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Baudoinia, a new genus to accommodate *Torula compniacensis*

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Abstract: *Baudoinia* gen. nov. is described to accommodate *Torula compniacensis*. Reported originally from the walls of buildings near brandy maturation warehouses in Cognac, France, species of *Baudoinia* are cosmopolitan colonists of exposed surfaces subjected to large diurnal temperature shifts, episodic high relative humidity and wetting, and ambient airborne ethanol. Morphologically *B. compniacensis* resembles some anamorphic Mycosphaerellaceae in possessing dark brown, nonseptate or uniseptate conidia with coarsely roughened walls that are borne acropetally in unbranched chains and released by schizolytic dehiscence. Analysis of partial nuclear rDNA SSU sequences positions *B. compniacensis* in the order Capnodiales and reveals that it is most closely related to the microcolonial genus *Friedmanniomyces*. Heat resistance is induced by brief sublethal temperature exposure.

Key words: *Capnobotryella*, *Knufia*, microcolonial fungi, sooty molds, systematics, warehouse staining fungus

INTRODUCTION

Several years ago we became involved in a project to characterize superficial fungal colonists on the exteriors of industrial buildings used to house barrels of spirits during the maturation period. The susceptibility of the exteriors of these buildings to dark, sooty, fungal growth, so-called ''warehouse staining'', has been well known anecdotally in the spirits industry for many years. During our investigation we reviewed a number of internal, industry-commissioned studies of this phenomenon from Asia, Europe and North America that attempted to ascertain the taxonomic composition of this material using culture-based techniques. Despite the distinctiveness of this habitat and the characteristic sooty appearance of the growth, these reports persistently implicated the same etiologically implausible set of ubiquitous environmental microfungi, chiefly *Aureobasidium pullulans* (de Bary) Arnaud, *Epicoccum nigrum* Link, and species of *Alternaria* Nees, *Aspergillus* P. Micheli ex. Haller, *Cladosporium* Link, and *Ulocladium* Preuss. A search of the post-1950s scientific literature indexed by ISI Web of Knowledge failed to yield references to this phenomenon. However a broader search of trade literature and the Web led us to the name *Torula compniacensis* Richon in reference to the warehouse staining fungus. Subsequently several profitable discussions with Dr Stanley Hughes provided historical references key to our rediscovery of this taxon.

In 1872 the French pharmacist Antonin Baudoin brought to the attention of Casimir Roumeguère and Charles Durieu de Maisonneuve a black, sooty growth found on the walls and roof tiles of buildings near distilleries in Cognac, France (Roumeguère 1881). Roumeguère and Durieu de Maisonneuve considered the fungus to be an undescribed species of *Xenodochus* Schltdl. and informally suggested the name ''*X. baudoinii*'' (Roumeguère 1881). A few years later Adolphe Chatin presented to a meeting of the Botanical Society of France colonized fragments of roofing tiles and stones collected from neighborhoods surrounding a distillery in Cognac (fide Malinvaud 1878). Later the same year Baudoin self-published a brochure describing the phenomenon in which he incorrectly attributed the growth to the cyanobacterial genus *Nostoc* Vaucher (fide Richon and Petit 1881). A more thorough examination of the fungus was undertaken by Richon and Petit (1881), who described it as *Torula compniacensis*. Stanley Hughes brought to our attention what seems to be the only modern investigation of this fungus, a study of germination patterns conducted by Kjøller (1961) published in a source not currently indexed by the ISI Web of Knowledge database.

The anamorph genus *Torula* Pers. : Fr., typified by *T. herbarum* (Pers.) Link : Fr., was conceived broadly to include mostly dematiaceous hyphomycetes with

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conidia borne in moniliform chains. Of the more than 300 taxa that have been included in this genus, all but a few since have been redisposed (Crane 2001). The modern concept of *Torula* is restricted to anamorphs with monoblastic or polyblastic conidiogenous cells that give rise to acropetal chains of dark, typically roughened phragmoconidia. The wall of the distal conidial cell is characteristically thinner and lighter in pigmentation than the rest of the conidium, often collapsing proximally to form a cupulate ''corona'' cell (Crane 2001).

In his recent nomenclator of *Torula*, Crane (2001) included both *T. compniacensis* and *T. conglutinata* Corda var. *compniacensis* (Richon) Sacc. in Roum. as *nomina nuda* but he did not reassign *T. compniacensis*. *Torula compniacensis* is not a species of *Torula* or *Hormiscium* Kunze, a taxonomic synonym of *Torula*. Furthermore it cannot be accommodated in the morphologically similar anamorph genera *Alysidiella* Crous, *Capnobotryella* Sugiyama, *Cladosporium* Link, *Devriesia* Seifert & N.L. Nickerson (based on *Hormodendrum staurophorum* Kendrick), *Knufia* Hutchison & Unter., *Trimmatostroma* Corda, or *Zasmidium* Fr. sensu de Hoog (=*Racodium cellare* Pers.).

We have obtained a number of darkly pigmented and slowly growing fungal isolates from environments near spirit aging facilities and also commercial bakeries. Colonies of these isolates are predominantly subsurface and radiating, producing densely roughened, unbranched aerial chains of schizolytically dehiscing blastoconidia that do not disarticulate readily. The present study describes our investigation of the relationship of our isolates to *T. compniacensis* and morphologically similar taxa based on morphotaxonomic methods and the comparison of sequences of the nuclear ribosomal small subunit (SSU) gene and proposes a modern reassignment of *T. compniacensis*.

MATERIALS AND METHODS

Isolation, culture and storage.—Cultures were obtained by streaking small amounts of powdered specimens on the surface of modified Leonian's agar (MLA) (Malloch 1981) solidified with 15 g L^{-1} agar. The medium used for primary isolations was amended with 10 ppm chloramphenicol and 5 ppm ethanol after autoclaving and before solidification. Single germinated hyphal fragments were recovered under low power transmitted light microscopy with the aid of a heat-sterilized insect pin (size 00, Fine Science Tools, Vancouver, British Columbia) and isolated in axenic culture. Slide cultures on filtered oatmeal agar (Gams et al 1987) and MLA were prepared following Malloch (1981). Cultures were stored on MLA slants overlain with sterile, heavy mineral oil.

Microscopic observations.—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. Digital images were acquired with a microscope-mounted Olympus C5050 digital camera.

Thermotolerance.—Stock cultures grown 14 d at 26 C on 90 mm Petri dishes containing MLA were used to prepare mycelial inoculum for liquid cultures by flooding log-phase agar cultures with sterile water and gently scraping surface mycelia into suspension. Suspensions were filtered under axenic conditions through sterile gauze in a thistle funnel covered with aluminum foil to remove large mycelial aggregates. Fragments in the resulting suspension were enumerated, their concentration adjusted with sterile distilled water to 150 000–250 000 elements mL^{-1} and stored at 4 C for up to 24 h until use.

Thermotolerance in liquid medium of isolates UAMH 10761 and 10762 was tested by inoculating flasks containing 50 mL of modified Leonian's broth (MLB) preconditioned to 26 (control), 37, 42 and 52 C with 200 μL mycelial suspension and incubated at the treatment temperature for 30 min. After incubation cells were harvested by centrifugation at 7000 rpm, gently washed with sterile distilled water, plated on MLA and incubated at 26 C for 14–21 d. Resulting colonies were counted, and the percentage viability for each treatment was calculated by comparison to controls maintained at 26 C. The thermotolerance assay was conducted in duplicate for 26, 37 and 42 C and in triplicate at 52 C. The effect on viability of a 30 min pretreatment regimen at 37 C before 15 min incubation at 52 C was tested in duplicate.

Thermotolerance under dry conditions also was tested by harvesting log-phase broth cultures developed at 26 C by vacuum filtration on sterile 25 mm diam 0.8 μm pore mixed cellulose esters membrane (MCEM) filters, drying the cells at temperatures up to 70 C for periods up to 21 d and reculturing cells on MLA at 26 C.

DNA isolation and amplification.—Mycelium was harvested from 5 d cultures grown in MLB, rinsed several times in sterile distilled water and stored at −20 C. Axenic culture was verified by incubating a small amount of the harvested mycelium on MLA. Cell walls of 15 mg thawed mycelial aliquots were disrupted by grinding with an equal volume sterile, powdered perlite (Dicaperl Minerals Inc., Socorro, New Mexico) (Scott et al 2000) and total DNA was isolated in a CTAB-based lysis buffer following the method of Scott et al (2004), modified from Weising et al (1995). Strain information, references and GenBank numbers for taxa used in this study are provided (TABLE I).

Portions of the nuclear ribosomal DNA SSU were amplified with the primers NS1, NS3, NS4 and NS8 (White et al 1990). PCR consisted of 1 unit of Taq DNA polymerase (Boeringher Mannheim, Laval, Quebec), 50 mM KCl, 2.0 mM $MgCl_2$, 250 μM of each dNTP, 0.2 mM of each primer and approximately 60 ng high molecular weight template in a total volume of 50 μL. Amplifications were carried out in a PTC-200 thermal cycler (MJ Research Inc./Bio-Rad Laboratories, Reno, Nevada) with this profile: 94 C for 30 s, 58 C for 30 s and 72 C for 30 s, repeated 30 cycles,

TABLE I. List of sequences and strains used in this study

Taxon	Strain	GenBank No.	Reference
Baudoinia compniacensis	UAMH 10808 T	EF494252	this study
B. compniacensis	UAMH 10762	EF137357	this study
B. compniacensis	UAMH 10764	EF137355	this study
Capnobotryella renispora J. Sugiyama	CBS 572.89	AY220614	Hambleton et al (2003)
Cap. renispora	CBS 214.90 T	EF137360	this study
Coccodinium bartschii A. Masall.	UME 30232	U77668	Winka et al (1998)
Delphinella strobiligena (Desm.) Sacc. ex E. Müll. & Arx	CBS 735.71	AY016341	Lumbsch and Lindemuth (2001)
Devriesia staurophora (W.B. Kendr.) Seifert & N.L. Nick.	CBS 374.81A	EF137358	this study
Dev. staurophora	CBS 375.81	EF137359	this study
Discospharina fagi (H.J. Huds.) M.E. Barr	CBS 171.93	AY016342	Lumbsch and Lindemuth (2001)
Dothidea sambuci (Pers.) Fr.	DAOM 231303	AY544722	Schoch et al (2006)
Dothiora cannabinae Froid.	CBS 737.71	DQ479933	Schoch et al (2006)
Endoconidioma populi A. Tsuneda et al	UAMH 10297 T	AY604526	Tsuneda et al (2004)
Friedmanniomyces endolithicus Onofri	CCFEE 522	DQ066715	Selbmann et al (2005)
F. simplex Selbmann et al	CCFEE 5184 T	DQ066716	Selbmann et al (2005)
Leptosphaeria maculans (Desm.) Ces. & De Not.	DAOM 229267	DQ470993	Schoch et al (2006)
Lophiostoma crenatum (Pers.) Fuckel	CBS 629.86	U42485	Berbee (1996)
Mycosphaerella punctiformis (Pers.) Starbäck	CBS 113265	DQ471017	Schoch et al (2006)
Pyrenophora tritici-repentis (Died.) Drechsler	OSC 100066	AY544716	Schoch et al (2006)
Scleroconidioma sphagnicola A. Tsuneda et al	UAMH 9731 T	AY220610	Hambleton et al (2003)
Trimmatostroma abietis Butin & Pehl	CBS 459.93 T	DQ678040	Schoch et al (2006)
Zasmidium cellare (Pers.) Fr.	CBS 892.85	EF137365	this study

Culture collection acronyms used: CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CCFEE = Culture Collection of Fungi from Extreme Environments, Viterbo, Italy; DAOM = National Mycological Herbarium/Canadian Collection of Fungal Cultures, Ottawa, Ontario, Canada; UAMH = University of Alberta Microfungus Collection and Herbarium, Edmonton, Alberta, Canada; UME = Herbarium, Umeå University, Umeå, Sweden). ''T'' denotes a culture derived from type material.

with a final extension at 72 C for 2 min. Yield was approximately quantified by electrophoresis on 1.2% agarose gels, ethidium bromide staining and UV visualization. PCR-amplified templates were purified with QIAquick PCR purification kit (QIAGEN Inc., Valencia, California).

Sequencing reactions were performed with a Taq Dye-Deoxy cycle sequencing kit (Applied Biosystems Inc., Foster City, California) with the above primers. Excess dye terminators were removed before analysis on an ABI-50 fluorescent automated sequencer (Applied Biosystems Inc.).

Sequence data analysis.—Sequence alignments were generated with SeqPup version 0.6d (Gilbert 1996) and Clustal X version 1.7 (Thompson et al 1997) and adjusted manually. Phylogenetic relationships were inferred from aligned sequences with the maximum parsimony method in PAUP* version 4.0b10 (Swofford 2003). An heuristic search of the dataset was performed employing tree bisection-reconstruction (TBR) branch-swapping using the MULTREES and steepest descent options. Bootstrap support for internal branches was evaluated from 1000 heuristic searches with TBR branch-swapping. Gaps were defined as a fifth character in all analyses. *Leptosphaeria maculans*, *Lophiostoma crenatum* and *Pyrenophora tritici-repentis* (Pleosporales) were used as outgroup taxa.

RESULTS

Baudoinia J.A. Scott & Unter., gen. nov.

Mycelium nigrum est, effusum, a velveteo ad crustaceum. Hyphae vegetativae fuscae, densae parietibus, frequenter moniliformes sunt, cum asperitatibus crassis. Conidiophoris egregiis cellulisque conidiogenosis caret. Cellulae conidiogenosae intra hyphas vegetativas iunguntur. Procreatio conidialis ambigua est, et ad summam explicatur disarticulatione hypharum vegetativarum in propagula germinabila oriri. Conidia in catenis acropetalibus quae frangendo etiam cum turbatione resistunt blasticaliter elaborantur. Dehiscentia conidialis schizolyse fit. Coloniae in cultura axenica in agaro Leoniani immutato lente crescentes, nigrae. Synanamorphos phialidicus absens. Teleomorphos ignotus.

Species typica: Baudoinia compniacensis (Richon) J.A. Scott & Unter.

Mycelium black, effused, velvety to crust-like. Vegetative hyphae dark brown, thick-walled, often moniliform. Distinctive conidiophores lacking. Conidiogenous cells integrated within vegetative hyphae. Conidia dry, nonseptate or uniseptate at the median,

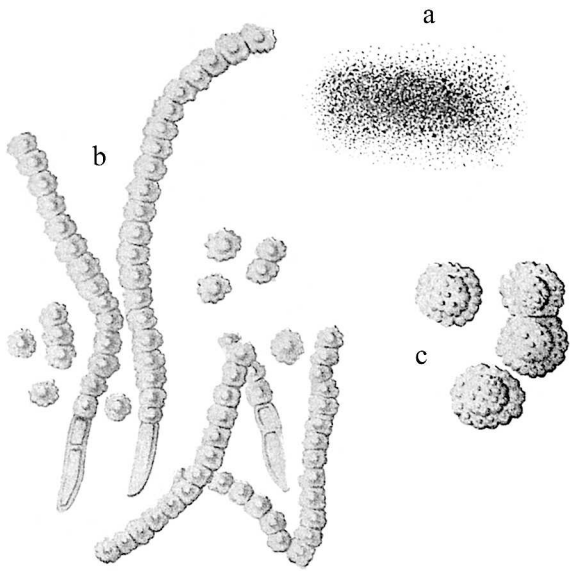


FIG. 1. Original illustration of *Torula compniacensis* reproduced from Richon and Petit (1881:Pl 5). a. Gross appearance. b. Conidia and conidiophores. c. Conidia. No magnification given.

thick-walled, globose to barrel-shaped, brown to black, typically with coarse surface ornamentation, dehiscencing by schizolysis. Ramoconidia absent. Colonies on MLA slow growing, darkly pigmented. Synanamorphs absent. Teleomorph unknown. MycoBank MB510726.

Type species: *Baudoinia compniacensis* (Richon) J.A. Scott & Unter.

Etymology: Named in honor of Antonin Baudoin.

Baudoinia compniacensis (Richon) J.A. Scott & Unter., comb. nov. FIGS. 1–7

Basionym: *Torula compniacensis* Richon, Rev. Mycol. (Paris) 3:17. 1881.

≡ *Torula conglutinata* Corda var. *compniacensis* (Richon) Sacc., Rev. Mycol. (Paris) 3:17. 1881.

= *Xenodochus baudoinii* Roum. & Durrieu, Mycol. Rev. Mycol. (Paris):16. 1881. *nom. nud.*

Illustrations. Richon and Petit (1881) Pl. 5; Richon in Roumeguère (1881) FIGS. 1–3; Kjøller (1961) FIGS. 1–3.

Colonies black, effuse to crusted-like, spreading to cover extensive areas of substrate with dense growth often with pronounced vertical streaking, becoming markedly thickened and friable in age. Vegetative hyphae dark brown, thick-walled, cylindrical, constricted at septa, with coarse, linear or tufted roughenings arising on proximal mature regions of the cell wall. Conidia borne in unbranched chains consisting of arcuate, lateral branches of vegetative hyphae that resist fragmentation. Conidia dry, 0–1 septate at the

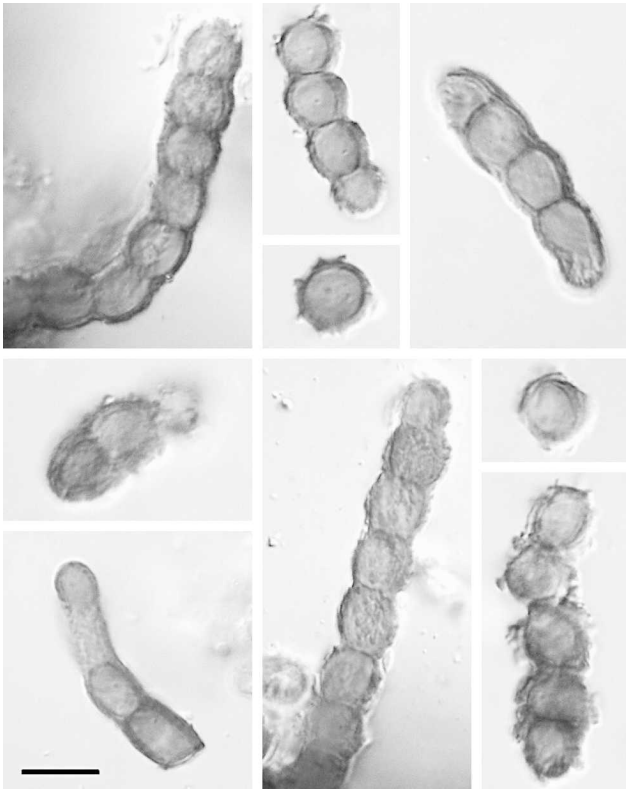
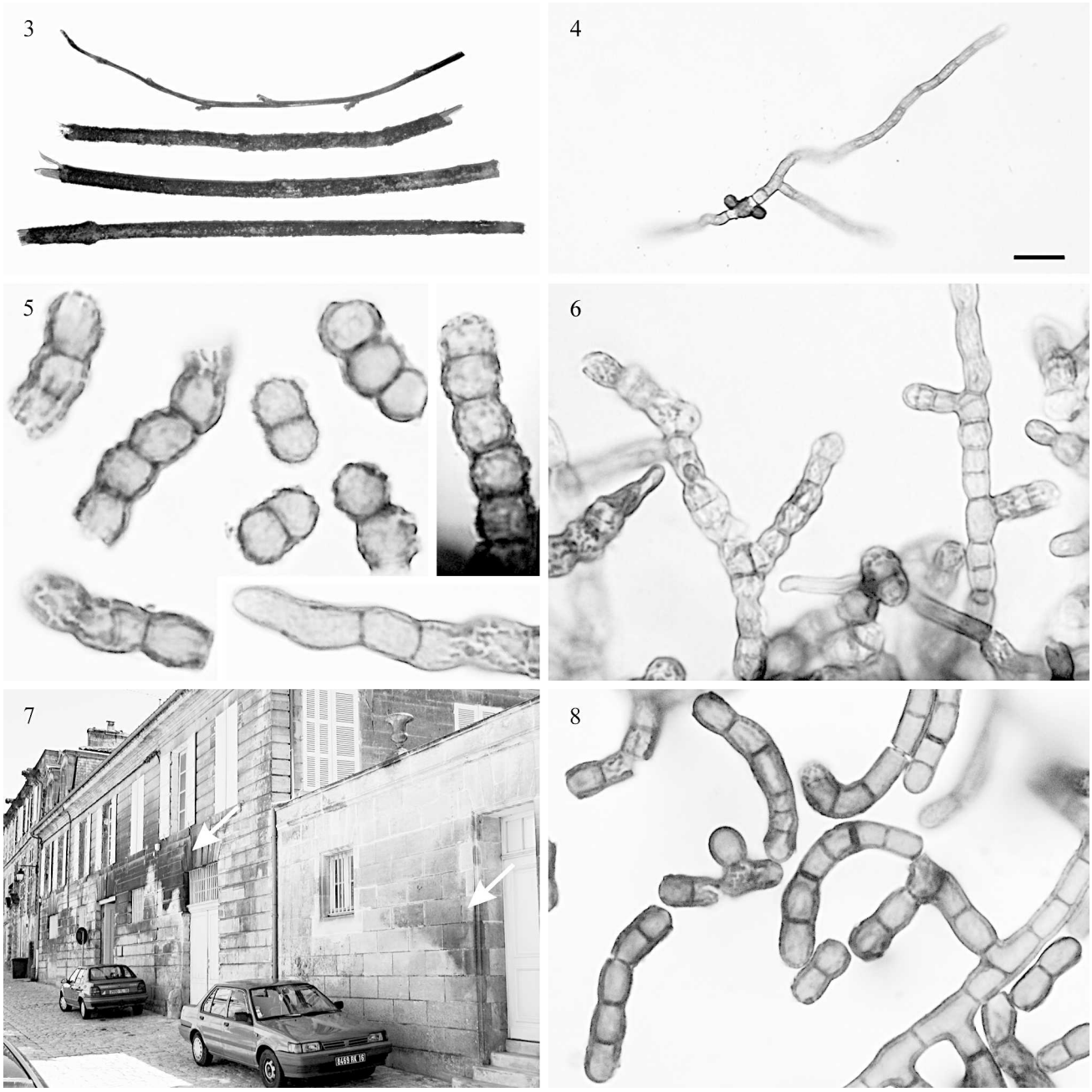


FIG. 2. *Baudoinia compniacensis* (DAOM 66898 lectotype). Conidia and hyphae showing characteristic hyperpigmented verrucose roughenings. Bar = 10 μm.

median, thick-walled, globose to barrel-shaped, (7)8–10(15) × 7–9 μm, brown to black with coarsely verrucose surface ornaments that often coalesce into tufts or short linear ridges, released by schizolysis, resulting in broad dehiscence scars, often revealing central dome-like herniations of the distal and proximal dehiscence regions of the cell wall that are paler and smoother than the lateral cell wall. Colonies on MLA slow growing, darkly pigmented (less than 10 mm diam in 28 d at room temperature), black, heaped to irregularly cerebriform, with radiating subsurface hyphae. Synanamorphs absent. Teleomorph unknown. MycoBank MB170778.

Distribution. Argentina, Canada, Denmark, France, United Kingdom (Scotland), USA.

Material examined. ARGENTINA: Bella Vista; Buenos Aires. On stone wall, 16 iii 2005, *C. Gomez*, det. *J.A. Scott* (DAOM 238779, UAMH 10813). CANADA. ONTARIO: Lakeshore, near Pike Creek. On bark of *Picea* sp., 12 iii 2002, *J.A. Scott* (DAOM 237861, UAMH 10762). Same location. On gravel, 12 iii 2002, *J.A. Scott* (DAOM 237860, UAMH 10763). QUEBEC: Valleyfield. On wall of distillery aging warehouse, vii 1967, *Collector unknown*, det. *S.J. Hughes* (DAOM 116544). DENMARK. SJÆLLAND: Dalby. On asbestos wallboard from a liquor factory, vi 1959, *M. Lange ex* Danish Fungi (DAOM 109435). Same location. On stone from roof of liquor factory, viii 1959, *M. Lange ex* Danish



FIGS. 3–8. Photographs of *Baudoinia compniacensis*. 3. Epitype specimen showing typical, dark, sooty colonies (DAOM 238773). 4. Streak preparation of field collection on MLA amended with 50 mL·L^{-1} Canadian whiskey showing germinating conidium (UAMH 10762). 5. Conidia and hyphae from field collection (DAOM 238773). 6. Conidia and hyphae from 14 d slide culture on MLA (UAMH 10809). 7. Sharply demarcated colonies on block wall, Cognac, France. 8. Hyphae and conidia from 14 d slide culture on MLA showing schizolytic dehiscence (UAMH 10761). Bars: 3 = 7 mm, 4 = 30 μm, 5 = 5 μm, 6 and 8 = 10 μm.

Fungi (DAOM 66897). FRANCE. Cognac, near the bank of the Charentes River. On affected walls of a building at a distillery of *eau-de-vie*, vii 1881, *A. Baudoin & P. Brunard ex C. Roumeguère, Fungi Gallici Exsiccati No. 1695* (LECTOTYPE DAOM 66898, designated here). Same location, near the Hennessy distillery. On paint chips peeled from a metal lamp standard, 17 v 2003, *J.A. Scott* (DAOM 237863). Merpins (4 km east of Cognac), near the Remy Martin distillery. On tree branches (species unknown), vii 2006, *R.C. Summerbell*, det. *J.A. Scott* (EPITYPE DAOM 238773, designated here, EX-EPITYPE living culture UAMH 10808). UNITED KINGDOM. SCOTLAND: Beith: Willowyard, near Kibirnie Loch. On mortar from a masonry wall, v 2005, *J. Spouge*, det. *J.A. Scott* (DAOM 237862, UAMH 10761).

Same location. On cloth covering of whisky barrel bung, iv 2006, *J. Spouge*, det. *J.A. Scott* (DAOM 238774, UAMH 10809). USA. KENTUCKY: Loretto. On a concrete wall, 24 v 2002, *D. Livermore*, det. *J.A. Scott* (DAOM 237864, UAMH 10764). NEW YORK: Olean, from a fallen tree branch (*?Acer* sp.) at a commercial bakery, vi 2005, *W. Burch*, det. *J.A. Scott* (DAOM 238776, UAMH 10811).

Additional material examined. Aureobasidium pullulans. USA. WISCONSIN: La Crosse. On exterior steel siding of a beer-aging warehouse, 24 vii 2005, *J.A. Scott* (UAMH 10765). *Capnobotryella renispora.* JAPAN. NAGANO PREF.: Sanada, Arboretum of Sugadaira Montane Research Center, alt. 1300 m. On subiculum of *Capnobotrys neesii* S. Hughes on living twig and leaf of *Abies veitchii* Lindley, ix 1982, *J. Sugiyama*, (EX-TYPE living culture CBS 214.90). *Coniosporium perforans* Sterflinger et al. GREECE. Isolated from marble, s.d., *K. Sterflinger*, (EX-TYPE living culture CBS 885.95). *Devriesia staurophora.* COLOMBIA. CUNDINAMARCA: Cruz Verde, ca. 3000 m alt. Isolated from páramo soil, vi 1981, *W. Gams* (CBS 375.81, as *Cladosporium staurophorum*). *Knufia cryptophialidica* Hutchison & Unter. CANADA. ALBERTA: near Whitecourt. Isolated from black gall on *Populus tremuloides* Michx., 13 v 1991, *P. Crane* (EX-TYPE living culture DAOM 216555). GERMANY. Lorch am Rhein. On wall of wine cellar, s.d., *M. Schlag* (CBS 892.85). s.l. On wall of wine cellar, vi 1936, *H. Schanderl* (CBS 146.36). *Zasmidium cellare.* s.l. On wall of wine cellar, s.d., *C.G.D. Nees v. Esenbeck ex herb. Persoon* no. 910.263-61 (EX-LECTOTYPE slide DAOM 57305, as *Racodium cellare* Fr. cited previously as *R. cellare* Pers.).

Commentary.—Richon and Petit (1881) described and illustrated *Torula compniacensis*, noting its microscopic similarity to *T. conglutinata* Corda. A specimen provided by Baudoin and Brunard was included later by Roumeguère in his *Fungi Gallici Exsiccati* Century 17, No. 1695 (Roumeguère 1881). In a footnote in the same publication, Roumeguère (1881) reduced Richon's species to a variety of *T. conglutinata*, an opinion, he noted, that was shared by Saccardo. Saccardo later accepted *T. compniacensis* Richon (Saccardo 1886). In the footnote Roumeguère (1881) made an orthographic error in the species epithet, substituting ''*conglomerata*'' for ''*conconglutinata*''. This error, in turn, was transcribed to the specimen packets of the exsiccata. Crane (2001) perpetuated the misspelling by listing ''*T. conglomerata nom. nud.* var. *compniacenis*'' as a nomenclatural synonym of *T. compniacensis*.

Thermotolerance.—In liquid culture, all isolates tested demonstrated optimal growth around 26 C, 50% lethality (LD_{50}) was observed at 37–52 C, and complete lethality was seen above 42 C. Typical temperature-viability profiles are provided (FIG. 9) (UAMH 10761 and 10762). A 30 min pre-incubation at 37 C before heat treatment at 52 C resulted in increased survival, from 0% to 25–60%. Under dry conditions all isolates tested remained viable after prolonged incubation at 70 C (up to 21 d).

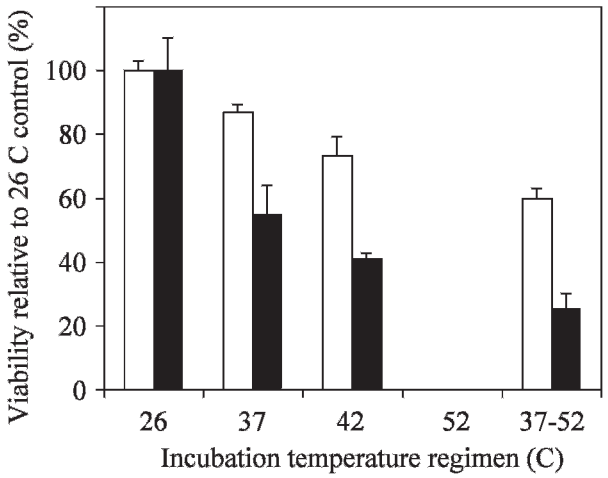


FIG. 9. Effect of different incubation temperature regimens on viability of UAMH 10761 (white histograms) and 10762 (black histograms). Values represent means of 2 or 3 replicates as a percentage of the mean control value (26 C), error bars indicate SD.

Phylogenetic analysis.—DNA sequence data are critical in the circumscription of the genus. Given our inability to infer these characters from lectotype material, we deem this specimen insufficient to interpret the taxon. In accordance with Art. 9.7 of the International Code of Botanical Nomenclature (McNeill et al 2006) we hereby designate an epitype specimen from which we have derived these characters.

SSU rDNA sequences of two isolates of *Baudoinia compniacensis* from the Americas (UAMH 10762 and 10764) were 1708 bp long and invariant. The SSU rDNA sequence of the ex-epitype strain of *B. compniacensis* (UAMH 10808) differed in at two bp positions and contained a 362 bp insertion. After the reduction of all multiple base indels to single characters, the final alignment for 22 taxa comprised 1707 characters of which 107 were parsimony informative. An heuristic analysis of this dataset produced three equally parsimonious trees 275 steps long with a consistency index (CI) of 0.804 and a retention index (RI) of 0.835. One of these three trees is shown (FIG. 10).

Baudoinia compniacensis is a member of a well supported clade (93% bootstrap support) corresponding to the order Capnodiales as circumscribed by Schoch et al (2006). Within the Capnodiales *B. compniacensis* forms a reasonably well supported clade (75% bootstrap support) with species of *Friedmanniomyces* Onofri. *Baudoinia* and *Friedmanniomyces* are most closely related to *Trimmatostroma abietis* Butin &

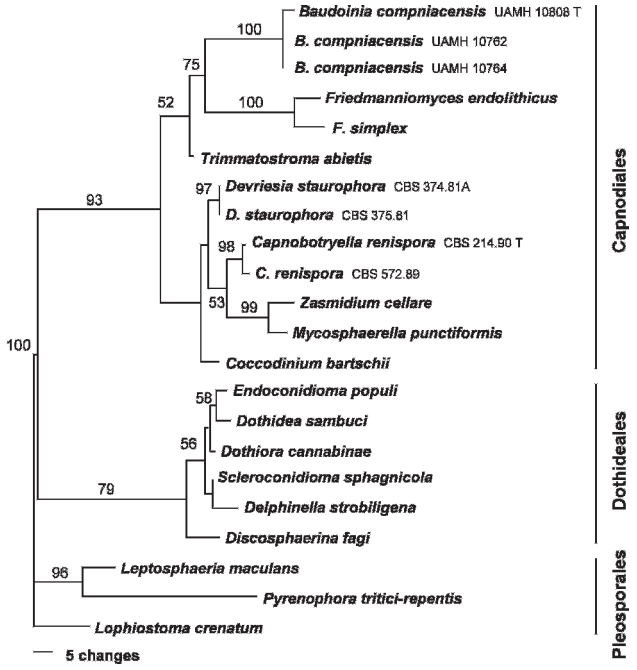


FIG. 10. Phylogenetic relationships of selected members of the orders Capnodiales, Dothideales and Pleosporales (Dothideomycetes) inferred from maximum parsimony analysis of SSU rDNA sequences. This is one of three equally parsimonious trees generated from an heuristic search of 1707 characters for 22 taxa (length = 275 steps, CI = 0.804, RI = 0.835). Bootstrap values greater than 50% calculated from 10 000 replicates are given either above or below branches. Outgroup taxa are *Leptosphaeria maculans*, *Lophiostoma crenatum* and *Pyrenophora tritici-repentis* (Pleosporales).

Pehl, but bootstrap support for this clade is weak (52%). The only strongly supported clade within the Capnodiales composed of more than one species is the group that includes *Zasmidium cellare* CBS 892.85 and *Mycosphaerella puntiformis* (99% bootstrap support). The Capnodiales is sister of a reasonably well supported clade (79% bootstrap support) comprising representatives of the order Dothideales. Groups within the Dothideales receiving greater than 50% bootstrap include the *Endoconidioma populi-Dothidea sambuci* clade (58%) and the clade encompassing these two taxa along with *Dothiora cannabinae*, *Scleroconidioma sphagnicola* and *Delphinella strobiligena* (56%).

DISCUSSION

The lack of distinctive conidiogenesis is itself a feature of *B. compniacensis*. All collections examined had similar schizolytic fragmentation of unspecialized hyphae into conidia (called ''chlamydospores'' by Kjøller 1961). These structures are consistent with both the ontogenic and propagative definitions of conidia, and we consider them to be conidia in the proper sense. On primary isolation the cell walls of *B. compniacensis* conidia swell and burst, giving rise to one or more lightly pigmented, smooth germ tubes within 2–3 d, frequently appearing greenish in transmitted light (FIG. 4). Kjøller (1961) noted two distinct patterns of germination of thick-walled conidia in primary cultures (i.e. those derived directly from naturally colonized substrata): (i) direct germination to produce filamentous germ tubes; and (ii) initial production of a mucilaginous yeast-like phase followed by filamentous growth after several months. We did not observe the latter pattern and suggest the possibility that Kjøller's observations of yeast-like growth in *B. compniacensis* were based erroneously on the yeast-like fungus, *Aureobasidium pullulans*, a contaminant we encountered frequently during this study, accidentally recovered during primary isolations. At maturity the latter species forms thick-walled melanized ''chlamydospores'' that are morphologically similar to the conidia of *B. compniacensis*.

Colonies of *B. compniacensis* on agar media are slow growing with broad, black, radiating, subsurface hyphae strongly reminiscent of those of *Knufia cryptophialidica*, but the latter species produces a phialidic synanamorph and endocondia (Hutchison et al 1995, Tsuneda and Currah 2004). Based on the comparison of micromorphological and cultural characteristics, Hutchison et al (1995) speculated that *K. cryptophialidica* was allied to the Metacapnodiaceae (Capnodiales); however our analyses of SSU rDNA sequences demonstrate that this species is closely related to *Coniosporium* (100% bootstrap support, data not shown). The latter genus has been inferred as sister of the Chaetothyriomycetes (Hambleton et al 2003; Sterflinger et al 1997, 1999).

Anamorphic Capnodiales resembling *Baudoinia compniacensis* include species of *Capnobotryella*, *Devriesia*, *Friedmanniomyces*, *Trimmatostroma* and *Zasmidium*. *Capnobotryella renispora*, a species documented from building materials in exposed conditions (Titze and de Hoog 1990), differs from *B. compniacensis* by its reniform conidia and *Capnophialophora* S. Hughes synanamorph (Sugiyama and Amano 1987). *Capnobotryella renispora* is inferred as a member of the Capnodiales in both our SSU rDNA phylogeny (FIG. 10) and the phylogeny of Hambleton et al (2003) but its position within this order is unresolved. Species of *Devriesia* produce muriform chlamydospores and mononematous conidiophores bearing conidia in branched chains (Seifert et al 2004). In contrast *Baudoinia* lacks chlamydospores and distinct ramoconidia and produces conidia predominantly in unbranched chains. *Zasmidium* Fr., a morphologically

and ecologically similar genus restricted by de Hoog (1977) to accommodate the well known wine cellar fungus, *Racodium cellare* Pers., differs from *Baudoinia* in possessing sclerotial bodies and a blastoconidial synanamorph. *Zasmidium cellare* and *B. compniacensis* also differ in habitat: the former is an indoor fungus associated with wine cellars, whereas the latter occurs only outdoors in association with low, intermittent levels of ethanol vapor. In our phylogeny a strain of *Z. cellare* from the wall of a wine cellar (CBS 892.85) was inferred as closely related to *Mycosphaerella punctiformis* (Mycospharellaceae, Capnodiales). A second strain of *Z. cellare* isolated from the same habitat (CBS 146.36, GenBank EF137362) was a member of a well supported clade (100% bootstrap support, data not shown) that included *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (GenBank DQ678004) and *Davidiella tassiana* (De Not.) Crous & U. Braun (GenBank DQ678022) (Davidiellaceae, Capnodiales).

Taxa inferred as most closely related to *Baudoinia compniacensis* in our SSU rDNA phylogeny are members of anamorph genera *Friedmanniomyces* and *Trimmatostroma*. *Friedmanniomyces* spp. are psychrophilic, dematiaceous, rock-inhabiting species known only from the Antarctic (Onifri et al 1999, Selbmann et al 2005). They are among a number of phylogenetically diverse, mostly melanized ascomycetes that manifest meristematic growth, many of which have been described from a variety of stone, marble, brick and block buildings or structures, and rocks, under conditions of low water availability, extreme temperatures and high UV radiation (Sterflinger et al 1999). In addition to their unique biogeography, microhabitat and inability to grow at 25 C (Selbmann et al 2005), species of *Friedmanniomyces* can be distinguished from *Baudoinia* by their meristematic growth habit, characterized by slowly expanding, cauliflower-like colonies that bear isodiametrically enlarging sclerotial bodies produced through a process of repeated cellular subdivision (Sterflingler et al 1999). Based on an analysis of SSU rDNA sequences, Selbmann et al (2005) inferred *F. endolithicus* and *F. simplex* as close to species of *Capnobotryella*, *Coccodinium* A. Massal., *Hobsonia* Berk. ex Massee, *Hortaea* Nishim. & Miyaji, *Mycocalicium* Vain. and *Trimmatostroma*, but support for this relationship was low. The sporodochial genus *Trimmatostroma* can be distinguished from *Baudoinia* by producing muriform conidia in easily disarticulating branched chains (Ellis 1971, 1976). *Trimmatostroma abietis* has been shown to be allied with members of the Mycosphaerellaceae (Selbmann et al 2005) and *Piedraia hortae* (Brumpt) Fonseca & Leão (Piedraiaceae, Capnodiales) (Schoch et al 2006).

Ecology.—We examined specimens of *B. compniacensis* from a range of geographic localities, primarily in association with the manufacture of distilled spirits. However *Baudoinia* species are not uniquely associated with spirit maturation because one collection originated from a commercial bakery. To date we have not observed *Baudoinia* spp. from nonindustrial habitats; however ambient ethanol vapor seems to be an important habitat determinant and it is reasonable to expect that *Baudoinia* spp. may occur in association with natural fermentative processes, such as seasonal fruit drops, bogs and natural composts.

In addition to ambient ethanol, *B. compniacensis* favors surfaces that are subjected to great environmental exposure, such as building exteriors and roofing materials that experience extreme diurnal fluctuations in ambient conditions. In parts of North America, the peak daytime temperatures of asphalt, roofing shingles with full sun exposure during the summer can exceed 65 C, while nighttime surface temperatures fall to as low as 15 C (J. Pogacar, Anderson Building Science Inc. pers comm; Winandy and Beaumont 1995). During the same time of year, morning dew may occur on these surfaces. Thus in 24 h temperatures can fluctuate up to 40–50 C, coincident with an extreme shift in moisture from total saturation to complete desiccation. The occurrence of *Baudoinia* in such habitats suggests a capacity for thermotolerance. Our results show this to be an inducible physiological feature of log-phase vegetative cells resulting from sublethal heat shock. This observation is in contrast to the mechanism of heat resistance reported for the genus *Devriesia*, in which high temperature pretreatment is required to initiate chlamydospore germination (Seifert et al 2004).

In our experience outdoor spore-trap air sampling from regions where heavy colonization was observed on building exteriors have rarely revealed conidia conforming morphologically to *B. compniacensis*. The apparent scarcity of conidial bioaerosol in these areas implies other modes of dispersal, such as rain splash or invertebrate-mediated dissemination. In an interesting note, we have observed in numerous, geographically distant localities signs of extensive invertebrate grazing of established colonies of *B. compniacensis* that suggest scraping by mollusk radulae. The dispersal of *B. compniacensis* remains an intriguing and unresolved aspect of the ecology of this ubiquitous fungus.

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