



# Multilocus DNA sequencing of the whiskey fungus reveals a continental-scale speciation pattern

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## Key words

Dothideomycetes  
Extremophilic fungi  
microcolonial fungi  
molecular phylogenetics  
warehouse staining

**Abstract** *Baudoinia* was described to accommodate a single species, *B. compniacensis*. Known as the 'whiskey fungus', this species is the predominant member of a ubiquitous microbial community known colloquially as 'warehouse staining' that develops on outdoor surfaces subject to periodic exposure to ethanolic vapours near distilleries and bakeries. Here we examine 19 strains recovered from environmental samples near industrial settings in North America, South America, the Caribbean, Europe and the Far East. Molecular phylogenetic analysis of a portion of the nuLSU rRNA gene confirms that *Baudoinia* is a monophyletic lineage within the *Teratosphaeriaceae* (*Capnodiales*). Multilocus phylogenetic analysis of nuITS rRNA (ITS1-5.8S-ITS2) and partial nuLSU rRNA, beta-tubulin (TUB) and elongation factor 1-alpha (TEF1) gene sequences further indicates that *Baudoinia* consists of five strongly supported, geographically patterned lineages representing four new species (viz. *Baudoinia antillensis*, *B. caledoniensis*, *B. orientalis* and *B. panamericana*).

**Article info** Received: 20 May 2015; Accepted: 12 July 2015; Published: 17 September 2015.

## INTRODUCTION

Dark staining on walls, roof tiles and vegetation in proximity to spirit maturation warehouses was first documented by Richon & Petit (1881), who recognised that the principal agent responsible was a single fungus, which they named *Torula compniacensis*. The fungus remained nearly forgotten by science for 80 years, being studied briefly by Scandinavian and French investigators in the 1960s. In the early 2000s, growing public awareness of health concerns associated with the growth of black moulds in water-damaged buildings stimulated interest in this soot-like growth around distilleries. This, in turn, prompted a re-examination of this fungus that led to its transfer to the genus *Baudoinia* (Scott et al. 2007).

*Baudoinia compniacensis* is a slow-growing, morphologically reduced, saprobic oligotroph that colonizes a wide range of materials, including man-made items such as construction materials, fences, road signs, outdoor furniture and even automobiles exposed to ethanol vapour. It also colonises a wide range of natural substrates including soil, rock and vegetation (Scott et al. 2007).

While the association between industrial ethanol emissions and the colonization of outdoor surfaces by *B. compniacensis* has been known since the initial description of the warehouse staining phenomenon (Richon & Petit 1881), the functional role of ethanol in facilitating fungal colonisation remained unclear until Ewaze et al. (2007, 2008a) reported on the multiple effects of ethanolic vapour on nutrition as well as the promotion of germination initiation and the induction of stress protective modifications. The underlying mechanisms responsible for these effects are unknown.

*Baudoinia compniacensis* grows primarily outdoors (Auger-Barreau 1966), typically on ethanol-exposed materials that are subject to cycles of stress from temperature and drought (Scott et al. 2007). Over the years, specimens of colonised materials have been collected and reported from a number of jurisdictions world-wide, including the Americas, Europe and Scandinavia (Richon & Petit 1881, Kjølner 1961, Auger-Barreau 1966, Scott et al. 2007). As part of a broad global survey of fungi associated with ethanol-emitting industrial processes, we obtained specimens of materials affected by warehouse staining from which we derived cultures that corresponded microscopically to *B. compniacensis*. Multilocus phylogenetic analysis revealed multiple genotypes among these collections, which we describe here as novel species.

## MATERIALS AND METHODS

### Culture acquisition and characterisation

Samples of building materials, equipment, landscaping materials and vegetation in proximity to distillery bond warehouses ( $n = 9$  representing 8 countries) and a commercial bakery ( $n = 1$ ) were collected either as bulk samples or scrapings. All of the materials sampled appeared visually to be colonized by dark growth, characteristic of *B. compniacensis*. Cultures were obtained by streaking small amounts of pulverized specimen on the surface of several growth media including modified Leonian's agar (MLA) (Malloch 1981) as described by Scott et al. (2007) and Ewaze's semi-selective *Baudoinia* medium (EBM) (Ewaze et al. 2008b). Cultures were incubated at room temperature for 12–14 d and examined daily in stereo microscopy for germinating cells suggestive of *B. compniacensis*. These were excised using a sterile, finely pointed tungsten needle (prepared according to the procedure described by McCrone et al. 1973), transferred to antibiotic-free MLA and incubated at 26 °C for microscopic examination.

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**Table 1** Sources and accession numbers of the isolates sequenced in this study.

Taxon	Strain No.*	Locality	Habitat	GenBank accession numbers			
				nuclTS	nuclSU	TUB	TEF1
<i>Austroafricana parva</i>	CBS 110503	Australia: Western Australia	<i>Eucalyptus globulus</i>	KT186488	KT186510	KT186532	KT186554
<i>Baudoinia antillensis</i>	UAMH 10810 T	Barbados	PVC pipe at a distillery	KT186481	KT186502	KT186524	KT186546
	UAMH 11552	Barbados	exhaust fan at a distillery	KT186480	KT186501	KT186523	KT186545
	UAMH 11555	Trinidad and Tobago: Trinidad	fire hydrant at a distillery	KT186477	KT186498	KT186520	KT186542
	UAMH 11556	Trinidad and Tobago: Trinidad	galvanized roofing of a distillery building	KT186478	KT186499	KT186521	KT186543
	UAMH 11557	Trinidad and Tobago: Trinidad	PVC gutter of a distillery building	KT186479	KT186500	KT186522	KT186544
<i>B. caledoniensis</i>	UAMH 10761 T	United Kingdom: Scotland	exterior of a distillery building	KT186473	KT186494	KT186516	KT186538
	UAMH 11553	United Kingdom: Scotland	brick distillery building exterior	KT186471	KT186492	KT186514	KT186536
	UAMH 11554	United Kingdom: Scotland	mortar joint of building exterior at a distillery	KT186472	KT186493	KT186515	KT186537
<i>B. compniacensis</i>	UAMH 10808 T	France	tree branches near a distillery	KT186468	KT186489	KT186511	KT186533
<i>B. orientalis</i>	UAMH 10814 T	Korea	exterior of a building at a distillery	KT186469	KT186490	KT186512	KT186534
	UAMH 11551	Korea	exterior of a building at a distillery	KT186470	KT186491	KT186513	KT186535
<i>B. panamericana</i>	UAMH 10762 T	Canada: Ontario	bark of <i>Picea</i> sp. at a distillery aging warehouse	KT186476	KT186497	KT186519	KT186541
	UAMH 10763	Canada: Ontario	gravel at a distillery aging warehouse	KT186474	KT186495	KT186517	KT186539
	UAMH 10764	United States: Kentucky	concrete wall at a distillery	KP990666	KT186504	KT186526	KT186548
	UAMH 10809	United Kingdom: Scotland	bung of a whisky barrel	KT186485	KT186507	KT186529	KT186551
	UAMH 10811	United States: New York	fallen tree branch near a bakery	KT186484	KT186506	KT186528	KT186550
	UAMH 10812	United States: Indiana	exterior at of a brick building at a distillery	KT186483	KT186505	KT186527	KT186549
	UAMH 10839	Canada: Ontario	concrete wall at a distillery aging warehouse	KT186475	KT186496	KT186518	KT186540
	UAMH 11550	Argentina	painted pipe and wall at a distillery	KT186482	KT186503	KT186525	KT186547
	<i>Devriesia staurophora</i>	CBS 374.81A	Colombia: Cundinamarca	páramo soil	KT186486	KT186508	KT186530
CBS 375.81		Colombia: Cundinamarca	páramo soil	KT186487	KT186509	KT186531	KT186553

\* 'T' denotes a type strain.

Mounts for microscopic examination of herbarium specimens and fresh collections were prepared in distilled water and examined in bright field and Nomarski Differential Interference Contrast microscopy on an Olympus BX-51 microscope. Replicate sets of cultures were deposited in the University of Alberta Microfungus Collection (UAMH) as well as stored locally on MLA slants overlaid with sterile, heavy mineral oil. Strain references and GenBank numbers for taxa examined in this study are given in Table 1.

#### DNA isolation and amplification

Mycelium was harvested from 5 d cultures grown in Modified Leonian's broth (MLB), rinsed several times in sterile distilled water and stored at  $-20^{\circ}\text{C}$ . Axenic culture was verified by incubating a small amount of the harvested mycelium on MLA. High molecular weight genomic DNA (gDNA) was isolated using the FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) and a FastPrep 24 Cell Disrupter (MP Biomedicals) following the manufacturer's instructions. The quality and concentration of gDNA were assessed and adjusted using a QuantiFluor dsDNA System (Promega, Madison, WI, USA).

A region of the nuclSU rRNA gene was amplified using the primers and procedure described by Gueidan et al. (2007). Primers used to amplify and sequence this region and three other loci included: nuclSU LR0R (Rehner & Samuels 1994), LR3R, LR5, LR7 (Vilgalys & Hester 1990); nuclITS ITS4, ITS5 (White et al. 1990); TEF1 EF1-728F (Carbone & Kohn 1999), EF1-983F, EF1-2218R (Rehner & Buckley 2005); TUB Bt2a, Bt2b (Glass & Donaldson 1995) and benA1 (Geiser et al. 1998). Descriptions of the PCR mixtures and thermocycling parameters are provided in Bogale et al. (2010). Yield was approximately quantified by electrophoresis on 1.2 % agarose gels, ethidium bromide staining and UV visualization. PCR products were cleaned using a QIAquick PCR Purification Kit (Qiagen, Mississauga, ON) or a GeneClean Turbo Kit (MP Biomedicals) in combination with the E-gel SizeSelect 2 %

agarose gel cutting system (Invitrogen, Carlsbad, CA, USA). Sequencing reactions were performed using a Taq DyeDeoxy cycle sequencing kit or a BigDye Terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA) using the above primers.

#### Sequence data analysis

Homologous sequences for each gene region were retrieved from GenBank based on the highest scores for identity using BLAST searches. Sequence alignments were generated using ClustalX v. 2.0 (Thompson et al. 1997, Larkin et al. 2007) and adjusted using Se-Al v. 2.0a11 (Rambaut 2008). All sequences were transcribed in 5'-3' orientation. Gaps were indicated by '#', and degenerated bases were indicated using standard nomenclature. Multiple base indels were reduced to single characters and all ambiguously aligned sequences were excluded. The position of isolates of *Baudoinia* within the *Teratosphaeriaceae* was inferred based on the analysis of nuclSU sequences of 38 taxa with *Ramularia aplospora* and *R. endophylla* (*Mycosphaerellaceae*) as outgroup. Relationships among strains identified as *B. compniacensis* from industrial settings in North America, South America, the Caribbean, Europe and the Far East were inferred from a combined dataset that included nuclSU, nuclTS, TUB and TEF1 sequences of 22 isolates. Outgroup taxa for this dataset included *Austroafricana parva* and *Devriesia staurophora*. Sequence alignments are deposited in TreeBASE (submission 17631).

Phylogenetic relationships were inferred from aligned sequences using the maximum parsimony method in PAUP v. 4.0b10 (Swofford 2002). Heuristic searches were performed employing tree bisection-reconstruction (TBR) branch-swapping with MulTrees and steepest descent options activated. Bootstrap support (BS) for internal branches was evaluated from 1 000 heuristic searches using TBR branch-swapping (MulTrees activated, steepest descent inactivated). The partition homogeneity test (PHT) (Farris et al. 1995) in PAUP was used to test combin-

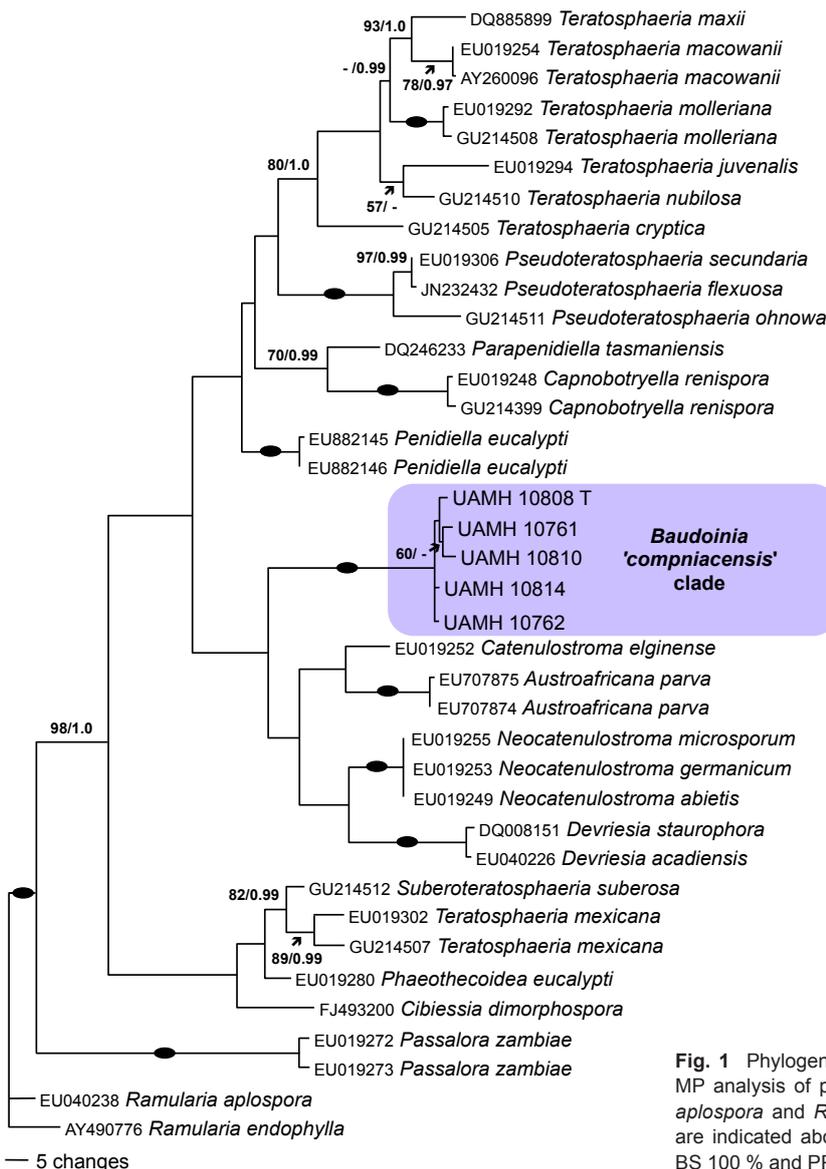
ability of the nuclITS, nuclLSU, TUB and TEF1 datasets based on heuristic searches employing 1 000 replications. Gaps were defined as a fifth character in all analyses.

The best-fit model of nucleotide substitution for each gene region was selected using jModelTest 2 (Darriba et al. 2012) under the Bayesian information criterion. Bayesian inference analyses were performed using MrBayes v. 3.2.5 (Ronquist & Huelsenbeck 2003). For the nuclLSU dataset for 38 taxa we used a GTR+I+G substitution model; for the combined dataset for 22 taxa we used a different model for each partition (nuclLSU GTR; nuclITS SYM+G; TUB HKY+I+G; TEF1 GTR+G). Multiple Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted using one cold and three heated Markov chains. Analyses were run for 5 million generations, with trees sampled every 100 generations. Bayesian posterior probabilities (PP) were calculated after the first 25 % of trees were discarded as burn-in. The remaining trees were retained to construct a 50 % majority rule consensus tree that was visualized using FigTree v. 1.4.2 (Rambaut 2009).

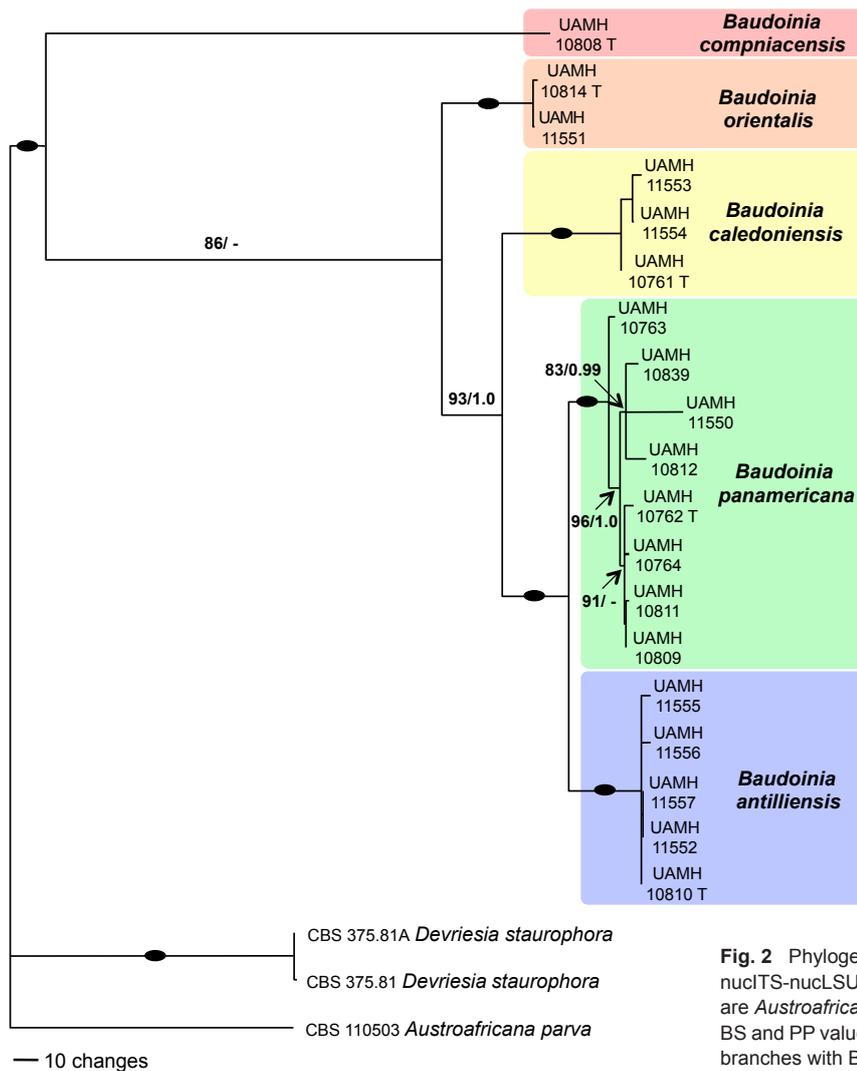
**RESULTS**

**Morphological observations**

The isolates of *Baudoinia* we examined were morphologically simple and lacked species-level diagnostic characters. In general, colonies are dark brown to black, heaped, and extremely slow-growing, reaching a diameter of 10 mm or less after 28 d incubation at 26 °C on MLA. The epitype strain of *B. compniacensis*, UAMH 10808, inconsistently produced small amounts of diffusible brown pigment in the growth medium. This feature was lacking in all other isolates and its diagnostic utility is unknown given the variable nature of pigment production in UAMH 10808. The vegetative hyphae of species of *Baudoinia* are pale brown, thick-walled and smooth when young becoming thick-walled, roughened and darkened with age. As cells mature they become centrally inflated and barrel-shaped. The roughened surface ornamentation appears due to longitudinal linear fracturing of the outer cell wall giving rise to thickened, darkened, irregular linear ridges that are initially greenish brown and become dark brown at maturity. Conidia are dry and range from 1-celled to phragmosporous with slight to prominent constrictions at septa (although 2-celled conidia tend to predominate) and are released mainly by thallic dehiscence but also arise through percurrent enteroblastic proliferation. This pattern of maturation, ornamentation and dehiscence is shared by all isolates we have examined.



**Fig. 1** Phylogenetic relationships of selected Capnodiales inferred from MP analysis of partial nuclLSU sequence. Outgroup taxa are *Ramularia aplospora* and *R. endophylla* (*Mycosphaerellaceae*). BS and PP values are indicated above the branches. Shaded ovals indicate branches with BS 100 % and PP 1.0.



**Fig. 2** Phylogeny of *Baudoinia* inferred from MP analysis of the combined nuclTS-nuclSU-TUB-TEF1 dataset ( $P$  value for PHT = 0.27). Outgroup taxa are *Austroafricana parva* and *Devriesia staurophora* (*Teratosphaeriaceae*). BS and PP values are indicated above the branches. Shaded ovals indicate branches with BS 100 % and PP 1.0.

### Phylogenetic analyses

Partial nuclSU sequences of five strains identified as *Baudoinia compniacensis* were characterized by a 13 base pair (bp) deletion located at position 522 of the nuclSU of *Ramularia endophylla* (GenBank AY490776) and the nuclSU of three isolates contained an insertion at position 913 that was 300 bp in length or longer (UAMH 10761 300 bp; UAMH 10808 302 bp; UAMH 10810 341 bp). Following the reduction of multiple base indels to single characters, the final alignment for 38 taxa in the nuclSU dataset comprised 1 422 characters of which 182 were parsimony informative. An heuristic analysis of this dataset produced 51 most parsimonious trees (MPT), 533 steps in length with a consistency index (CI) of 0.568 and a retention index (RI) of 0.776. One of these trees is shown in Fig. 1. In this phylogeny, isolates of *B. compniacensis* form a strongly supported clade (BS 100 %, PP 1.0) within a lineage (BS 98 %, PP 1.0) corresponding to the *Teratosphaeriaceae*. Within this family, the *Baudoinia compniacensis* clade is positioned as sister to a lineage that includes *Austroafricana parva*, *Catenulostroma elginense*, *Devriesia staurophora*, and species of *Neocatenulostroma*, but support for this relationship is poor (BS < 50 %, PP < 0.95).

The final alignment of the combined dataset for 19 isolates of *Baudoinia* and the three strains representing the outgroup consisted of 3 628 characters (of which 357 were parsimony-informative) representing the nuclTS (479 bp), nuclSU (1 361 bp), TUB (937 bp) and TEF1 (851 bp). Results of 1 000 heuristic searches implementing the PHT ( $P = 0.270$ ) indicated that

the four datasets for these taxa were congruent. An heuristic search of this dataset produced 522 MPT 730 steps in length (CI = 0.871, RI = 0.902); one of these trees is presented in Fig. 2. In this phylogeny, *Baudoinia* forms a strongly supported lineage (BS 100 %, PP 1.0) that comprises isolates divided between two clades. The first is represented by the ex-epitype strain of *B. compniacensis* (UAMH 10808) isolated from tree branches near a distillery in France. The second clade (BS 86 %, PP < 0.95) includes a subclade (BS 100 %, PP 1.0) consisting of two strains (UAMH 10814 and UAMH 11551) isolated from building exteriors at a distillery in Korea and a larger group (BS 93 %, PP 1.0) divided into three strongly supported lineages (all BS 100 %, PP 1.0). The first contains three isolates from building exteriors and other surfaces at distilleries, aging warehouses and a bakery in Scotland (UAMH 10761, 11553, 11554). The second lineage includes isolates from North America, South America and Scotland (UAMH 10762, 10763, 10764, 10809, 10811, 10812, 10839, 11550) while the third lineage encompasses isolates from the Antillean Islands (UAMH 10810, 11552, 11555, 11556, 11557). All isolates positioned in the clade consisting solely of strains from Scotland contained a 300 bp insertion in the nuclSU identical to that seen in UAMH 10761. Similarly, the 341 bp insertion identified in the nuclSU of UAMH 10810 is present in all isolates from the Antillean Islands. We recognise each of the robustly supported clades inferred from the combined dataset as new species for which descriptions are given below. Species-level molecular diagnostic characters are summarised in Table 2–4.

**Table 2** Molecular identification of *Baudoinia* species using polymorphisms in the nuclTS gene.

Taxon	Nucleotide position*				
	4274–4272	4260–4250	4245–4243	4055–4053	4044–4021
<i>B. panamericana</i>	TTT	CTTTGATAAA#T	TCA	GCC	CACCGCGCGCTTCATGTCCCCCG
<i>B. antilliensis</i>	---	-----	---	---	-----C-----
<i>B. caledoniensis</i>	---	-----	-T-	---	-G---A---CT-----
<i>B. compniacensis</i>	---	---#-----	---	---	-----C-----
<i>B. orientalis</i>	-C-	---#-----A-	---	-T-	-----C-----

\* Reference sequence *B. panamericana* UAMH 10762T (GenBank NW006911274.1); # denotes a gap. Reference sequence in reverse orientation.

**Table 3** Molecular identification of *Baudoinia* species using polymorphisms in the TUB gene, intron 3.

Taxon	Nucleotide position*
	1145307–1145253
<i>B. panamericana</i>	GTATGTTGACGTGTWTTATGGACGGACGTAGAGGATCAGTGCTTACGCTGGGCAG
<i>B. antilliensis</i>	-----A-G-----
<i>B. caledoniensis</i>	-----A-CCGCGGAC###-TGAT-G--CAG-G-----T-T-----
<i>B. compniacensis</i>	---G--A-GAC-C###-A---AGA-G--G--G-T-----AT-C-----
<i>B. orientalis</i>	-----AA-C-AGGATC-T-TGT-GG-----G-----T-----

\* Reference sequence *B. panamericana* UAMH 10762T (GenBank NW006911257.1); W = A or T; # denotes a gap. Reference sequence in reverse orientation.

**Table 4** Molecular identification of *Baudoinia* species using polymorphisms in the TEF1 gene.

Taxon	Nucleotide position*								
	606647–606649	606690–606692	606704–606706	606752–606754	606782–606787	606881–606883	606947–606949	607184–607186	
<i>B. panamericana</i>	CGA	GTT	CAG	TGA	CAGTCA	CGA	GGG	GAT	
<i>B. antilliensis</i>	-A-	---	---	-A-	---CG-	-A-	-A-	-G-	
<i>B. caledoniensis</i>	---	-C-	-G-	---	---A-	-C-	-C-	---	
<i>B. compniacensis</i>	---	-C-	-G-	---	--ACA-	---	-A-	-C-	
<i>B. orientalis</i>	-A-	-C-	-G-	---	-GACA-	---	-A-	---	

\* Reference sequence *B. panamericana* UAMH 10762T (GenBank NW006911257.1). Reference sequence in forward orientation

**Taxonomy**

***Baudoinia antilliensis*** J.A. Scott & Unter., *sp. nov.* — MycoBank MB812512

*Etymology.* From the Latin name of the island archipelago bordering the Caribbean Sea, the type locality.

Nucleotides at the following positions are fixed for *B. antilliensis* relative to the numerical location identifiers given in the following GenBank sequences: TUB (NW006911257.1) at positions 1145306–1145255 (TATGTTGACGTGTATGATGACGGACGTAGAGGATCAGTGCTTACGCTGGGC); TEF1 (NW006911257.1) at positions 606648 (A), 606691 (T), 606705 (A), 606753 (A), 606782–606787 (CAGCGA), 606882 (A), 606948 (A), 607185 (G); and nuclITS (NW006911274.1) at positions 4273 (T), 4260–4250 (CTTTGATAAA#T) and 4044–4021 (CACCGCGCGCTTCATGTCCCCCG).

*Specimens examined.* BARBADOS, St. Michael, Bridgetown, on PVC pipe, north face of bond warehouse, 5 Apr. 2005, *J. Edwards* (holotype Herb. UAMH 10810, culture ex-type UAMH 10810); same location, on exhaust fan of warehouse, 5 Apr. 2005, *J. Edwards*, UAMH 111552. — TRINIDAD AND TOBAGO, Trinidad, Laventille, on fire hydrant, July 2007, *V. Doodnath*, UAMH 11555; same location, on galvanised roofing, July 2007, *V. Doodnath*, UAMH 11556; same location, on PVC gutter, July 2007, *V. Doodnath*, UAMH 11557.

***Baudoinia caledoniensis*** J.A. Scott & Unter., *sp. nov.* — MycoBank MB812513; Fig. 3a–c, e

*Etymology.* From the Latin name applied to the region now known as Scotland, the type locality.

Nucleotides at the following positions are fixed for *B. caledoniensis* relative to the numerical location identifiers given in the following GenBank sequences: TUB (NW006911257.1) at positions 1145306–1145255 (TATGTTGACAT###CCGCG

GACGTGATTGGACAGTGAGTGCTTATGTTGGGC); TEF1 (NW006911257.1) at positions 606648 (G), 606691 (C), 606705 (G), 606753 (G), 606782–606787 (CAGTAA), 606882 (C), 606948 (C), 607185 (A); and nuclITS (NW006911274.1) at positions 4273 (T), 4260–4250 (CTTTGATAAA#T) and 4044–4021 (CGCCGACGCTCTATGTCCCCCG).

*Specimens examined.* UK, Scotland, Beith, Willowyard, near Kibirnie Loch, on exterior siding of a building, 1 m above ground, May 2005, *J. Spouge* (holotype Herb. UAMH 10761, culture ex-type UAMH 10761); same location, on a brick surface of a building, May 2005, *J. Spouge*, UAMH 11553; same location, on mortar from a masonry wall, May 2005, *J. Spouge*, UAMH 11554.

***Baudoinia compniacensis*** (Richon) J.A. Scott & Unter., Mycologia 99: 594. 2007, amended — MycoBank MB170778; Fig. 3f

*Basionym.* *Torula compniacensis* Richon, Brébissonia 3: 155. 1881.  
= *Torula conglutinata* Corda var. *compniacensis* (Richon) Sacc., Rev. Mycol. (Toulouse) 3: 17. 1881.  
= *Xenodochnus baudoinii* Roum. & Durrieu, Rev. Mycol. (Toulouse) 3: 16. 1881, *nom. nud.*

Nucleotides at the following positions are fixed for *B. compniacensis* relative to the numerical location identifiers given in the following GenBank sequences: TUB (NW006911257.1) at positions 1145306–1145255 (TAGGTTAAGACG###CATA GACGAGAGGAGGGGATGATTGCTTACATTCCGC); TEF1 (NW006911257.1) at positions 606648 (G), 606691 (C), 606705 (G), 606753 (G), 606782–606787 (CAACAA), 606882 (G), 606948 (A), 607185 (C); and nuclITS (NW006911274.1) at positions 4273 (T), 4260–4250 (CTT#GATAAA#T) and 4044–4021 (CACCGCGCGCTCCATGTCCCCCG).

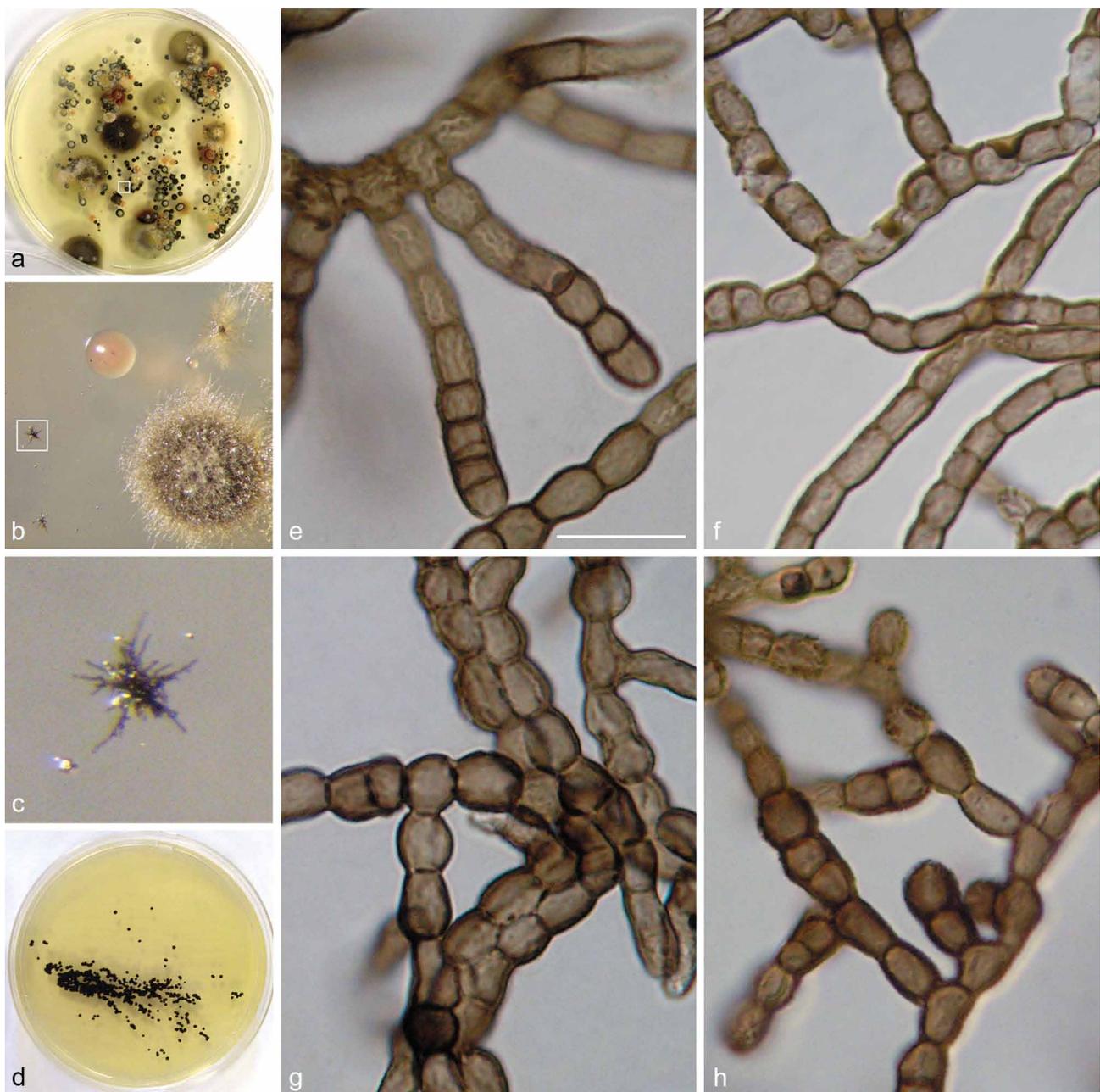
*Specimens examined.* FRANCE, Cognac, near the bank of the Charentes River, on affected walls of a building at a distillery of *eaux-de-vie*, July 1881, *A. Baudoin* & *P. Brunaud* ex *C. Roumeguère*, *Fungi Gallici Exsiccati* No.

1695 (lectotype Herb. DAOM 66898); Merpins, 4 km east of Cognac, near the Remy Martin distillery, on tree branches (species unknown), July 2006, R.C. Summerbell, epitype Herb. DAOM 238773, ex-epitype culture UAMH 10808.

Notes — Scott et al. (2007) described *Baudoinia* to accommodate *Torula compniacensis* and named the genus in recognition of Antonin Baudoin's early contributions to the study of the organism responsible for warehouse staining. They provided a synonymy based on Crane (2001), but as noted by Illana-Esteban (2013), both Crane (2001) and Scott et al. (2007) incorrectly cited the journal reference for the basionym when they listed it as Rev. Mycol. (Paris). This abbreviation refers to Revue de Mycologie (Paris) rather than to Revue Mycologique (Toulouse), which included the article in question. The latter is also the place of publication for the *nomen nudum* *Xenodochus baudoinii* (Illana-Esteban 2013). Finally, Illana-

Esteban (2013) observed that *T. compniacensis* was published initially by Richon & Petit (1881) in the journal *Brébissonia* in February 1881 and reprinted by Roumeguère in the *Revue Mycologique* later that same year. Illana-Esteban (2013) incorrectly attributed authorship of Richon's reprinted diagnosis to Baudoin. The last sentence of Roumeguère's article reads: "Je distribue la nouvelle variété dans ma centurie XVI. M. Baudoin et M."; implying that Baudoin and an unnamed individual, 'M.', authored the article. However, this sentence was truncated, with the remainder transposed to the head of the following page. The final sentence of the article reads: "M. Baudoin et M. Paul Brunaud après lui, ont bien voulu m'en approvisionner, C.R.", confirming Roumeguère as the article's author.

*Baudoinia compniacensis* is known from a single culture that was the subject of whole-genome sequencing and annotation (Ohm et al. 2012).



**Fig. 3** a. Scrapings from mortar joint collected at a spirit aging warehouse in Kilmalid, Dumbarton, Scotland (parent specimen of *B. caledoniensis*, UAMH 11554) cultured on EBM at 26 °C for 18 d; b. enlargement of 5 × 5 mm square elaborated from (a) showing developing microcolonies of *B. caledoniensis* (e.g., inset square); c. enlargement of 500 × 500 µm square elaborated from (b) depicting microcolony of *B. caledoniensis*; d. pure culture of *B. panamericana* (UAMH 10764) on MLA from a streaked spore suspension incubated at 26 °C for 28 d; e–h. colonies of *Baudoinia* species photographed in Nomarski Differential Interference Contrast microscopy from slide cultures grown on MLA; e. *Baudoinia caledoniensis* (UAMH 10761 T); f. *Baudoinia compniacensis* (UAMH 10808 T); g. *Baudoinia orientalis* (UAMH 10814 T); h. *Baudoinia panamericana* (UAMH 10809). — Scale bar = 10 µm.

***Baudoinia orientalis*** J.A. Scott & Unter., *sp. nov.* — MycoBank MB812514; Fig. 3g

*Etymology.* From the Latin, *orientalis*, 'of the east', in reference to the type locality.

Nucleotides at the following positions are fixed for *B. orientalis* relative to the numerical location identifiers given in the following GenBank sequences: TUB (NW006911257.1) at positions 1145306–1145255 (TATGTTGACAAGCAAGGAT-CATGTGTGGGGAGGATGAGTGCTTACTCTGGGC); TEF1 (NW006911257.1) at positions 606648 (A), 606691 (C), 606705 (G), 606753 (G), 606782–606787 (CGACAA), 606882 (G), 606948 (A), 607185 (A); and nuclITS (NW006911274.1) at positions 4273 (C), 4260–4250 (CTT#GATAAAAT) and 4044–4021 (CACCGCGCGCCTCCATGTCCCCCG).

*Specimens examined.* KOREA, Seoul, Kyonggi-Do, on building exterior of spirit aging warehouse, 2008, *S.P. Chang* (holotype Herb. UAMH 10814, culture ex-type UAMH 10814); same location, on building exterior of spirit aging warehouse, 2008, *S.P. Chang*, UAMH 11551.

***Baudoinia panamericana*** J.A. Scott & Unter., *sp. nov.* — MycoBank MB812515; Fig. 3d, h

*Etymology.* In reference to the continental Americas, the type locality.

Nucleotides at the following positions are fixed for *B. panamericana* relative to the numerical location identifiers given in the following GenBank sequences: TUB (NW006911257.1) at positions 1145306–1145255 (GTATGTTGACGTGTWT-TATGGACGGACGTAGAGGATCAGTGCTTACGCTGGGCAG); TEF1 (NW006911257.1) at positions 606648 (G), 606691 (T), 606705 (A), 606753 (G), 606782–606787 (CAGTCA), 606882 (G), 606948 (G), 607185 (A); and nuclITS (NW006911274.1) at positions 4273 (T), 4260–4250 (CTTTGATAAA#T) and 4044–4021 (CACCGCGCGCCTTCATGTCCCCCG).

*Specimens examined.* ARGENTINA, Buenos Aires, Bella Vista, on stone wall, 16 Mar. 2005, *C. Gomez*, UAMH 10813; same location, on painted pipe and wall, 16 Mar. 2005, *C. Gomez*, UAMH 11550. — CANADA, Ontario, Lakeshore, near Pike Creek, on bark of *Picea* sp., 12 Mar. 2002, *J.A. Scott* (holotype Herb. UAMH 10762, culture ex-type UAMH 10762); same location, on gravel, 12 Mar. 2002, *J.A. Scott*, UAMH 10763; same location, on concrete wall, 12 Mar. 2002, *J.A. Scott*, UAMH 10839; same location, unknown substrate, 12 Mar. 2002, *J.A. Scott*, UAMH 11558. — UK, Scotland, Beith, Willowyard, near Kibirnie Loch, on cloth covering of whisky barrel bung, Apr. 2006, *J. Spouge*, UAMH 10809. — USA, Indiana, Lawrenceburg, on brick on north face of spirit maturing warehouse, 9 Aug. 2005, *J.A. Scott*, UAMH 10812; Kentucky, Loretto, on a concrete wall, 24 May 2002, *D. Livermore*, UAMH 10764; New York, Olean, from a fallen tree branch (?*Acer* sp.) at a commercial bakery, June 2005, *W. Burch*, UAMH 10811.

## DISCUSSION

*Baudoinia* is a member of the *Teratosphaeriaceae* (*Capnodiales*) but its position within this family is unresolved based on a comparison of nuclLSU sequences. *Baudoinia* was inferred as sister to *Friedmanniomyces* based on the comparison of nucSSU sequences (Scott et al. 2007); this relationship was not investigated in the present study because these genera were not identified as close relatives in BLAST searches and preliminary analyses of nuclLSU sequence data (results not shown).

Species of *Baudoinia* resemble those of *Catenulostroma* and *Neocatenulostroma* in possessing dark brown, thick-walled hyphae, reduced, integrated conidiogenous cells and dry, thick-walled septate conidia that are formed in simple to irregular chains. *Catenulostroma* includes species with muriform conidia that occur on leaves of *Proteaceae* whereas *Neocatenulostroma* are plant-associated or saxicolous species with variously shaped conidia that are transversely, longitudinally or obliquely

septate (Crous et al. 2007, Quaedvlieg et al. 2014). Species of *Baudoinia* are also reminiscent of *Capnobotryella renispora*, a species with thick-walled hyphae and ellipsoidal 0–1 septate phialoconidia (Crous et al. 2009) and *Elasticomyces elasticus*, a lichenicolous extremotolerant species that forms cylindrical, septate arthroconidia (Selbmann et al. 2008). Given that none of the aforementioned taxa have been identified as close relatives to *Baudoinia* in our BLAST searches and phylogenetic analyses, it appears that the micromorphological characters shared by these and likely other anamorphic *Capnodiales* are of limited value in species identification and differentiation. Indeed, since it is challenging to differentiate among species of *Baudoinia* using cultural and micromorphological characters, we have elected to recognize species in the genus based on distinctive sequence motifs of the nuclITS rRNA, beta-tubulin and elongation factor 1-alpha genes.

Although associated only with industrial pollution, *Baudoinia* probably exists in nature as isolated microcolonies where its growth may be favoured by the presence of natural ethanol emissions associated with decomposition or in microenvironments where it is not subject to competition from other microbes. Its conidia (modified cells) probably break free from these microcolonies, becoming distributed in the air, although it is not a common airborne fungus even in areas with extensive *Baudoinia* growth (J.A. Scott, unpubl.). *Baudoinia* is also present on used whisky barrels (Scott et al. 2007) suggesting that the transport of whisky barrels provides inoculum to seed its establishment in new habitats. However, it is only in the presence of the unnatural, superfluous ethanol emissions of industry that these fungi grow luxuriantly, producing thick, confluent, crust-like colonies indiscriminately on nearly every surface, causing extensive aesthetic damage.

**Acknowledgements** Specimens from which cultures were derived for this study were kindly arranged/collected by David Doyle (Pernod Ricard, USA), Dr. Patrice Robichon (Pernod Ricard, Paris, France), Dr. Jeremy Spouge (Chivas Bros. Ltd., Dumbarton, Scotland, UK), Vidia Doodnath (Angostura Ltd., Laventille, Trinidad and Tobago), William Burch (formerly MacTec Federal Programs Inc., Herndon, VA, USA), Jerry Edwards (Mount Gay Distillers Ltd., St Michael, Barbados), Jean-François Lavigne (Pernod Ricard, Cadiz, Spain), Don Livermore and Dan DeMarco (formerly Hiram Walker & Sons Ltd., Windsor, Ontario, Canada), C. Gomez (Bella Vista, Buenos Aires, Argentina), S.P. Chang (Jinro Distillers Co, Kyonggi-Do, Korea). Financial support for this study was generously provided by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to WAU and by Sporometrics Inc. The Canada Foundation for Innovation is also gratefully acknowledged for infrastructure awards to WAU.

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