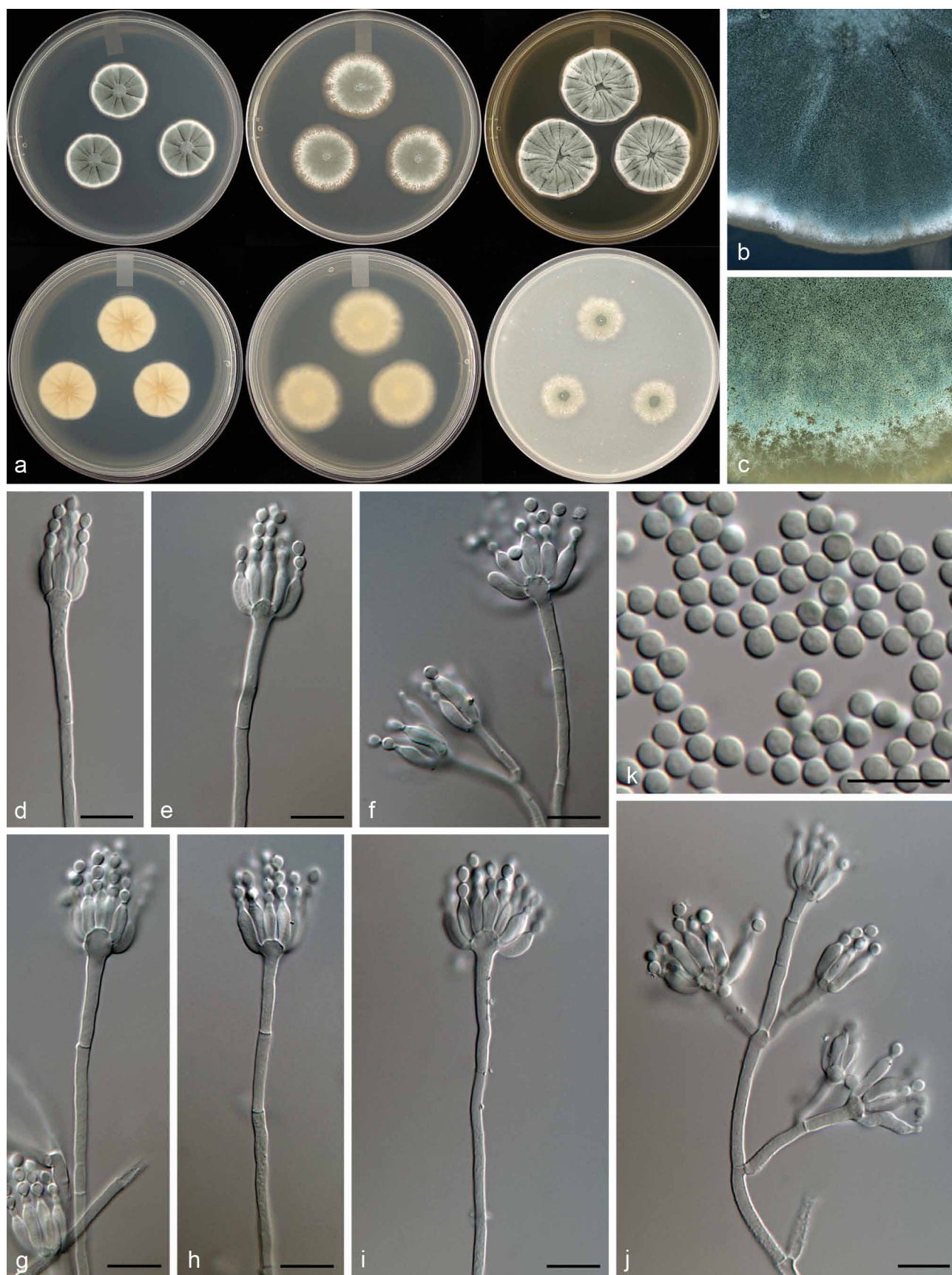
tion moderately dense, conidia *en masse* greyish turquoise to greyish green (24D4–E5–25E5); soluble pigments absent; exudates absent; reverse greyish yellow (2B3), greenish grey to dull green (1B2–29D4). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise (24D5–E5); soluble pigments absent; exudates absent; reverse greyish yellow (3B6), pale yellow (3A3). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise (24D4–E6); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid weakly produced.

*Conidiophores* monoverticillate, sometimes divaricate; *stipes* smooth, some roughened hyphae present, 40–200(–330) × 2.5–3.5 µm; *vesicle* 4.5–6.5 µm; *branches* 13–32.5 µm; *phialides* ampulliform, (3–)5–12 per stipe, 7.5–10.5 × 2.5–3.5 µm (8.9 ± 0.8 × 3.0 ± 0.2); *conidia* smooth walled, globose to subglobose, 2.5–3 × 2.5–3 µm (2.8 ± 0.1 × 2.8 ± 0.1), average width/length = 0.96, n = 43.

Extrolites — citrinin, isofumigaclavine A, isofumigaclavine B.

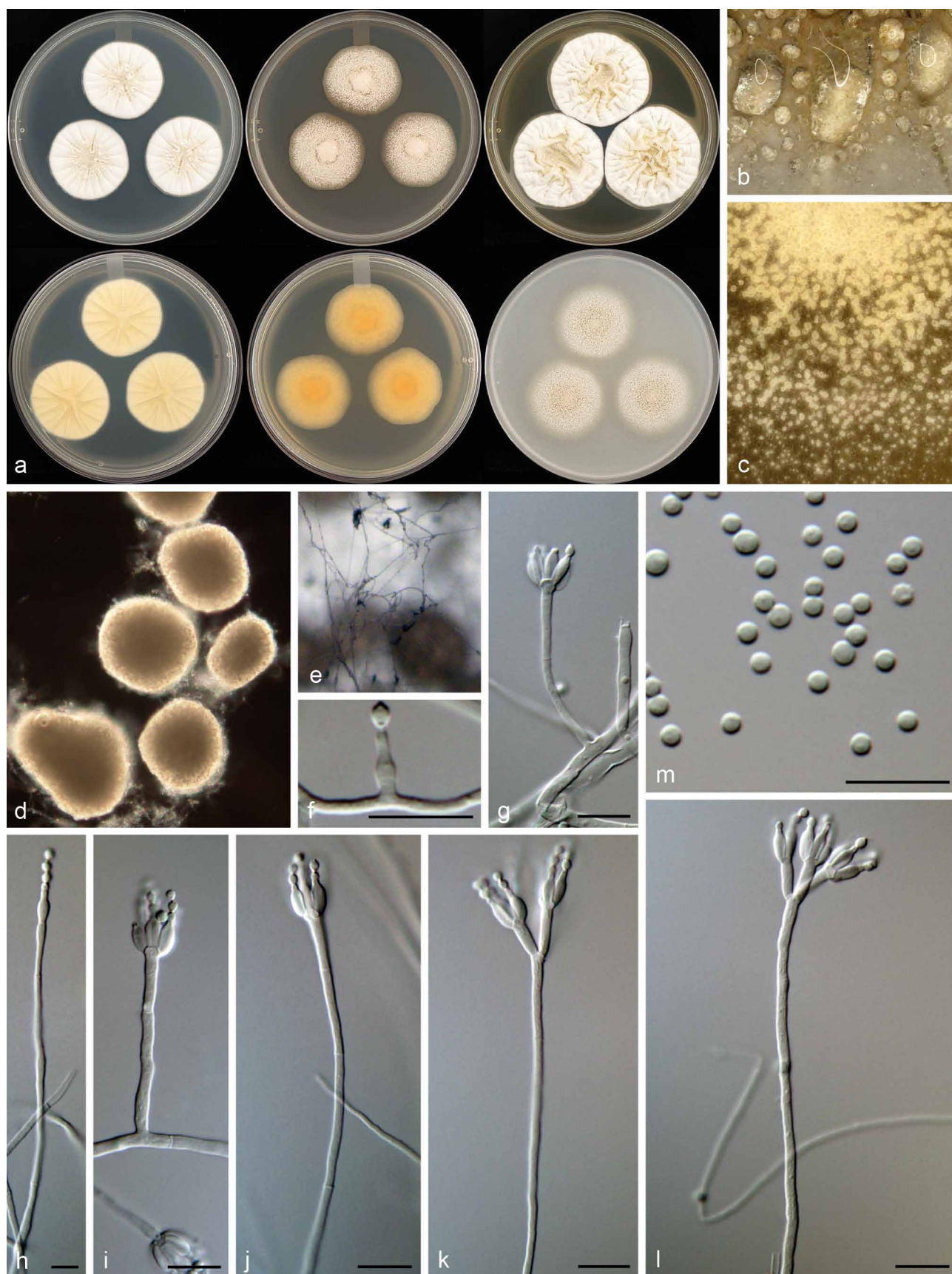
*Additional material examined.* CANADA, Niagara falls, soil from oak/maple/ash forest, 28 Sept. 1996, *D. Malloch*, culture DAOMC 250546 (= CBS 140993 = KAS 2381 = W 155), DAOMC 250548 (= CBS 140995 = KAS 2393 = W 154).

Notes — Phylogenetic analyses resolved *P. improvisum*, classified in section *Aspergilloides*, in a unique clade distinct from the 12 clades recognised by Houbraken et al. (2014) (Fig. 1, 2). Section *Aspergilloides* species are typically monoverticillate, but some do produce extra subterminal branches as observed in some conidiophores of *P. glabrum*, *P. armarii*, *P. athertonense* and *P. bussumense*. In addition, more complex patterns occur in *P. saturniforme* (biverticillate), *P. verhagenii* (biverticillate and divaricate) and *P. ranomafanaense* (divaricate). In common with the latter two, *P. improvisum* produces



**Fig. 10** Morphological characters of *Penicillium improvisum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10 µm.





**Fig. 11** Morphological characters of *Penicillium corvianum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d. sclerotia; e–l. conidiophores; m. conidia. — Scale bar: e–k = 10 µm.

divaricate conidiophores. However, *P. ranomafanaense* and *P. verhagenii* produce rough-walled conidia in contrast to the smooth-walled conidia of *P. improvisum*. Additionally, *P. verhagenii* produces larger conidia (3.5–4 µm) than *P. improvisum*, while *P. ranomafanaense* has more ellipsoidal conidia in comparison to the globose conidia of the new species.

### *Penicillium* section *Canescentia* — Fig. 3

*Penicillium corvianum* Visagie & Seifert, *sp. nov.* — MycoBank MB815770; Fig. 11

In: subgenus *Penicillium*, section *Canescentia*.

ITS barcode: KT887875. Alternative identification markers: *BenA* = KT887836, *CaM* = KT887797.

*Etymology.* Latin, *corvianum*, named after Corviano, Italy, where the isolates originate.

*Type specimen.* ITALY, Tuscany, Natural Monument Corviano, soil, 27 Aug. 2008, K.A. Seifert (holotype DAOM 695764, culture ex-type DAOMC 250517 = CBS 141000 = KAS 3618 = IT-2008-4-D).

Colony morphology — Colony diam, 7 d, in mm: CYA 30–35; CYA 37 °C no growth; MEAb1 (17–)28–32; YES 36–40; OA 30–32; CREA 21–23.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate, brown to brownish yellow sclerotia present; margins moderately deep, wide, entire; mycelia white; texture floccose; sporulation absent to sparse, conidia *en masse* dull green (26D4); soluble pigments absent; exudates clear; reverse yellowish white to pale yellow (2A2–3), orange (5A5). MEA 25 °C, 7 d: Colonies low, plane, creamish to brown sclerotia; margins low, narrow, entire; mycelia white; texture floccose; sporulation absent, sparse in some colonies, conidia *en masse* dull green (26D4); soluble pigments absent; exudates absent; reverse pale yellow to light orange (4A3–5A4), orange (6B7) in some colony areas. YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, crateriform, brownish sclerotia sparsely produced; margins low, narrow, entire; mycelia white; texture floccose; sporulation absent to sparse, conidia *en masse* greenish grey (26B2); soluble pigments absent; exudates clear; reverse brownish orange to light brown (5C4–7C8–D8), pale yellow to light yellow (4A3–4). OA 25 °C, 7 d: Colonies low, plane, cream to brown sclerotia produced; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse, conidia *en masse* greenish grey (26B2); soluble pigments absent; exudates clear. CREA 25 °C, 7 d: Weak growth, acid not produced.

*Conidiophores* on MEA very sparse, monoverticillate, minor proportion biverticillate and also phialides born directly on hyphae, on CYA biverticillate, sometimes monoverticillate; *stipes* smooth, (20–)100–400 × 1.5–2.5 µm; *metulae* divergent, 2–4 per stipe, (7.5–)9.5–15 × 2–3 (–3.5) µm; *phialides* ampulliform, when not monophialidic 3–8 per metula/stipe, 6–9 × 2–3 µm (7.3 ± 0.7 × 2.7 ± 0.3); average length metula/phialide 1.6; *conidia* smooth walled, globose to subglobose, 2–3 × 2–3 µm (2.1 ± 0.2 × 2.1 ± 0.2), average width/length = 0.96, n = 41. *Sclerotia* 130–265 × 110–230 µm.

Extrolites — fiscalin C, trichoderamide C, mevastatin, uncharacterised C<sub>18</sub>H<sub>22</sub>O<sub>5</sub>, C<sub>29</sub>H<sub>20</sub>O<sub>7</sub>S<sub>2</sub>, C<sub>11</sub>H<sub>15</sub>O<sub>3</sub>N.

*Additional material examined.* ITALY, Tuscany, Natural Monument Corviano, soil, 27 Aug. 2008, K.A. Seifert, culture DAOMC 250518 (= CBS 141001 = KAS 3622 = IT-2008-4-A).

*Notes* — *Penicillium corvianum* is classified in section *Canescentia*. Phylogenies (Fig. 3) resolve it in a larger clade with *P. canescens*, *P. jensenii* and *P. janczewskii*. Colonies of the new species are dominated by the presence of abundant cream to brown sclerotia. Sclerotia in the section are not a common

feature and are only known from *P. coralligerum* and *P. novae-zeelandiae*. However, sclerotia generally do not dominate colony appearance in the latter two species. Also, their conidiophores are consistently symmetrically biverticillate compared to the more diverse range of conidiophore branching observed in *P. corvianum*.

*Penicillium nucicola* Visagie, Malloch & Seifert, *sp. nov.* — MycoBank MB815771; Fig. 12

In: subgenus *Penicillium*, section *Canescentia*.

ITS barcode: KT887860. Alternative identification markers: *BenA* = KT887821, *CaM* = KT887782.

*Etymology.* Latin, *nucicola* (from *nux*), meaning living on nuts.

*Type specimen.* CANADA, Ontario, Niagara falls, fallen nuts of *Carya ovata*, May 1996, D. Malloch (holotype DAOM 695770, culture ex-type DAOMC 250522 = CBS 140987 = W 59 = KAS 2203).

Colony morphology — Colony diam, 7 d, in mm: CYA 20–23; CYA 37 °C no growth; MEAb1 12–14; YES 37–40; OA 17–20; CREA 13–15.

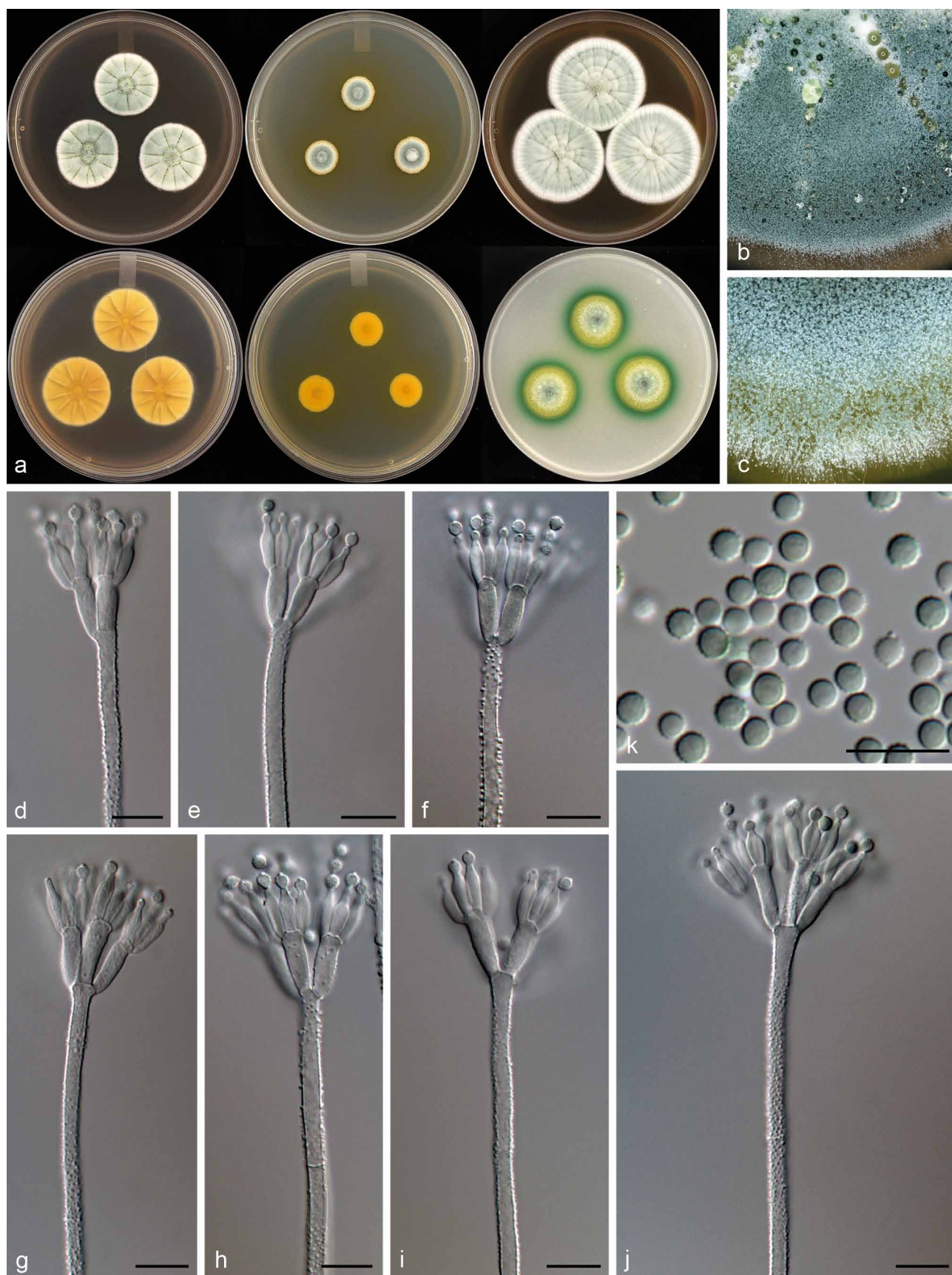
CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins moderately deep, wide, entire; mycelia white, inconspicuously yellow; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (26D5–E5–E6); soluble pigments reddish pink, yellow halo close to colonies, sometimes absent; exudates clear; reverse greyish yellow to olive (4B4–D7), pale yellow (4A3), sometimes deep green (27E8) at centre. MEA 25 °C, 7 d: Colonies low, plane; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (26D5–E5–E6); soluble pigments deep yellow, inconspicuous sometimes; exudates absent; reverse olive brown (4D6–E6), light yellow to greyish yellow (4A4–B6). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (26D5–E5–E6); soluble pigments reddish brown, yellow halo close to colonies, absent sometimes; exudates absent; reverse light yellow (3A5), greyish yellow (4C4). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white, yellow; texture floccose; sporulation moderately dense, conidia *en masse* greyish green (26D5–E5–E6); soluble pigments yellow close to colonies, deep green surrounding yellow pigment; exudates absent. CREA 25 °C, 7 d: Weak growth, acid moderately produced. *Conidiophores* biverticillate; *stipes* rough, some warted, 180–890 × 2.5–4 µm; *metulae* divergent, 2–6 per stipe, 10.5–15 × 3–4 µm; *phialides* ampulliform, 4–10 per metula, 7.5–10.5 × 2.5–3.5 µm (8.9 ± 0.8 × 2.9 ± 0.2); average length metula/phialide 1.4; *conidia* rough walled, some finely rough, globose to subglobose, 2.5–3 × 2.5–3 µm (2.8 ± 0.2 × 2.7 ± 0.2), average width/length = 0.97, n = 56.

Extrolites — andrastin A, andrastin B, andrastin C, andrastin D, austin, uncharacterised C<sub>20</sub>H<sub>20</sub>O<sub>6</sub>.

*Additional material examined.* CANADA, Ontario, 5 km NW of Milton, fallen nuts of *Fagus grandifolia*, 24 Oct. 1995, D. Malloch, DAOMC 250521 (= CBS 140973 = KAS 2101 = W 109).

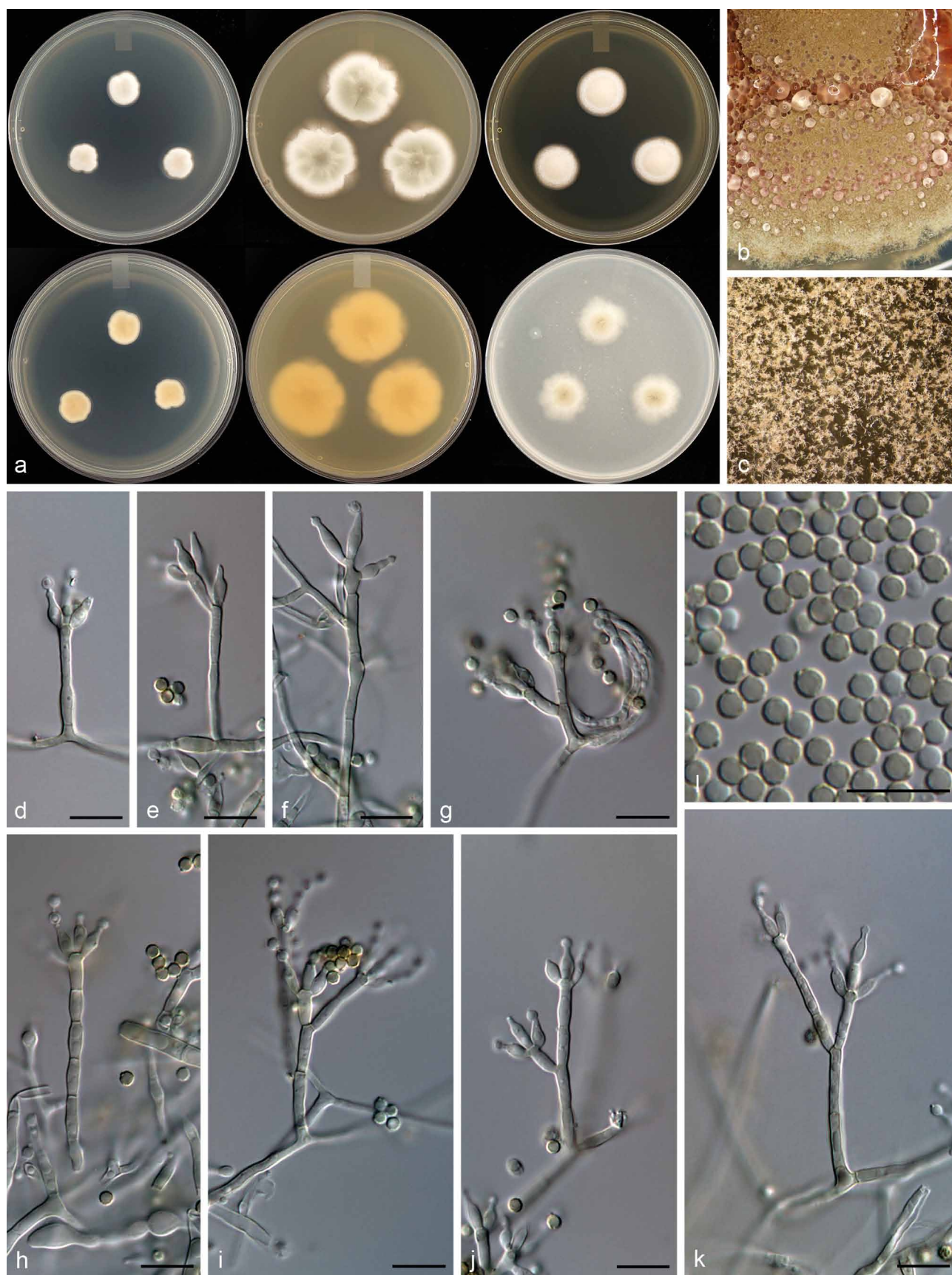
*Notes* — Classified in section *Canescentia*, *P. nucicola* sits on a well-supported branch distinct from all other species (Fig. 3). Morphologically it resembles *P. atrovenetum*, *P. antarcticum*, *P. coralligerum*, *P. novae-zeelandiae* and *P. herquei* (the latter classified in section *Sclerotiora*). These species commonly produce broad, symmetrical biverticillate conidiophores with bright yellow and green colours often evident in colonies, as observed for *P. nucicola* on OA (Fig. 12). However, the restricted growth on MEA (12–14 mm diam) easily distinguishes *P. nucicola* from all of these morphologically similar species.



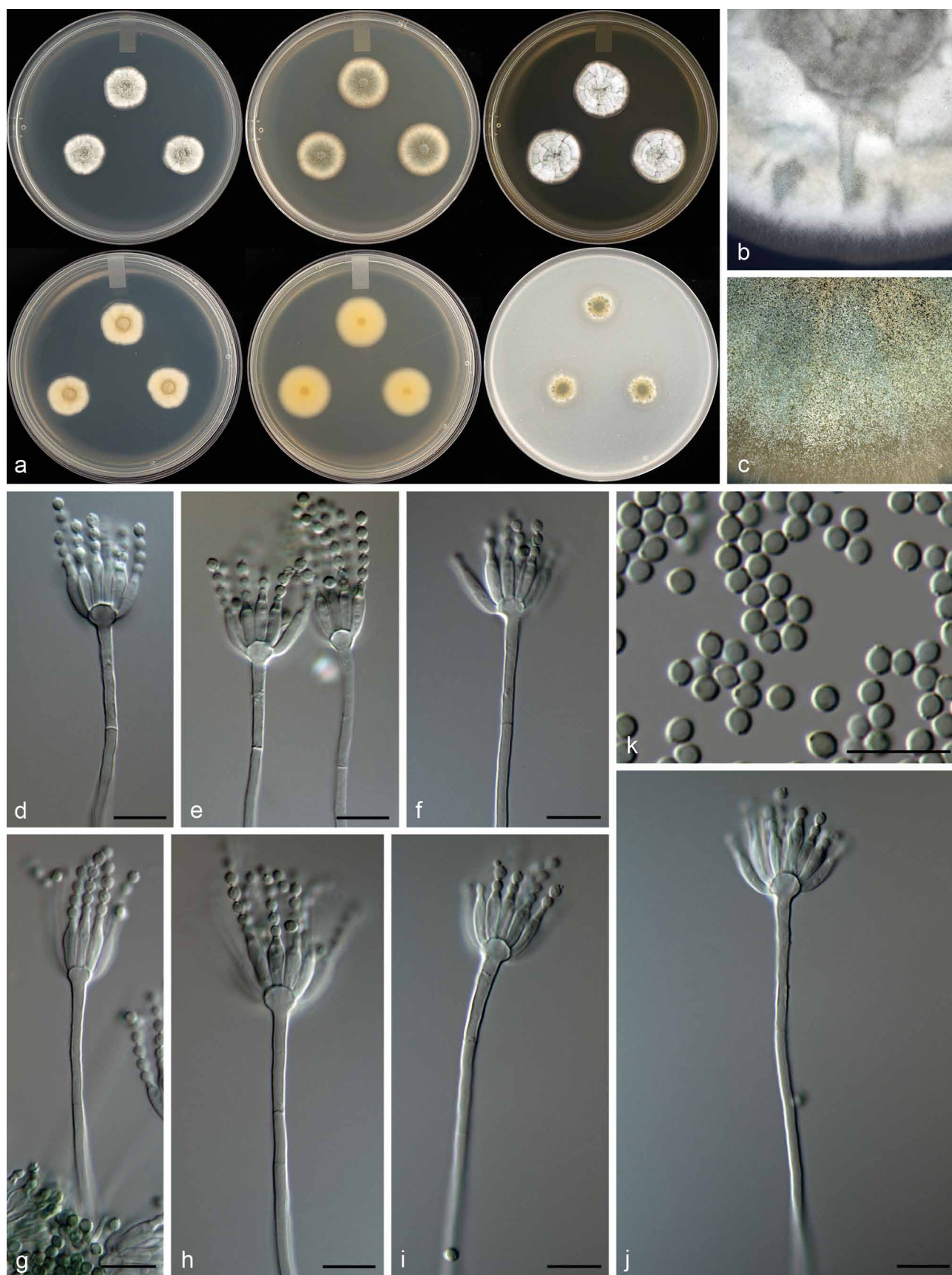


**Fig. 12** Morphological characters of *Penicillium nucicola*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10  $\mu$ m.





**Fig. 13** Morphological characters of *Penicillium alogum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–k. conidiophores; l. conidia. — Scale bar: d–l = 10 µm.



**Fig. 14** Morphological characters of *Penicillium costaricense*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10  $\mu$ m.



***Penicillium* section *Charlesia* & *Stolkia* — Fig. 4**

***Penicillium alogum*** Visagie, Frisvad & Seifert, *sp. nov.* — MycoBank MB815772; Fig. 13

*In:* subgenus *Aspergilloides*, section *Stolkia*.

*ITS barcode:* KT887869. Alternative identification markers: *BenA* = KT887830, *CaM* = KT887791.

*Etymology.* Latin, *alogum*, meaning irrational, irregular, nonsensical. Named for the irregular conidiophore branching observed in the species.

*Type specimen.* USA, Wyoming, Middle Mountain, Wind River Range, Tundra wetland soil, June 2001, *M. Christensen* (holotype DAOM 695759, culture ex-type DAOMC 250543 = CBS 140996 = IBT 23947 = KAS 2475).

**Colony morphology** — Colony diam, 7 d, in mm: CYA (15–)19–22; CYA 37 °C no growth; MEAbI 31–35; YES 21–22(–29); OA 22–25; CREA 12–14.

CYA 25 °C, 7 d: Colonies deep, radially and concentrically sulcate, crateriform in some isolates; margins low, narrow, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* greenish grey to dull green (29D2–3); soluble pigments absent; exudates absent, after two weeks inconspicuously red present; reverse dull to greyish yellow (3B4–C4), yellowish white (3A2). MEA 25 °C, 7 d: Colonies low, plane, sometimes slightly raised centrally; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense to sparse, conidia *en masse* greyish green to dark green (29E5–F5), in some a lighter greyish green (29D4); soluble pigments absent; exudates absent, after two weeks inconspicuously red present; reverse greyish green (29E5), pale yellow (2A3), yellowish grey (2B2). YES 25 °C, 7 d: Colonies deep, plane; margins low, narrow, entire; mycelia white, orange white (5A2–6A2); texture floccose; sporulation absent, conidia *en masse* not determined; soluble pigments absent; exudates absent; reverse greyish orange (5B5), yellowish white (4A2). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense near centre, conidia *en masse* greyish green (30E6); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

**Conidiophores** monoverticillate and divaricate, sometimes solitary phialides present; *stipes* smooth, inconspicuous greenish brown pigment, 10–43 × 2–3 µm; *metulae* divergent, 2 when present, of unequal length on the same conidiophore, 7–12(–20) × 2–3 µm; *phialides* ampulliform, sometimes solitary and elongated to almost acerose, mostly 3–5 per metula, 5.5–7(–8) × 2.5–3 µm (6.4 ± 0.5 × 2.7 ± 0.2); average length metula/phialide 1.7; *conidia* thick walled, finely rough walled, globose, 2.5–3 × 2.5–3 µm (2.7 ± 0.1 × 2.7 ± 0.1), average width/length = 0.96, *n* = 64.

**Extrolite** — uncharacterised C<sub>21</sub>H<sub>28</sub>O<sub>8</sub>.

**Additional material examined.** USA, Wind River Mountain, Wyoming, Tundra wetland soil, 1998, *M. Christensen*, culture DAOMC 250544 (= CBS 126173 = IBT 23948 = KAS 2476); Middle Mountain, Wind River Mountain, Wyoming, Tundra wetland soil, 1998, *M. Christensen*, culture DAOMC 250545 (= CBS 126150 = IBT 23950 = KAS 2477).

**Notes** — *Penicillium alogum* belong to section *Stolkia* and is closely related to *P. stolksiae*, *P. subarcticum*, *P. boreae*, *P. pullum* and *P. canariense* (Fig. 4). These species all produce conidiophores with some brown pigmentation. *Penicillium boreae* and *P. canariense* typically produce biverticillate conidiophores, while the others mainly have monoverticillate/simple conidiophore branching patterns. *Penicillium pullum* produces large, conspicuously rough walled conidia (3.5–4.5 µm) that turn dark with age, which easily distinguish it from *P. alogum*. On the other hand, *P. subarcticum* produces bigger conidia than the

new species (3–4 µm) (Peterson & Sigler 2002). From a morphological perspective, *P. alogum* is most similar to *P. stolksiae*, having similar colony colours, growth rates and conidiophore branching patterns. However, *P. stolksiae* produces longer phialides (7–10 µm) and slightly larger conidia (2.8–3.5 µm) fide Pitt (1980).

***Penicillium costaricense*** Visagie, M. Urb & Seifert, *sp. nov.* — MycoBank MB815773; Fig. 14

*In:* subgenus *Aspergilloides*, section *Charlesia*.

*ITS barcode:* KT887873. Alternative identification markers: *BenA* = KT887834, *CaM* = KT887795.

*Etymology.* Latin, *costaricense*, named after Costa Rica, the origin of the isolate.

*Type specimen.* COSTA RICA, Area de Conservación Guanacaste, Santa Rosa, intestines of *Rothschildia lebeau* (01-CACG-589) feeding on *Spondias mombin*, 19 Oct. 2001, *J. Diaz* (holotype DAOM 695765, culture ex-type DAOMC 250520 = CBS 140998 = KAS 2597 = 01-RGTHC-294).

**Colony morphology** — Colony diam, 7 d, in mm: CYA 15–17; CYA 37 °C no growth; MEAbI 19–21; YES 21–22; OA 11–12; CREA 6–7.

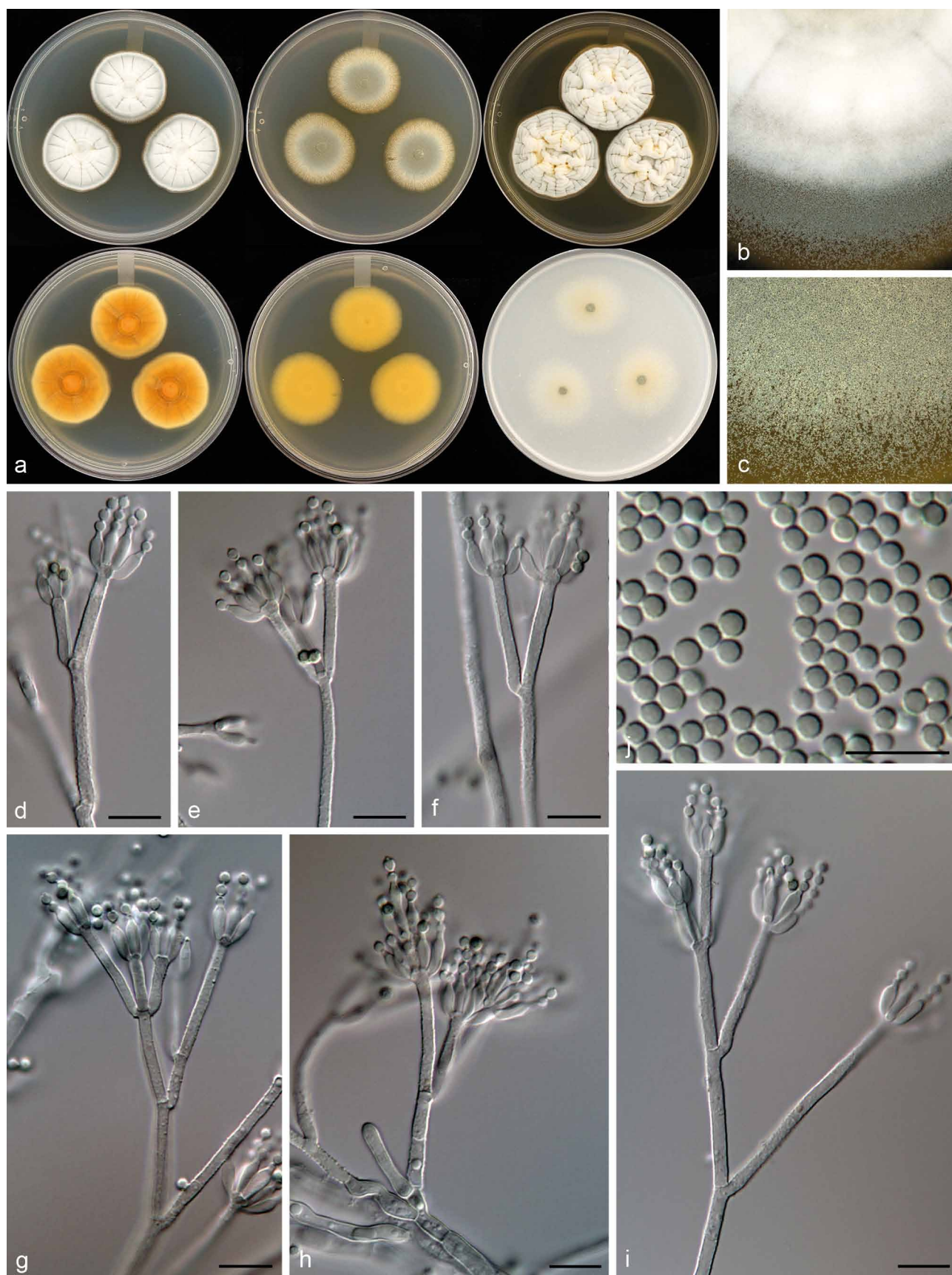
CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate, crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish turquoise to dull green (25C4–D3); soluble pigments absent; exudates absent; reverse greyish green to olive (1D4–2D4), yellowish white to pale yellow (3A2–3). MEA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green to dark green (25E5–F5); soluble pigments absent; exudates absent; reverse greyish yellow (2B3–C4–3C5). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish green to dull green (25C4–D4); soluble pigments absent; exudates absent; reverse olive (2D3–E3), light yellow to dull yellow (3A4–B3). OA 25 °C, 7 d: Colonies low, plane; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish green to dark green (25E5–F5); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

**Conidiophores** monoverticillate; *stipes* smooth, 50–280 × 2–3 µm; *vesicle* 4–6.5 µm; *phialides* ampulliform, 10–20 per stipe, 8.5–11.5 × 2–3.5 µm (9.7 ± 0.7 × 2.7 ± 0.3); *conidia* smooth walled, subglobose, 2.5–3 × 2–3 µm (2.6 ± 0.1 × 2.4 ± 0.1), average width/length = 0.94, *n* = 72.

**Extrolites** — andrastin A, andrastin C, uncharacterised C<sub>11</sub>H<sub>15</sub>O<sub>3</sub>N.

**Notes** — *Penicillium costaricense* is known from only the one isolate and forms a subbasal branch distinct from the main body of section *Charlesia* and *P. georgiense*, the basal species in the section (Fig. 4). The new species produces monoverticillate conidiophores, readily distinguished from the biverticillate to divaricate conidiophores of *P. georgiense*. The same character also distinguishes it from *P. charlesii* and *P. fellutanum*. The remaining species, *P. coffeae*, *P. phoeniceum* and *P. indicum* all produce monoverticillate conidiophores. However, *P. indicum* (20–30 mm diam) and *P. phoeniceum* (5–8 mm diam) are able to grow on CYA at 37 °C, in contrast to *P. costaricense*, which does not grow at 37 °C. Microscopically, *P. costaricense* most closely resembles *P. coffeae*, but the latter grows much slower on CYA and MEA (11–17 mm, 11–13 mm diam) than the new species.





**Fig. 15** Morphological characters of *Penicillium aotearoae*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–i. conidiophores; j. conidia. — Scale bar: d–j = 10 µm.

***Penicillium* section *Exilicaulis* — Fig. 5, 6**

***Penicillium aotearoae*** Visagie & Seifert, *sp. nov.* — MycoBank MB815774; Fig. 15

*In:* subgenus *Aspergilloides*, section *Exilicaulis*.

*ITS barcode:* KT887874. Alternative identification markers: *BenA* = KT887835, *CaM* = KT887796.

*Etymology.* Latin, *aotearoae*, from the Maori name for New Zealand, the origin of the isolate.

*Type specimen.* NEW ZEALAND, South Island, Arthurs Pass National Park, Devil's Punchbowl, isol. ex clay, light yellow brown, beneath moss and *Nothofagus*, 18 Feb. 2003, K.A. Seifert (holotype PDD 107543, culture ex-type DAOMC 250538 = CBS 140999 = KAS 3088).

Colony morphology — Colony diam, 7 d, in mm: CYA 30–32; CYA 37 °C no growth; MEAbI 29–30; YES 35–37; OA 33–35; CREA 18–19.

CYA 25 °C, 7 d: Colonies low to moderately deep, radially and concentrically sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* greyish turquoise to greyish green (24D4–25D4); soluble pigments absent; exudates clear to absent; reverse brown (6D7), greyish orange (5B6), light yellow (4A5), greyish green (28C4). MEA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* greyish turquoise to greyish green (24D4–25D4); soluble pigments inconspicuously yellow; exudates absent; reverse dull yellow to olive yellow (3B4–D6). YES 25 °C, 7 d: Colonies deep, radially and concentrically sulcate, crateriform; margins low, narrow, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* greyish green (25B3–C4); soluble pigments inconspicuously yellow; exudates absent; reverse brown (7E7), orange to brownish orange (6B8–C8), yellowish orange (4A6). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* dull green (26E4); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Strong growth, acid weakly produced.

*Conidiophores* biverticillate, divaricate, some monoverticillate; *stipes* rough to finely rough, 115–180 × 2–3 µm; *branches* 16.5–46 µm; *metulae* divergent, 2–5 per stipe, 9.5–24 × 2–3 µm; *phialides* ampulliform, 6–20 per metula, 6.5–9 × 2–3 µm (7.5 ± 0.6 × 2.4 ± 0.2); average length metula/phialide 2.1; *conidia* finely rough walled, globose, 2–3 × 2–3 µm (2.5 ± 0.1 × 2.4 ± 0.1), average width/length = 0.96, n = 67.

Extrolites — uncharacterised C<sub>19</sub>H<sub>24</sub>O<sub>8</sub>.

*Notes* — *Penicillium aotearoae* is classified in section *Exilicaulis* and is closely related to *P. melinii*, *P. 'fynbos1'* (an undescribed species from the fynbos in South Africa, Visagie et al. 2016b) and *P. diabolicalicense* described below (Fig. 5, 6). The new species can be distinguished from the others based on colony growth rates. It grows more restrictedly than the fynbos species on MEA, but faster on MEA compared to *P. melinii*, and faster on CYA but more restricted on MEA compared to *P. diabolicalicense*.

***Penicillium diabolicalicense*** Visagie & Seifert, *sp. nov.* — MycoBank MB815775; Fig. 16

*In:* subgenus *Aspergilloides*, section *Exilicaulis*.

*ITS barcode:* KT887840. Alternative identification markers: *BenA* = KT887801, *CaM* = KT887762.

*Etymology.* Latin, *diabolicalicense*, meaning Devil's Punchbowl, the origin of type strain – diabolus, diaboli = devil; calix, calicis = drinking bowl.

*Type specimen.* NEW ZEALAND, Arthurs Pass National Park, Devil's Punchbowl, light yellow brown, beneath moss and *Nothofagus*, 18 Feb. 2003, Keith Seifert (holotype PDD 107542, culture ex-type DAOMC 250542 = CBS 140967 = KAS 1726).

Colony morphology — Colony diam, 7 d, in mm: CYA 20–25; CYA 37 °C no growth; MEAbI 36–40; YES 31–34; OA 30–31; CREA 20–21.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, narrow, entire; mycelia white, deep yellow; texture floccose; sporulation absent to sparse, conidia *en masse* not determined; soluble pigments absent; exudates absent; reverse brown (5F6), light brown (5D3), pale yellow to greyish yellow (4A3–B3). MEA 25 °C, 7 d: Colonies moderately deep, plane; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dark green (25F6–26F6); soluble pigments absent; exudates absent; reverse dull yellow to greyish green (3B3–29B3–C3), yellowish white (2A2–3A2). YES 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins moderately deep, narrow, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* greenish grey to greyish green (25B2–D4); soluble pigments absent; exudates absent; reverse greyish orange (5B6), pale yellow to light yellow to yellowish orange (4A3–6). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish green (25E5); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Strong growth, acid strongly produced.

*Conidiophores* biverticillate, often divaricate; *stipes* rough, 200–800 × 2–3 µm; *branches* 20–68 µm; *metulae* divergent, 2–4 per stipe, unequal length on same conidiophore, 12–26 × 2–3 µm; *phialides* ampulliform, 4–8 per metula, (5.5–)6.5–8(–9) × 2.5–3 µm (7.2 ± 0.7 × 2.7 ± 0.2); average length metula/phialide 2.1; *conidia* rough walled, subglobose, minor proportion globose, 2–3 × 2–2.5 µm (2.6 ± 0.2 × 2.5 ± 0.0), average width/length = 0.96, n = 41.

Extrolites — N,N-dimethylrestricticin (CYA), penitrem A (CYA), restricticin.

*Notes* — *Penicillium diabolicalicense* is classified in section *Exilicaulis* and is closely related to *P. melinii*, *P. 'fynbos1'* (Visagie et al. 2016b) and *P. aotearoae* described above (Fig. 5, 6). The new species can easily be distinguished from its close relatives based on its more restricted growth on CYA at 25 °C compared to the fynbos species, faster growth on MEA compared to *P. melinii*, and growth slower on CYA but faster growth on MEA compared to *P. aotearoae*.

***Penicillium fundyense*** Visagie, David Clark & Seifert, *sp. nov.* — MycoBank MB815776; Fig. 17

*In:* subgenus *Aspergilloides*, section *Exilicaulis*.

*ITS barcode:* KT887853. Alternative identification markers: *BenA* = KT887814, *CaM* = KT887775.

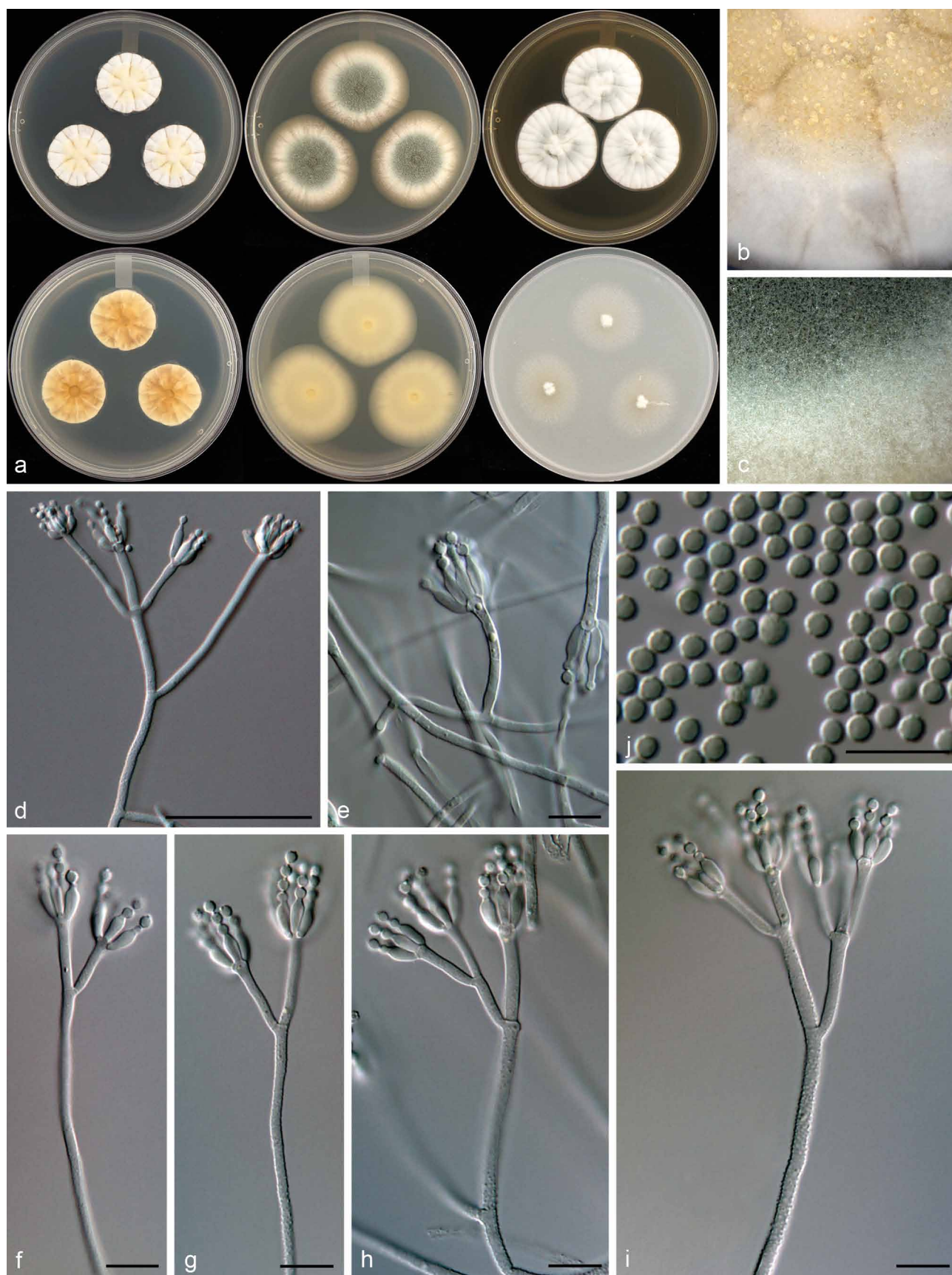
*Etymology.* Latin, *fundyense*, named after the Bay of Fundy, origin of the strain.

*Type specimen.* CANADA, New Brunswick, St. Andrews, Carpenter ants (*Camponotus pennsylvanicus*), Aug. 2000, D. Clark (holotype DAOM 695767, culture ex-type DAOMC 250519 = CBS 140980 = NBBR-2-3 = W466 = KAS 2174).

Colony morphology — Colony diam, 7 d, in mm: CYA 20–21; CYA 37 °C no growth; MEAbI 22–24; YES 28–30; OA 25–26; CREA 14–15.

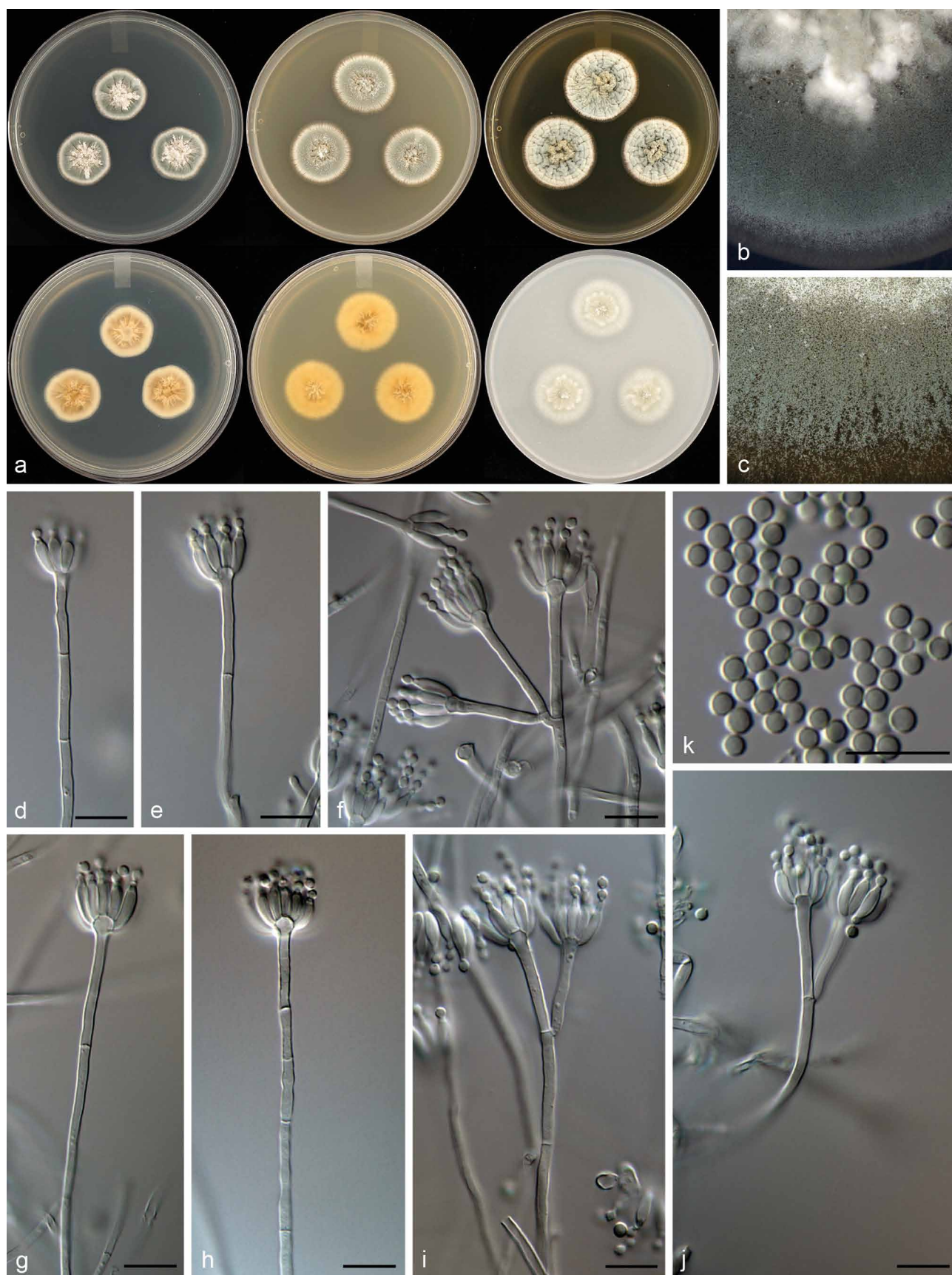
CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate, crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse*



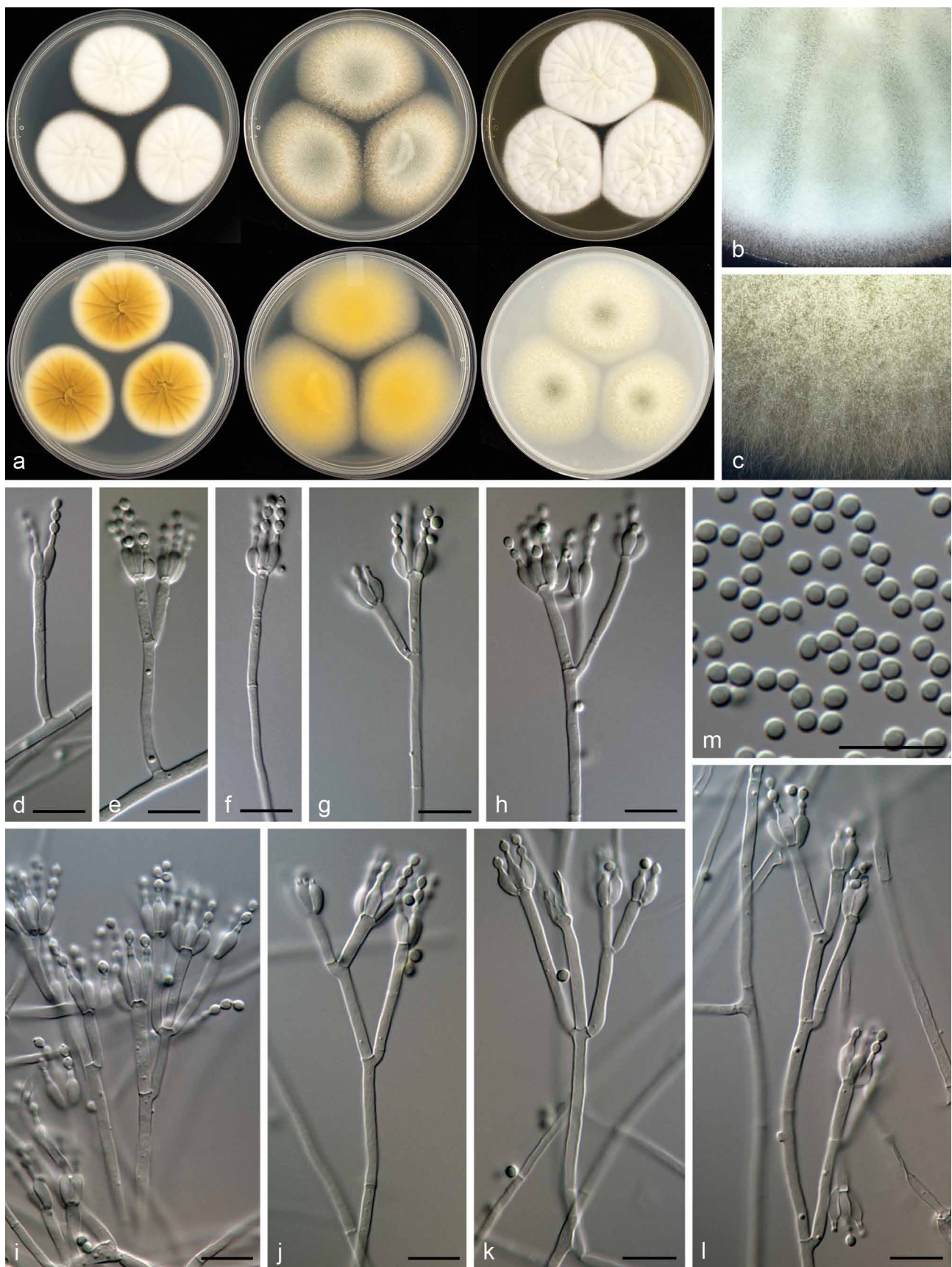


**Fig. 16** Morphological characters of *Penicillium diabolicalicense*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–i. conidiophores; j. conidia. — Scale bar: d = 50 µm, e–j = 10 µm.





**Fig. 17** Morphological characters of *Penicillium fundyense*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10  $\mu$ m.



**Fig. 18** Morphological characters of *Penicillium amphipolaria*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–l. conidiophores; m. conidia. — Scale bar: d–m = 10  $\mu$ m.



dull green to greyish green (25E4–5); soluble pigments absent; exudates absent; reverse olive (3D4), yellowish white (2A2). MEA 25 °C, 7 d: Colonies low, lightly sulcate centrally; margins low, narrow, entire; mycelia white; texture velutinous; sporulation moderately dense, conidia *en masse* dull green to greyish green (25E4–5); soluble pigments absent; exudates absent; reverse olive (3E4), greyish yellow (3B4–C4). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, crateriform; margins low, narrow, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish green (25B3–C4); soluble pigments absent; exudates deep yellow, inconspicuous; reverse brownish orange (5C6), light yellow to greyish yellow (4A4–B5). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous; sporulation sparse to moderately dense, conidia *en masse* greyish green to dull green (26C3–D3); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Strong growth, acid not produced.

*Conidiophores* monoverticillate, biverticillate and divaricate both common, often difficult to distinguish between branches and metulae; *stipes* smooth, 15–200(–400) × 2–2.5 µm; *branches* 16–30(–47) µm; *metulae* divergent, 2–4 per stipe, 16–30 × 2–2.5 µm; *phialides* ampulliform, 8–16 per metula, 6–8.5 × 2–2.5 µm (7.4 ± 0.7 × 2.4 ± 0.2); average length metula/phialide 3.3; *conidia* smooth walled, globose, 2–3 × 2–3 µm (2.4 ± 0.2 × 2.4 ± 0.2), average width/length = 0.96, n = 46.

Extrolites — dehydrohistidyltryptophanyldiketopiperazine, meleagrin, N6-formyl-roquefortine C, neoxaline, oxaline, roquefortine C, roquefortine M.

Notes — *Penicillium fundyense* is classified in section *Exiliacaulis*, with phylogenies resolving it in a clade with *P. cinerascens*, *P. citreonigrum* and *P. citreosulfuratum*. The species of this complex are almost impossible to distinguish morphologically, but chemically they represent distinct species, which resulted in Visagie et al. (2016b) suggesting the use of ITS and *BenA* sequences for identifications of strains in this clade. This approach is followed here, because *P. fundyense* shows only minor morphological differences from its close relatives, but is phylogenetically distinct from the other three.

### *Penicillium* section *Lanata-Divaricata* — Fig. 7, 8

*Penicillium amphipolaria* Visagie, Malloch & Seifert, *sp. nov.* — MycoBank MB815777; Fig. 18

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*.

ITS barcode: KT887872. Alternative identification markers: *BenA* = KT887833, *CaM* = KT887794.

*Etymology*. Latin, *amphipolaria*, meaning both poles, named to indicate the origin of the two strains on opposite sides of the Earth, close to the north and south poles.

*Type specimen*. ANTARCTICA, Quartermain Mountains of Antarctic Dry Valleys (–77.842741° 161.122229°), soil, Jan. 1998, W.C. Mahaney (holotype DAOM 695760, culture ex-type DAOMC 250551 = CBS 140997 = W 284 = KAS 2555).

Colony morphology — Colony diam, 7 d, in mm: CYA (30–)36–39; CYA 37 °C 5–7; MEAbI 50–52; YES 48–51; OA 45–50; CREA 24–29.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white, inconspicuously yellow; texture floccose; sporulation very sparse, moderate in the ex-type, conidia *en masse* dull green (26D4) in the ex-type; soluble pigments absent; exudates clear to absent; reverse greyish yellow to olive brown (4C6–D6), light yellow (2A4), pale yellow (3A3) in DAOMC 250550. MEA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white;

texture floccose and velutinous; sporulation moderately dense, conidia *en masse* dull green (26D3–4); soluble pigments deep yellow, absent in DAOMC 250550; exudates absent; reverse yellow to olive yellow (3B6–C6), greenish grey (1B2) in KAS1396. YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, raised at centre; margins low, wide, entire; mycelia white, inconspicuously yellow; texture floccose; sporulation very sparse, moderately dense in the ex-type, conidia *en masse* greenish grey to dull green (26B3–D4); soluble pigments absent; exudates absent; reverse greyish yellow to olive brown (4C6–D6), light yellow (2A4), pale yellow (3A3) in DAOMC 250550. OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture floccose and velutinous; sporulation moderately dense, conidia *en masse* greenish white to dull green (26B2–D3–E3); soluble pigments yellow, absent in DAOMC 250550; exudates absent. CREA 25 °C, 7 d: Strong growth, acid moderately produced.

*Conidiophores* biverticillate and divaricate, minor proportion monoverticillate; *stipes* smooth, 240–460 × 2–2.5 µm; *branches* 12–42 µm; *metulae* divergent, 2–4 per stipe, 9.5–21 × 2–3 µm; *phialides* ampulliform, 3–6 per metula, 6.5–10 × 2–3 µm (7.4 ± 0.8 × 2.5 ± 0.8); average length metula/phialide 2.1; *conidia* smooth walled, mainly broadly ellipsoidal, some globose, 2–3 × 2–2.5 µm (2.4 ± 0.1 × 2.2 ± 0.1), average width/length = 0.9, n = 78.

Extrolites — fumitremorgin B, fusaperazine E, fusaperazine related (C<sub>20</sub>H<sub>27</sub>N<sub>3</sub>O<sub>4</sub>S), oxaline, uncharacterised C<sub>22</sub>H<sub>23</sub>O<sub>4</sub>N<sub>3</sub>, C<sub>27</sub>H<sub>31</sub>O<sub>7</sub>N<sub>3</sub>.

*Additional material examined*. CANADA, Quebec, peat moss factory, 2001, C. Duchaine, cultures DAOMC 250549 (= CBS 140965 = KAS 1392 = R-273), DAOMC 250550 (= CBS 140966 = KAS 1396 = R-293).

Notes — *Penicillium amphipolaria* is classified in section *Lanata-Divaricata* and is a close relative of *P. singorense* (Fig. 7, 8). The new species can easily be distinguished from its close relatives by its poor growth on CYA at 37 °C, in contrast to the strong growth of *P. singorense* (40–43 mm diam) (Visagie et al. 2014a). Also, *P. amphipolaria* has a more complex conidiophore branching pattern compared to the generally simple conidiophores of *P. singorense* (Visagie et al. 2014a).

*Penicillium bissettii* Visagie & Seifert, *sp. nov.* — MycoBank MB815778; Fig. 19

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*.

ITS barcode: KT887845. Alternative identification markers: *BenA* = KT887806, *CaM* = KT887767.

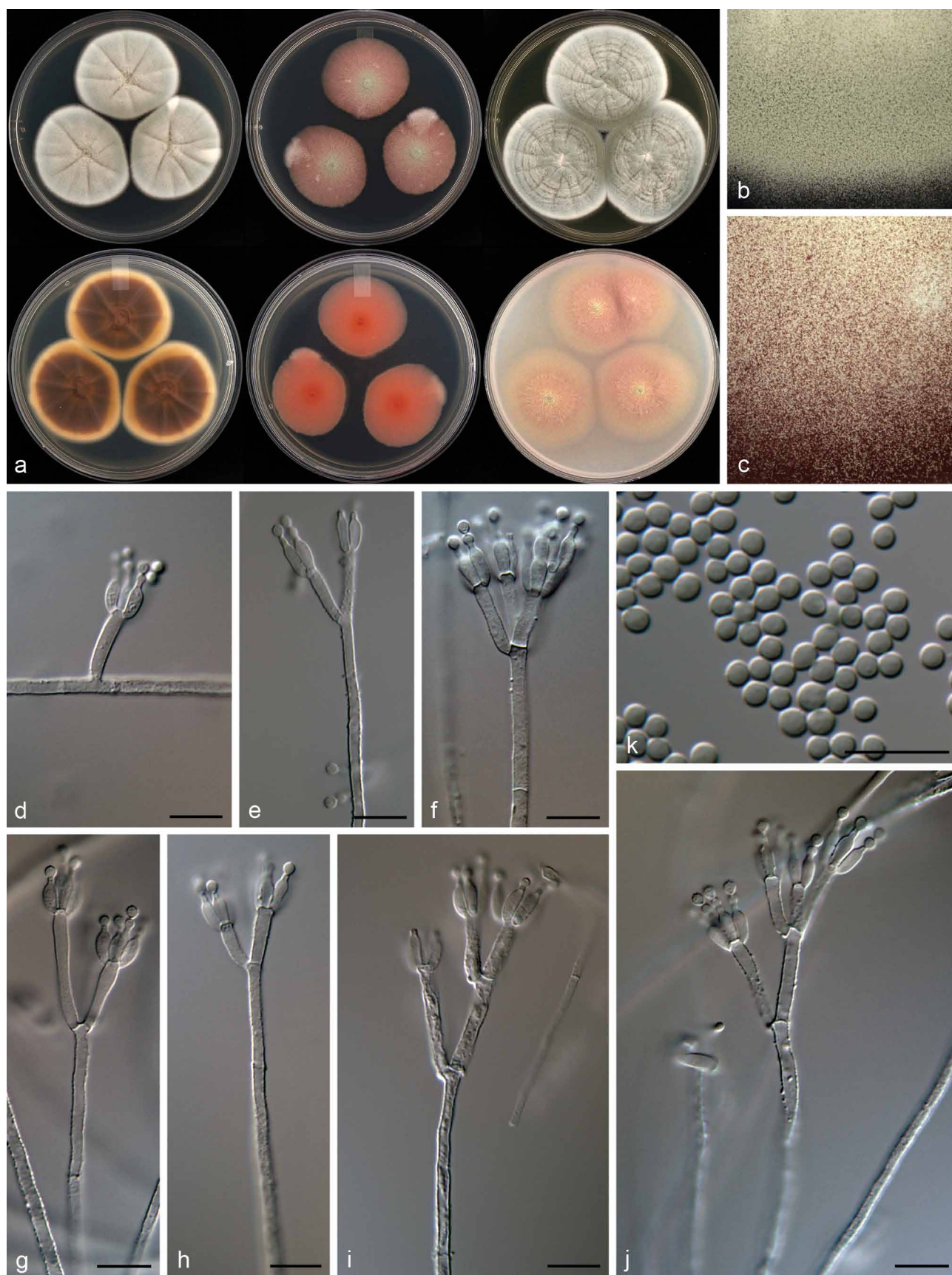
*Etymology*. Latin, *bissettii*, named after John Bissett, recently retired mycologist, AAFC Ottawa, who collected and isolated the strains, in honour of his contributions to soil fungus ecology.

*Type specimen*. CANADA, Quebec, near Lacolle, soil from Spruce forest, 5 Apr. 1977, J. Bissett (holotype DAOM 695761, culture ex-type DAOMC 167011 = CBS 140972 = KAS 1951).

Colony morphology — Colony diam, 7 d, in mm: CYA 42–43; CYA 37 °C no growth; MEAbI 35–43; YES 47–52; OA 45–48; CREA 23–26.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, sparse in DAOMC 167033, conidia *en masse* greyish green to dull green (26C3–D4), greenish grey (26B2); soluble pigments absent; exudates absent; reverse brown to dark brown (7E6–F6), yellowish white to greyish yellow (4A2–B4). MEA 25 °C, 7 d: Colonies low, plane, pinkish red underneath mycelial and sporulating areas; margins low, wide, entire; mycelia white, red submerged in media; texture floccose and velutinous; sporulation absent, sparse in some colonies, conidia *en masse* greyish green to dull green (26D4–E5),





**Fig. 19** Morphological characters of *Penicillium bissettii*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10 µm.

greenish grey (26B2); soluble pigments absent; exudates absent; reverse greyish red to reddish brown (8B6–D6). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, some strains sporulating in concentric rings; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, absent in some strains, conidia *en masse* dull green (26D4–E4), greenish white (26B2); soluble pigments absent; exudates absent; reverse brownish orange (6C5), light yellow to greyish yellow (4A3–B5). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture velutinous and floccose; sporulation sparse, conidia *en masse* greenish grey to greyish green (26B2–3); soluble pigments pink, absent in DAOMC 167033; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

*Conidiophores* biverticillate, terverticillate, minor proportion divaricate; *stipes* rough, 190–670 × 2.5–3.5 µm; *branches* 14–25 µm; *metulae* divergent, 3–4 per stipe or branch, 11–15.5 × 2.5–3.5 µm; *phialides* ampulliform, 3–6 per metula, 6.5–9 × 2.5–3 µm (7.5 ± 0.7 × 2.6 ± 0.2); average length metula/phialide 1.8; *conidia* smooth walled, globose to subglobose, 2–3 × 2–3 µm (2.6 ± 0.2 × 2.4 ± 0.1), average width/length = 0.92, n = 72.

Extrolites — DAOMC 167033: penicillic acid, uncharacterised C<sub>14</sub>H<sub>19</sub>O<sub>4</sub>N. DAOMC 167011: aurantioclavine, dehydrois-tidyltryptophanyldiketopiperazine, glandicoline B, meleagrin, oxalicine B, roquefortine C, roquefortine M, roquefortine N.

*Additional material examined.* CANADA, Quebec near Lacolle, soil from Spruce forest, 5 Apr. 1977, J. Bissett, culture DAOMC 167033 (= CBS 140971 = KAS 1949).

**Notes** — *Penicillium bissettii* is classified in section *Lanata-Divaricata* in a clade distantly related to all other species in the section. The multigene phylogeny places it closest to *P. vasconiae* and *P. annulatum* (Fig. 7, 8), but on a poorly supported backbone. *Penicillium bissettii* and *P. vasconiae* share the production of red mycelia submerged in the media (Ramírez & Martínez 1980). However, *P. vasconiae* has smooth walled conidiophores, in comparison with the roughened conidiophores of the new species. *Penicillium annulatum* grows well on CYA at 37 °C in contrast to *P. bissettii*, which does not grow at this temperature.

***Penicillium camponotum*** Visagie, David Clark & Seifert, *sp. nov.* — MycoBank MB815779; Fig. 20

*In:* subgenus *Aspergilloides*, section *Lanata-Divaricata*.

*ITS barcode:* KT887855. Alternative identification markers: *BenA* = KT887816, *CaM* = KT887777.

*Etymology.* Latin, *camponotum*, named in relation to genus of Carpenter ants that the species was isolated from.

*Type specimen.* CANADA, New Brunswick, St. Andrews, Carpenter ants (*Camponotus pennsylvanicus*), Aug. 2000, D. Clark (holotype DAOM 695762, culture ex-type DAOMC 250557 = CBS 140982 = NBBR-2-1 = W 471 = KAS 2177).

**Colony morphology** — Colony diam, 7 d, in mm: CYA (23–)37–45; CYA 37 °C no growth to microcolonies; MEAbI 48–55(–60); YES 53–60; OA 49–52; CREA 20–25.

CYA 25 °C, 7 d: Colonies moderately deep, lightly radially sulcate; margins low, narrow, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, absent in some strains, conidia *en masse* greyish green to dull green (25C4–E4–26E4); soluble pigments reddish brown, absent in some isolates; exudates clear; reverse greyish yellow (4B4–5), yellowish white to pale yellow (3A2–3), brownish orange (5C5–6C5) in isolates with soluble pigments. MEA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (26D3–4–E4); soluble pigments absent; exu-

dates absent; reverse greenish grey to greyish green (30B2–B3), yellowish white (2A2). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, wide, entire; mycelia white, inconspicuously pinkish grey; texture floccose; sporulation moderately dense, conidia *en masse* dull green to greyish green (25D4–26D4–E5); soluble pigments absent; exudates absent; reverse brownish orange (5C3), greyish orange (5B4–5), pale yellow to light yellow (4B3–5). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture floccose; sporulation absent to sparse to moderately dense, conidia *en masse* greyish green to dull green (26C3–D3–4); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid moderately produced.

*Conidiophores* biverticillate, minor proportion divaricate; *stipes* rough, 220–620 × 2.5–3.5 µm; *branches* 13–44 µm; *metulae* divergent, 2–4 per stipe, sometimes of unequal length on same conidiophore, 12–26 × 2.5–4 µm; *phialides* ampulliform, 4–8 per metula, 8–11.5 × 2.5–3.5 µm (9.5 ± 0.8 × 2.9 ± 0.2); average length metula/phialide 1.7; *conidia* rough to finely rough walled, globose, 2.5–3.5 × 2.5–3.5 µm (2.9 ± 0.1 × 2.9 ± 0.1), average width/length = 0.97, n = 72.

Extrolites — andrastin B, andrastin C, andrastin D, uncharacterised C<sub>15</sub>H<sub>22</sub>O<sub>2</sub> (only New Brunswick strains), andrastin A (only German strains), marcfortine A, marcfortine B (all strains).

*Additional material examined.* CANADA, New Brunswick, St. Andrews, Carpenter ants (*Camponotus pennsylvanicus*), Aug. 2000, D. Clark, culture DAOMC 250552 (= CBS 140975 = NBGP-17-4 = W 447 = KAS 2166), DAOMC 250553 (= CBS 140977 = NBGP-9-1 = W 451 = KAS 2169), DAOMC 250554 (= CBS 140978 = NBGP-1-1 = W 452 = KAS 2170), DAOMC 250555 (= CBS 140979 = NBGP-17-4B = W 463 = KAS 2172), DAOMC 250556 (= CBS 140981 = NBGP-23-1 = W 469 = KAS 2176), DAOMC 250558 (= CBS 140984 = NBGP-5-1 = W 477 = KAS 2182). — GERMANY, Grosse-Pfahl, Bayern, ant nest in *Picea abies*, Aug. 2000, D. Clark, DAOMC 250559 (= CBS 140985 = TP-4-1 = W 498 = KAS 2193); Pfahl Viechtach, Bayern, Carpenter ants (*Camponotus herculeanus*), Aug. 2000, D. Malloch, culture DAOMC 250560 (= CBS 140986 = TPF-8-3 = W 512 = KAS 2199).

**Notes** — *Penicillium camponotum* is classified in section *Lanata-Divaricata* as a close relative to *P. subrubescens* (Fig. 7, 8). However, the new species produces weak acid on CREA, which is absent in *P. subrubescens* (Mansouri et al. 2013). Also, *P. camponotum* has larger globose conidia compared to the smaller subglobose to broadly ellipsoidal conidia of *P. subrubescens*.

***Penicillium cataractum*** Visagie, Malloch & Seifert, *sp. nov.* — MycoBank MB815780; Fig. 21

*In:* subgenus *Aspergilloides*, section *Lanata-Divaricata*.

*ITS barcode:* KT887847. Alternative identification markers: *BenA* = KT887808, *CaM* = KT887769.

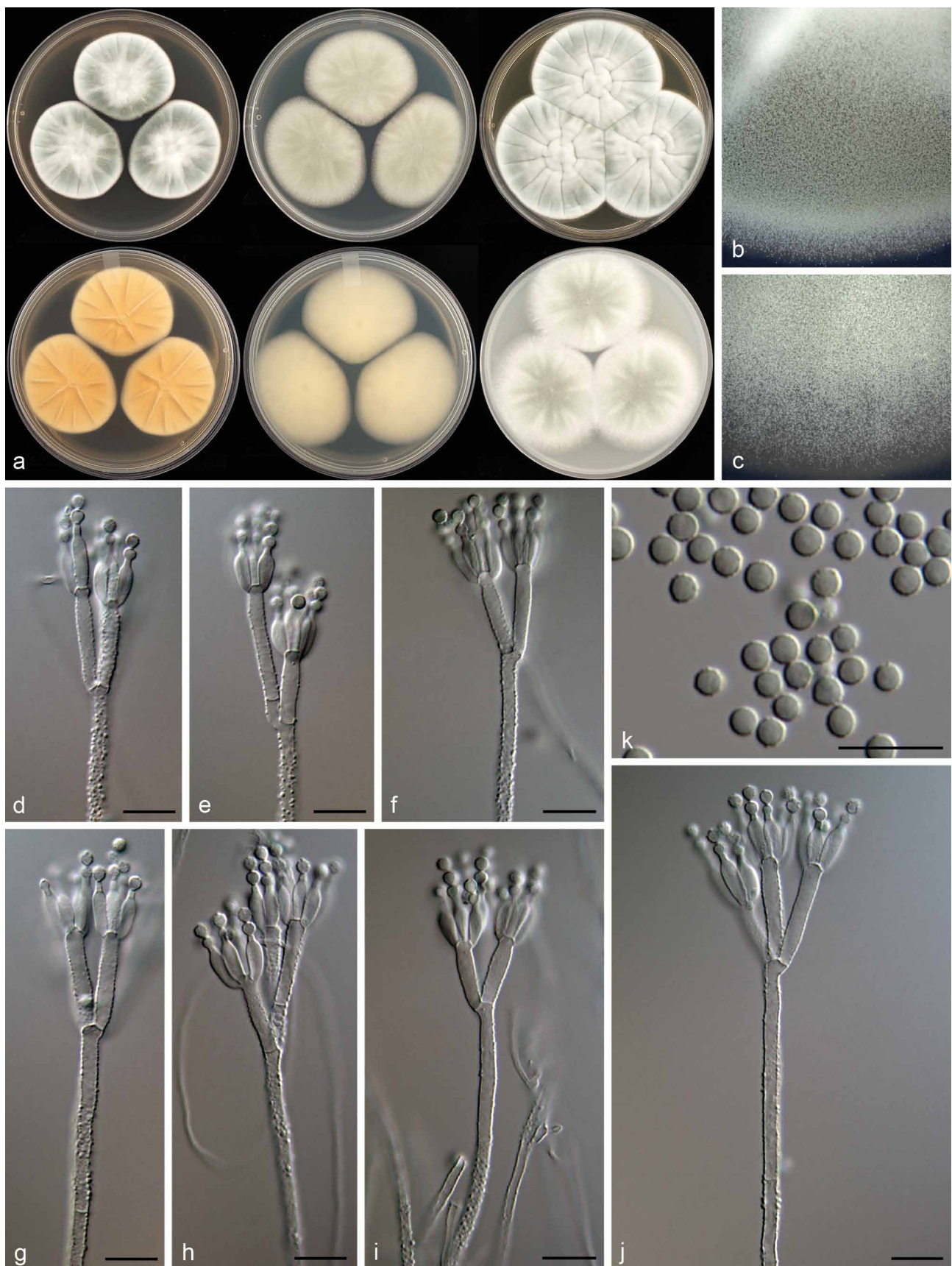
*Etymology.* Latin, *cataractum*, meaning waterfall, reflecting the origin of the strains, Niagara Falls, Ontario.

*Type specimen.* CANADA, Niagara falls, on fallen nuts of *Carya cordiformis*, May 1996, D. Malloch (holotype DAOM 695763, culture ex-type DAOMC 250534 = CBS 140974 = W 4 = KAS 2145).

**Colony morphology** — Colony diam, 7 d, in mm: CYA 34–42; CYA 37 °C no growth; MEAbI 15–25; YES 45–60; OA 28–37; CREA 13–15.

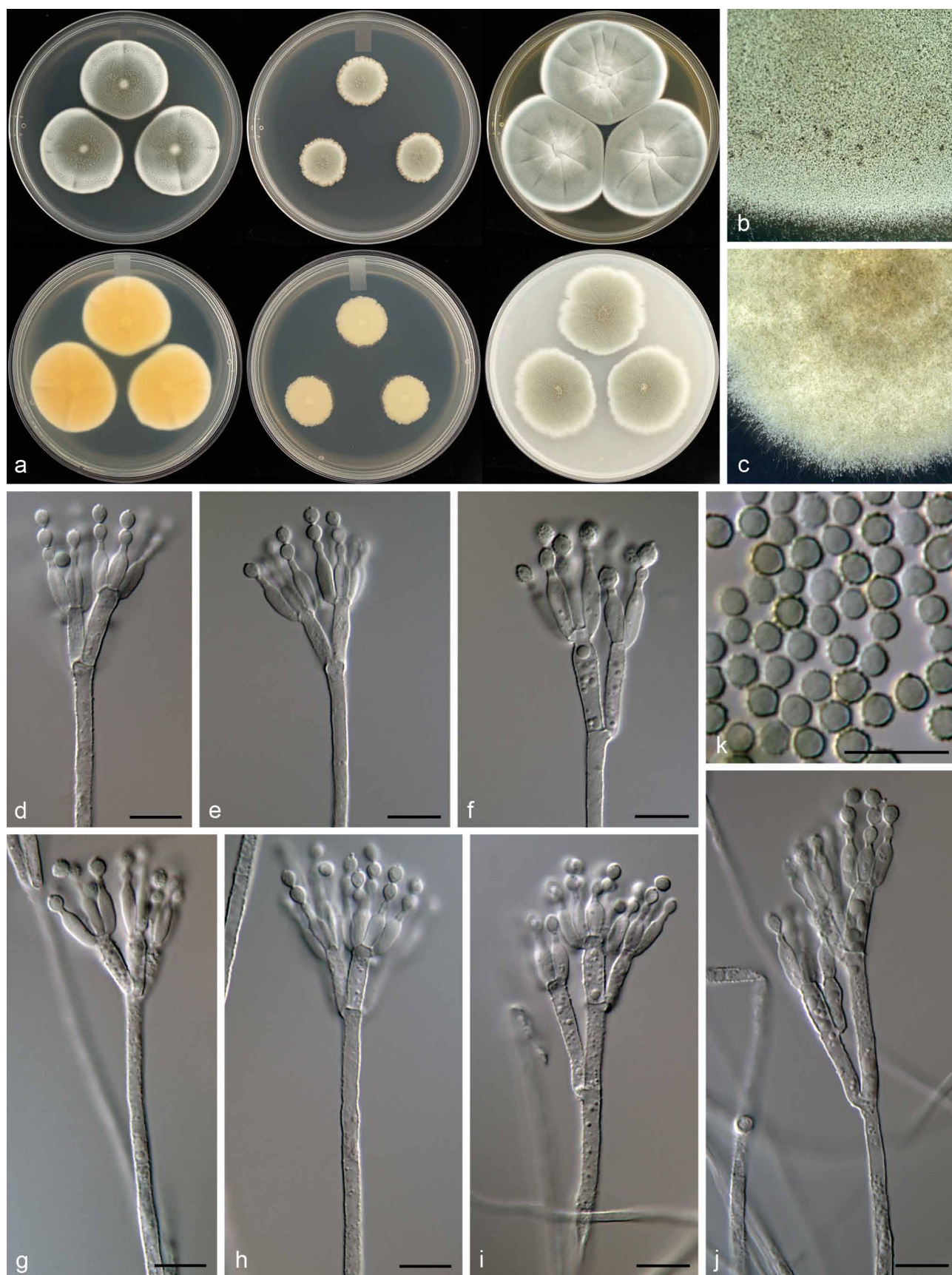
CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* dull green (26E4), greyish green (25C3); soluble pigments absent; exudates clear; reverse greyish yellow to greyish orange (4B4–5B4), pale yellow (4A2). MEA 25 °C, 7 d: Colonies low to moderately deep, plane; margins low, wide, irregular; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* dull green (25D4–E4); soluble pigments absent;



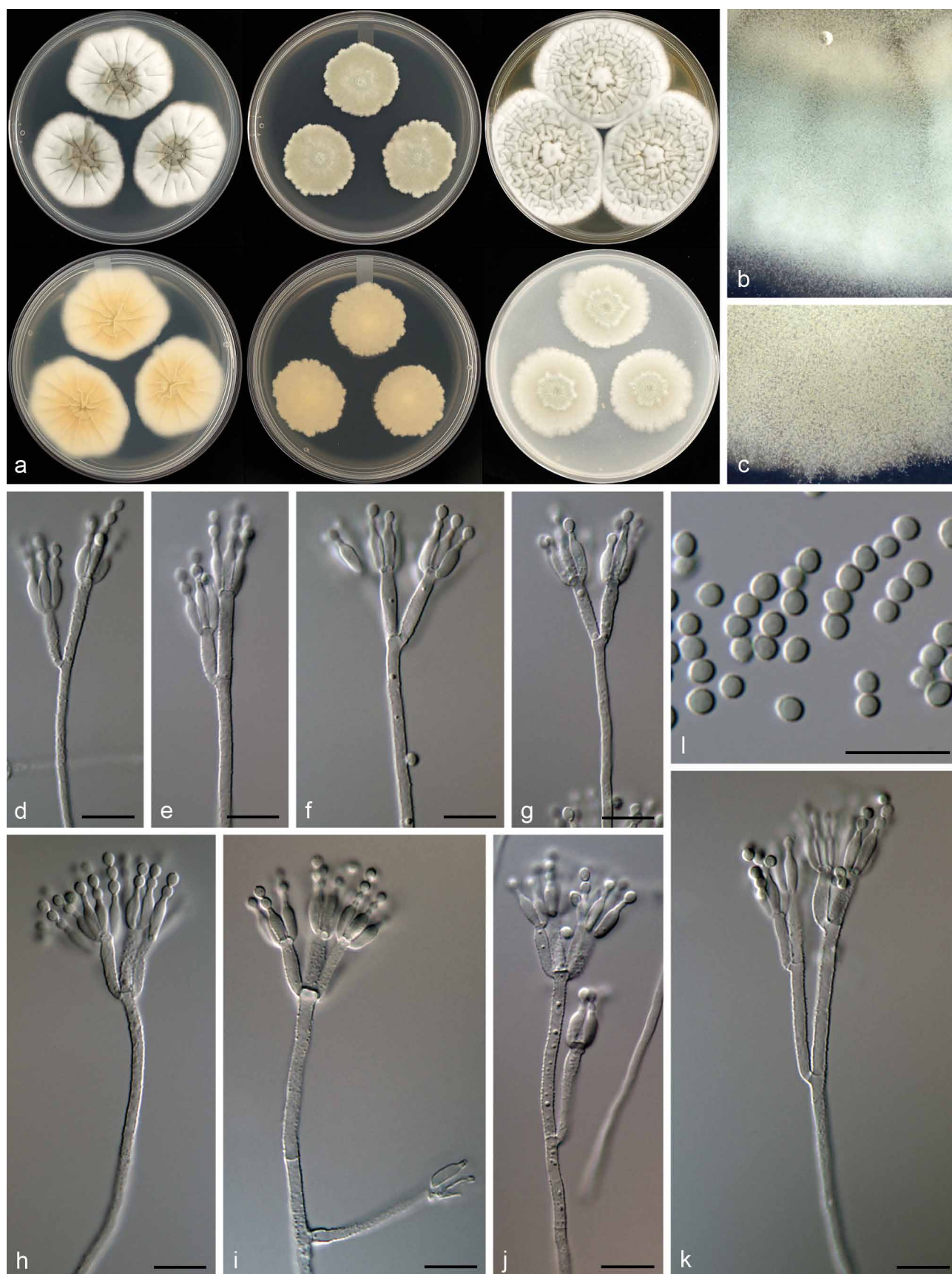


**Fig. 20** Morphological characters of *Penicillium camponotum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10  $\mu$ m.





**Fig. 21** Morphological characters of *Penicillium cataractum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10 µm.



**Fig. 22** Morphological characters of *Penicillium infrabuccalum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–k. conidiophores; l. conidia. — Scale bar: d–l = 10  $\mu$ m.



exudates absent; reverse greyish yellow to greyish orange (4B4–5B4), pale yellow (4A2), yellowish grey (4B2). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, raised at centre; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green to dull green (25C4–E5); soluble pigments absent; exudates absent; reverse greyish yellow (4B5), pale yellow to light yellow (4A3–4). OA 25 °C, 7 d: Colonies low, plane, orange colour in some isolates underneath sporulation; margins low, wide, entire; mycelia white; texture floccose and velutinous; sporulation sparse to moderately dense, conidia *en masse* dull green (26D4–E4); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Moderate growth, acid strongly produced.

*Conidiophores* biverticillate, terverticillate, minor proportion divaricate; *stipes* rough, (120–)200–650 × 3–4 µm; *branches* 13–30 µm; *metulae* divergent, 2–5 per stipe or branch, 11–20 × 3–4 µm; *phialides* ampulliform, 5–10 per metula, 8–10.5 × 3–3.5 µm (9.5 ± 0.7 × 3.1 ± 0.2); average length metula/phialide 1.4; *conidia* rough walled, globose to minor proportion broadly ellipsoidal, 3–3.5 × 3–3.5 µm (3.3 ± 0.1 × 3.3 ± 0.1), average width/length = 0.95, n = 60.

Extrolites — andrastin A, andrastin B, andrastin C, andrastin D, pulvilloric acid, trichodermamide A, trichodermamide C, uncharacterised C<sub>29</sub>H<sub>20</sub>O<sub>7</sub>S<sub>2</sub>, C<sub>20</sub>H<sub>30</sub>O<sub>6</sub>N<sub>2</sub>.

**Additional material examined.** CANADA, Niagara falls, on fallen nuts of *Carya ovata*, May 1996, D. Malloch, culture DAOMC 250533 (= CBS 140991 = W 6 = KAS 2270); Niagara falls, on fallen nuts of *Carya cordiformis*, May 1996, D. Malloch, culture DAOMC 250535 (= CBS 140992 = W 7 = KAS 2271).

**Notes** — *Penicillium cataractum* belongs to section *Lanata-Divaricata* and is a close relative of *P. mariae-crucis* and the new species *P. infrabuccalum* (Fig. 7, 8). *Penicillium mariae-crucis* produces sclerotia and reddish brown colonies, which are absent in both of the closely related new species. *Penicillium cataractum* has more restricted growth on MEA and has larger conidia than *P. infrabuccalum*.

***Penicillium infrabuccalum*** Visagie, David Clark & Seifert, *sp. nov.* — MycoBank MB815782; Fig. 22

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*.

**ITS barcode:** KT887856. Alternative identification markers: *BenA* = KT887817, *CaM* = KT887778.

**Etymology.** Latin, *infrabuccalum*, meaning from infrabuccal pockets of the Carpenter ant *Camponotus pennsylvanicus*, the niche of this fungus.

**Type specimen.** CANADA, New Brunswick, St. Andrews, Carpenter ants (*Camponotus pennsylvanicus*), Aug. 2000, D. Clark (holotype DAOM 695769, culture ex-type DAOMC 250537 = CBS 140983 = NBSM-6-2 = W 475 = KAS 2181).

**Colony morphology** — Colony diam, 7 d, in mm: CYA 37–45; CYA 37 °C no growth; MEAbI 29–31; YES 54–58; OA 35–37; CREA 28–30.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* dull green (26E4–27E3–4), greenish white (25A2); soluble pigments absent; exudates clear; reverse greyish orange (5B3), greyish yellow (4B3), pale yellow (3A2). MEA 25 °C, 7 d: Colonies low to moderately deep, plane; margins low, narrow, irregular; mycelia white; texture floccose; sporulation moderately dense to dense, conidia *en masse* dull green (26D3–27D3–4); soluble pigments absent; exudates absent; reverse greenish white (30A2), greenish grey to greyish green (30B2–3). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate; margins low, wide, entire; mycelia

white; texture floccose; sporulation sparse to moderately dense, conidia *en masse* greyish green (25B3–4–26B3–4); soluble pigments absent; exudates absent; reverse brownish orange (5C6), greyish yellow to greyish orange (4C3–5C3). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, irregular; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green to dull green (26C3–D4); soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

*Conidiophores* biverticillate, terverticillate, minor proportion divaricate; *stipes* rough, 210–640 × 2–3 µm; *branches* 20–30(–48) µm; *metulae* divergent, 2–5 per stipe or branch, 10–19 × 2.5–3.5 µm; *phialides* ampulliform, 5–10 per metula, 7.5–9.5 × 2–3 µm (8.3 ± 0.5 × 2.4 ± 0.2); average length metula/phialide 1.5; *conidia* finely rough walled, subglobose to broadly ellipsoidal, 2.5–3 × 2–3 µm (2.7 ± 0.2 × 2.4 ± 0.1), average width/length = 0.89, n = 49.

Extrolites — andrastin A, andrastin B, andrastin C, andrastin D, oxalicine A, oxalicine B, pulvilloric acid.

**Additional material examined.** CANADA, New Brunswick, St. Andrews, Carpenter ants (*Camponotus pennsylvanicus*), Aug. 2000, D. Clark, culture DAOMC 250536 (= CBS 140976 = NBSM-4-4 = W 449 = KAS 2168).

**Notes** — *Penicillium infrabuccalum* is classified in section *Lanata-Divaricata*. The single gene phylogenies resolved it in a clade with *P. mariae-crucis* and the new species *P. cataractum* (Fig. 7, 8). However, *P. mariae-crucis* produces sclerotia and reddish brown colonies, which are absent in both of the closely related new species. *Penicillium infrabuccalum* grows faster on MEA and has smaller conidia than *P. cataractum*.

***Penicillium panissanguineum*** Visagie, Ketch & Seifert, *sp. nov.* — MycoBank MB815783; Fig. 23

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*.

**ITS barcode:** KT887862. Alternative identification markers: *BenA* = KT887823, *CaM* = KT887784.

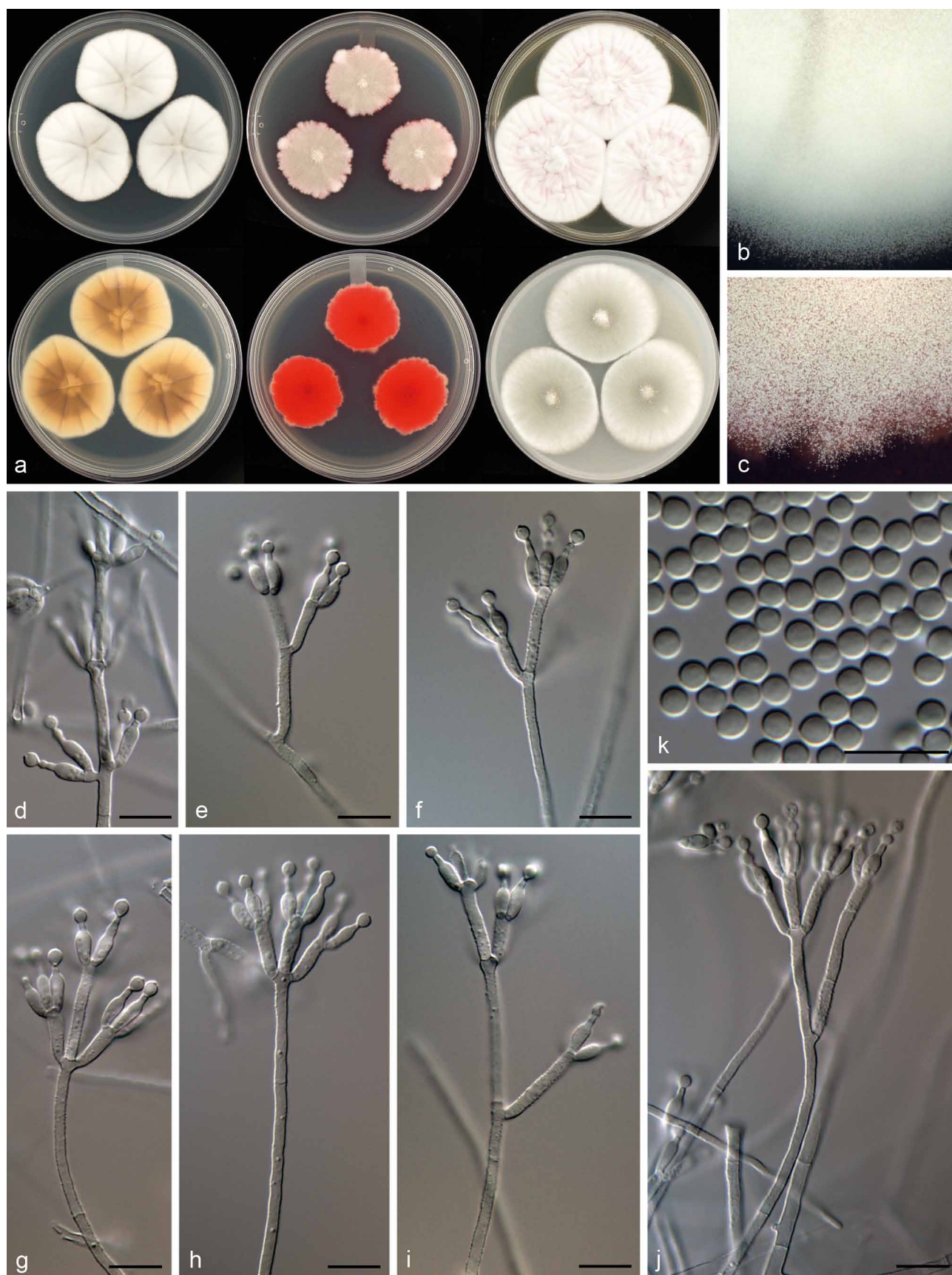
**Etymology.** Latin, *panissanguineum*, meaning blood of a chimpanzee, named in relation to chimpanzees that ate the soil from where strains were isolated and the red colony reverse on MEA.

**Type specimen.** TANZANIA, Kigoma region, soil related with termite mounds, Oct. 1996, coll. M.A. Huffman, isol. L. Ketch (holotype DAOM 695771, culture ex-type DAOMC 250562 = CBS 140989 = W 93 = KAS 2209).

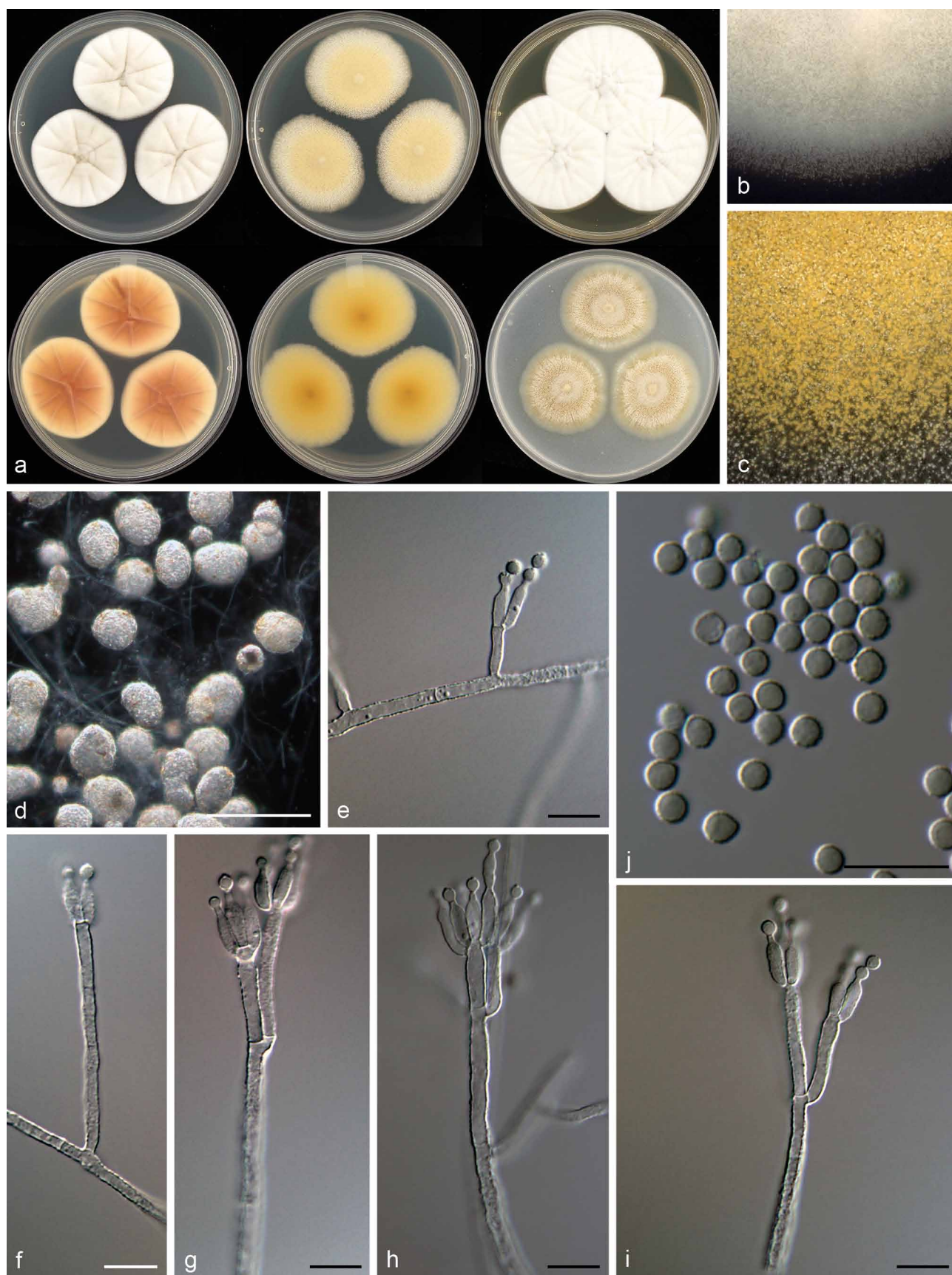
**Colony morphology** — Colony diam, 7 d, in mm: CYA 41–44; CYA 37 °C no growth; MEAbI 30–35; YES 48–55; OA 40–45; CREA 20–21.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greyish green to dull green (26C3–D4), greenish grey (26B2); soluble pigments absent; exudates absent; reverse brown to dark brown (7E6–F6), yellowish white to greyish yellow (4A2–B4). MEA 25 °C, 7 d: Colonies low, plane, pinkish red underneath mycelial and sporulating areas; margins low, wide, irregular; mycelia white; texture floccose and velutinous; sporulation moderately dense, conidia *en masse* greyish green to dull green (26D4–E5), greenish grey (26B2); soluble pigments pink; exudates absent; reverse greyish red to deep red (10B5–C8). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, raised at centre; margins low, wide, entire; mycelia white, pinkish areas present; texture floccose; sporulation very sparse, conidia *en masse* light grey; soluble pigments absent; exudates absent; reverse greyish red to red (10B5–7), pale yellow (4A3). OA 25 °C, 7 d: Colonies low, plane; margins low, wide, entire; mycelia white; texture floccose; sporulation moderately dense, conidia *en masse* greenish white to dull green (26B2–D3–E3);





**Fig. 23** Morphological characters of *Penicillium panissanguineum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d–j. conidiophores; k. conidia. — Scale bar: d–k = 10 µm.



**Fig. 24** Morphological characters of *Penicillium tanzanicum*. a. Colonies from left to right (top row) CYA, MEA, YES; (bottom row) CYA reverse, MEA reverse, YES reverse; b. colony texture on CYA; c. colony texture on MEA; d. sclerotia; e–i. conidiophores; j. conidia. — Scale bar: d = 50  $\mu$ m, e–j = 10  $\mu$ m.



soluble pigments pink and absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

*Conidiophores* biverticillate, minor proportion terverticillate and divaricate, solitary phialides sometimes present; *stipes* rough, 200–730 × 2–2.5 µm; *branches* 19.5–34 µm; *metulae* divergent, 2–4 per stipe or branch, 10–16.5(–21) × 2.5–3 µm; *phialides* ampulliform, 3–7 per metula, 6.5–10 × 2.5–3.5 µm (8.2 ± 0.8 × 2.8 ± 0.2); average length metula/phialide 1.6; *conidia* smooth walled, globose to broadly ellipsoidal, 2.5–3.5 × 2.5–3.5 µm (3.1 ± 0.2 × 3 ± 0.1), average width/length = 0.95, n = 76.

Extrolites — andrastin A, andrastin B, andrastin C, andrastin D, pulvilloric acid.

*Additional material examined.* TANZANIA, Kigoma region, soil related with termite mounds, Oct. 1996, coll. M.A. Huffman, isol. L. Ketch, culture DAOMC 250561 (= CBS 140988 = W 85 = KAS 2208).

Notes — *Penicillium panissanguineum* is classified in section *Lanata-Divaricata* and is in a clade of species that typically produce rough-walled conidiophores, such as *P. simplicissimum*. However, the inability of the new species to grow on CYA at 37 °C and its bright red colony reverse colour on MEA distinguish it from all other species in this clade.

***Penicillium tanzanicum*** Visagie, Ketch & Seifert, sp. nov. — MycoBank MB815781; Fig. 24

In: subgenus *Aspergilloides*, section *Lanata-Divaricata*.

ITS barcode: KT887841. Alternative identification markers: *BenA* = KT887802, *CaM* = KT887763.

*Etymology.* Latin, *tanzanicum*, named after Tanzania, origin of the strains.

*Type specimen.* TANZANIA, Kigoma region, soil related with termite mounds, Oct. 1996, coll. M.A. Huffman, isol. L. Ketch (holotype DAOM 695766, culture ex-type DAOMC 250514 = CBS 140968 = 50.118 = KAS 1946).

Colony morphology — Colony diam, 7 d, in mm: CYA 36–40; CYA 37 °C no growth; MEAb1 41–43; YES 47–50; OA 39–40; CREA 28–31.

CYA 25 °C, 7 d: Colonies moderately deep, radially sulcate; margins low, wide, entire; mycelia white, inconspicuously yellowish grey; texture floccose; sporulation sparse, conidia *en masse* greenish grey (27B2–D2); soluble pigments absent; exudates absent; reverse brownish orange (6C4–7C4), yellowish white to orange white (4A2–5A2). MEA 25 °C, 7 d: Colonies low, plane, yellow to greyish yellow colour due to abundant sclerotia dominating colony appearance; margins low, wide, entire; mycelia white, inconspicuously yellow; texture floccose; sporulation absent to very sparse, conidia *en masse* indeterminate; soluble pigments absent; exudates absent; reverse yellowish brown (5D5), pale yellow to greyish yellow (4A2–B3–5). YES 25 °C, 7 d: Colonies moderately deep, radially and concentrically sulcate, raised at centre; margins low, wide, entire; mycelia white; texture floccose; sporulation very sparse, conidia *en masse* not determined; soluble pigments absent; exudates absent; reverse yellowish brown (5D5), pale yellow to greyish yellow (4A2–B3–5). OA 25 °C, 7 d: Colonies low, plane, yellow to greyish yellow colour due to abundant sclerotia; margins low, wide, entire; mycelia white, inconspicuously yellow; texture floccose; sporulation very sparse, conidia *en masse* not determined; soluble pigments absent; exudates absent. CREA 25 °C, 7 d: Weak growth, acid not produced.

*Conidiophores* biverticillate, divaricate; *stipes* rough, (80–)200–875 × 2.5–3.5 µm; *branches* 12.5–13.5; *metulae* divergent, 2–4 per stipe, unequal length on same conidiophore, 11.5–26 × 2.5–3.5 µm; *phialides* ampulliform, 4–8 per metula, 8–11 × 2.5–3.5 µm (9.5 ± 0.0.7 × 3.0 ± 0.3); average length metula/phialide 1.9; *conidia* rough walled, some finely rough, globose

to subglobose, often pointed on one side with connective still visible, 2.5–3.5 × 2.5–3.5 µm (3.1 ± 0.2 × 3.0 ± 0.1), average width/length = 0.96, n = 55; *sclerotia* 33–105 × 32–96 µm.

Extrolites — bisdechlorogeodin, fiscalin A, fiscalin C, pulvilloric acid.

*Additional material examined.* TANZANIA, Kigoma region, soil related with termite mounds, Oct. 1996, coll. M.A. Huffman, isol. L. Ketch, cultures DAOMC 250515 (= CBS 140969 = 51.92 = KAS 1947), DAOMC 250516 (= CBS 140990 = 50.11 = KAS 2256).

Notes — *Penicillium tanzanicum* is classified in section *Lanata-Divaricata*. The phylogeny assigns it to a clade of species typically having roughened conidiophores, i.e. *P. simplicissimum*, *P. araracuaraense* and *P. wotroi* (Fig. 7, 8). *Penicillium tanzanicum* does not grow on CYA at 37 °C, in contrast with *P. wotroi* and *P. simplicissimum*, which grow at 37 °C. It should be noted that this character has been considered unstable for delineating species in the *P. simplicissimum* complex (Pitt 1980, Houbraken et al. 2011b). Additionally, *P. tanzanicum* also grows more restrictedly than *P. simplicissimum*. *Penicillium araracuaraense* produces a weak acid on CREA, whereas acid production is absent in *P. tanzanicum*. The main character distinguishing the new species from all three of its close relatives, is the abundant yellowish sclerotia on MEA, which are absent in close relatives.

## DISCUSSION

*Penicillium* species are reported often in ecological surveys. If identified to the species rank, they are usually determined using micromorphological and colony characters. Although there are some recent comprehensive monographs of some subgenera or sections of *Penicillium* (Frisvad & Samson 2004, Houbraken et al. 2011a, b, 2014, Visagie et al. 2013, 2016a, b, Peterson et al. 2015), the available generic monographs are now 35 years out of date and the species concepts employed were biased heavily towards isolates from food or other niches of particular interest to humans. Species with apparently diverse ecologies, such as e.g. *P. citrinum* (according to Pitt 1980 one of the most common eukaryotes on Earth), *P. glabrum*, *P. janthinellum* and *P. simplicissimum*, incorporate great phenotypic diversity. Variability in growth, form and ecology made reliable identification of new isolates very difficult. The consequence is that before the development of a standardised molecular approach and extrolite profiling, species with more restricted ecological niches that do not intrude on the human economy tended to be lumped into other species, their unique genetic or biochemical status unrecognised. Many of the strains described as new in this paper were identified originally as known species using micromorphology before molecular data indicated the significance of some of the deviating or inconspicuous characters.

There is increasing evidence of ecological specialisation in *Penicillium*. Several of the new species described in this paper come from studies of esoteric ecological niches, such as soil eaten by chimpanzees, infrabuccal cavities of carpenter ants, caterpillar intestines, nuts in temperate forests, or leaf surfaces in tropical rain forests. It seems likely that several of our new species are actual functional components and not incidental contaminants of the ecosystem samples. There was no particular effort to target *Penicillium* specifically in most of these studies, so the strains were isolated among other fungi at differing frequencies. The Tanzanian soil eaten by chimpanzees, for example, yielded a broad diversity of hyphomycetes, including many *Penicillium* species that were a significant part of the mycota (Ketch 1998). Even though it is likely that some penicillia are actually true soil fungi or associates of roots, we speculate that many species have other primary habitats where they may perform various functions, similar to the close

association that a number of subgenus *Penicillium* species have with food related products (Frisvad & Samson 2004) such as grains, dairy products or fruits. This may explain why it is often difficult to obtain numerous strains of one species when making isolations from soil.

Previous studies hint at possible interactions between *Penicillium* and insect hosts or habitats. *Penicillium brocae* has a very close association with coffee-berry borers (Peterson et al. 2003) while *P. mallochii* and *P. guanacastense* are known only from the intestines of Costa Rican caterpillars (Rivera et al. 2012). *Penicillium herquei* and the leave-rolling weevil (*Euops chinensis*) have a close association (Kobayashi et al. 2008, Li et al. 2012), and mites are suspected dispersal vectors for some *Penicillium* species in *Protea repens* infructescences (Visagie 2012). The dominant fungi in buccal cavities of carpenter ants were species of *Mortierella* (Clark 2002), but our new species *P. camponotum* seems to be a consistent inhabitant of this niche, and occurred in both Canada and Germany. The other two species, *P. infrabuccalum* with two isolates and *P. fundyense* with one, are known only from this niche but with so few strains available, it is difficult to interpret the significance of the association.

Whatever the actual ecological roles of the species described in this paper, it seems likely that the extrolites they produce will have crucial ecological functions. From the 15 species described here, 36 known extrolites were identified and 11 uncharacterised compounds detected (Table 3). As shown repeatedly in *Penicillium*, extrolite profiling is a valuable addition to morphological characterisation and gene comparisons for species delimitation (e.g. Frisvad & Filtenborg 1990, Sonjak et al. 2007, Tuthill et al. 2001), although chemotaxonomic comparisons with close relatives were not possible for most of our new species because of the lack of data for sister species. Among the species we studied, *P. diabolicalicense*, *P. improvisum*, *P. alogum* and *P. aotearoae* produced unique, previously unknown extrolites. Isofumigaclavine A and citrinin were extrolites of *P. improvisum*. Restricticins are broad spectrum antifungal polyenes previously only identified in cultures of *P. restrictum* (Hensens et al. 1991, Schwartz et al. 1991) and *Penicillium* sp. NR6564 (Matsukuma et al. 1992). *Penicillium diabolicalicense* also produces these compounds, but the less active N,N-dimethylrestrictin occurred in greater concentrations. In this study, *P. diabolicalicense* (section *Exilicaulis*) was the only producer of the neurotoxin penitrem A (on CYA), which is otherwise well-known among species of subgenus *Penicillium*. *Penicillium alogum* and *P. aotearoae* produced uncharacterised extrolites of formula  $C_{21}H_{28}O_8$  and  $C_{19}H_{24}O_8$ , respectively. *Penicillium amphipolaria* was the only producer of fusaperazine E, fumitremorgin B and several related, uncharacterised metabolites. Andrastins were the extrolites of *P. nucicola*, *P. cataractum*, *P. infrabuccalum*, *P. panissanguineum* and some *P. camponotum*. Despite these similarities, all species produced unique combinations of additional extrolites (Table 3).

Intraspecific variation of extrolite production was observed for *P. camponotum*, *P. cataractum* and *P. bissettii*. All strains of *P. camponotum* produced marcfortine A and marcfortine B, yet the predominant extrolites produced by Canadian and German strains differed significantly. The seven Canadian strains isolated in New Brunswick from carpenter ants (*Camponotus pennsylvanicus*) produced an uncharacterised compound of formula  $C_{15}H_{22}O_2$  that was absent in the German strains isolated from *Camponotus herculeanus*, which produced andrastin A. The greatest intraspecific variation of extrolites occurred within strains of *P. bissettii*, both of which were isolated from soil samples collected from a spruce forest in Quebec, Canada on the same date. DAOMC 167011 produced high amounts of meleagrins and aurantioclavine in all three growth media tested

whereas these extrolites were absent in DAOMC 167033, which produced penicillic acid.

Pulvilloric acid ( $C_{15}H_{18}O_5$ ) was first identified in cultures of *P. pulvillum* (Brian et al. 1957), and its structure was elucidated (Barber et al. 1986, Barret et al. 1969, McOmie et al. 1966) following isolation under acidic conditions (pH = 1). Recently, pulvilloric acid was identified in the related *P. wotroi* and *P. araracuaraense* (Houbraken et al. 2011b). Using LC-high resolution mass spectrometry (LC-HRMS) in negative ionization mode, we've identified a similar compound of chemical formula  $C_{15}H_{20}O_6$  ( $m/z$  295.1186) as an extrolite of *P. panissanguineum*, *P. cataractum*, *P. infrabuccalum* and *P. tanzanicum*. An isochroman similar to pulvilloric acid, with an identical chemical formula to that reported in this study, was characterised in cultures of the phylogenetically related species *P. simplicissimum* and patented as an amyloid aggregation inhibitor (Hirai et al. 2001). Interestingly, when these strains were screened by positive mode LC-HRMS, the  $m/z$  of the major peak was 279.1226, which corresponds to the chemical formula of pulvilloric acid ( $C_{15}H_{18}O_5$ ) (Fig. 9). The LC conditions were adjusted to ensure that the two contradictory signals observed in positive and negative ionization modes were indeed from the same compound. Non-aqueous extraction solutions were also used to determine if the extraction method was causing an undesired chemical reaction. We determined that during ionization in positive mode, protonation of this compound causes it to immediately dissociate by neutral loss of water, producing a  $[C_{15}H_{18}O_5+H]^+$  product ion that can be mistaken as the molecular ion. This is a charge-directed fragmentation phenomenon known to occur when the protonated, gas-phase ion is highly unstable (Renaud et al. 2013). In negative mode ionization, the molecular charge is obtained through the deprotonation of the carboxylic acid and the compound does not undergo extensive in-source fragmentation, exemplifying the benefits of LC-MS screening with both polarities. Our results suggest that 'pulvilloric acid' which was previously identified in cultures of *P. wotroi* and *P. araracuaraense* and quite possibly *P. pulvillum* itself, is not the structure as it exists on artificial solid and liquid growth media; it is the patented isochroman isolated from cultures of *P. simplicissimum*. The originally reported pulvilloric acid structure is known to be unstable and require careful extraction and work-up methodology, to avoid rearrangement and decomposition reactions from occurring during isolation (Tanenbaum & Nakajima 1969). Therefore, it is reasonable to believe that the two structures could be inter-convertible due to an increased stability of the molecule provided by the aromatisation of the originally proposed structure of pulvilloric acid to its isochroman. However, the true form of pulvilloric acid within the natural environments of its producers remains unknown. In solid and liquid growth media, the formula of this extrolite is  $C_{15}H_{20}O_6$  and we will simply maintain the name of pulvilloric acid.

**Acknowledgements** This paper is warmly dedicated to Dr Robert A. Samson on the occasion of his 70th birthday. Rob is a teacher, mentor and colleague to the senior authors of this paper and we celebrate the remarkable achievements of his career, in particular his contributions to *Penicillium* taxonomy and phylogeny, with this paper. KAS thanks Peter Johnston, Mycology & Bacteriology, Landcare Research, Auckland, New Zealand, for his assistance in arranging a collecting permit for Arthur's Pass National Park, where two of the new species in this paper were collected in 2003. Walter Gams assisted with collection of soil samples in Italy in 2008. Walter Gams and Shaun Pennycook assisted with the derivation of appropriate epithets for some of our species. KAS, RA, GL-S, JR and MS received operating support from several competitive grants offered by Agriculture & Agri-Food Canada Science and Technology Branch. DWM acknowledges nearly three decades of support from the Natural Sciences and Engineering Research Council of Canada (grants OGP000145 and STR0149534). LK was supported by the Natural Science and Engineering Research Council of Canada (NSERC) postgraduate scholarship program. MU acknowledges support from NSERC



and the US National Science Foundation (NSF) to R. Greg Thorn, from NSF to Daniel H. Janzen and from CR-USA to Costa Rican parataxonomists at the Area de Conservación Guanacaste who collected and reared the caterpillar host of *P. costaricensis* and isolated and sent the fungi to the Thorn lab in London, Ontario. We are grateful to the Molecular Microbiology Technology Laboratory group at ORDC for their support with DNA sequencing.

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