Blastobotrys americana sp. nov., *Blastobotrys illinoisensis* sp. nov., *Blastobotrys malaysiensis* sp. nov., *Blastobotrys muscicola* sp. nov., *Blastobotrys peoriensis* sp. nov. and *Blastobotrys raffinosifermentans* sp. nov., novel anamorphic yeast species

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The genus *Blastobotrys*, which now includes species previously assigned to the synonymous genera *Arxula* and *Sympodiomyces*, represents the anamorph of the ascosporeogenous genus *Trichomonascus*. Six novel species are proposed for assignment to *Blastobotrys*. They were detected from their unique nucleotide sequences in large-subunit rDNA, ITS1–5.8S–ITS2 rDNA, mitochondrial small-subunit rDNA and the cytochrome oxidase II gene. The proposed novel species are *Blastobotrys americana* sp. nov. (type strain NRRL Y-6844^T = CBS 10337^T; substrate unknown; Kansas, USA), *Blastobotrys illinoisensis* sp. nov. (type strain NRRL YB-1343^T = CBS 10339^T; from forest debris; Illinois, USA), *Blastobotrys malaysiensis* sp. nov. (type strain NRRL Y-6417^T = CBS 10336^T; from soil; Malaysia), *Blastobotrys muscicola* sp. nov. (type strain NRRL Y-7993^T = CBS 10338^T; from moss; Louisiana, USA), *Blastobotrys peoriensis* sp. nov. (type strain NRRL YB-2290^T = CBS 10340^T; from a fungus; Peoria, IL, USA) and *Blastobotrys raffinosifermentans* sp. nov. (type strain NRRL Y-27150^T = CBS 6800^T; substrate unknown).

The dimorphic genus *Blastobotrys* was originally described as a hyphomycete (von Klopotek, 1967), but phylogenetic analysis of domains D1/D2 of the large-subunit (LSU) rDNA demonstrated that assigned species are actually anamorphic members of the Saccharomycetales (Kurtzman & Robnett, 1995, 1998). The D1/D2 LSU rDNA analysis also showed close relatedness between *Blastobotrys*, *Arxula*, *Sympodiomyces* and several *Candida* species. More recently, Kurtzman & Robnett (2007) re-examined relationships among these taxa with a more robust dataset consisting of nucleotide sequences from the nearly complete LSU rDNA, mitochondrial small-subunit (SSU) rDNA and the cytochrome oxidase II (COXII) gene. This multigene analysis demonstrated *Blastobotrys*, *Arxula*, *Sympodiomyces* and the above-noted *Candida* species to be members of the same clade, which was interpreted as a single genus. Of described genera, *Blastobotrys* (von Klopotek, 1967) has taxonomic priority for this clade over *Arxula* (van der Walt *et al.*, 1990)

and *Sympodiomyces* (Fell & Statzell, 1971), resulting in the transfer of species assigned to the latter two genera to *Blastobotrys*. The multigene sequence analysis also showed *Trichomonascus* to represent the ascosporeic state of this clade. During the course of that study, six novel species of *Blastobotrys* were detected based on their unique nucleotide sequences in LSU rDNA, ITS rDNA, mitochondrial SSU rDNA and the COXII gene. In this report, descriptions are given for these six novel species.

Strains of the proposed novel species and reference taxa are given in Table 1 along with GenBank accession numbers for the genes sequenced. The strains are maintained in the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL, USA. Sources of the novel species are given in each species description. The composition of culture media used in this study, as well as the methods for fermentation and assimilation tests, was given previously (Yarrow, 1998).

Methods for DNA isolation and sequencing of domains D1/D2 of the LSU rDNA, as well as the nearly entire LSU rDNA, ITS1–5.8S–ITS2 rDNA, mitochondrial SSU rDNA and the COXII gene, were given by Kurtzman & Robnett

Abbreviations: LSU, large-subunit; SSU, small-subunit.

The GenBank/EMBL/DDBJ accession numbers for the LSU and mitochondrial SSU rDNA sequences, ITS sequences and COXII sequences of the proposed type strains and related strains are listed in Table 1.

Table 1. GenBank accession numbers for the strains compared

Accession numbers are for the nearly complete LSU (26S) rDNA sequence (LSU), the ITS1–5.8S–ITS2 region (ITS), the mitochondrial SSU rDNA (MtSm) and the cytochrome oxidase II gene (COXII). T, Type strain; I, isotype strain.

Strain	LSU	ITS	MtSm	COXII
<i>Blastobotrys americana</i> sp. nov. NRRL Y-6844 ^T = CBS 10337 ^T	DQ442699		DQ442778	DQ443106
<i>Blastobotrys illinoisensis</i> sp. nov. NRRL YB-1343 ^T = CBS 10339 ^T	DQ442696	DQ898169	DQ442775	DQ443103
<i>Blastobotrys malaysiensis</i> sp. nov. NRRL Y-6417 ^T = CBS 10336 ^T	DQ442695	DQ898170	DQ442774	DQ443102
<i>Blastobotrys muscicola</i> sp. nov. NRRL Y-7993 ^T = CBS 10338 ^T	DQ442680		DQ442759	DQ443087
<i>Blastobotrys peoriensis</i> sp. nov. NRRL YB-2290 ^T = CBS 10340 ^T	DQ442700		DQ442779	DQ443107
<i>Blastobotrys raffinosifermentans</i> sp. nov. NRRL Y-27150 ^T = CBS 6800 ^T	DQ442698	DQ898172	DQ442777	DQ443105
<i>Blastobotrys adeninivorans</i> NRRL Y-17692 ^T = CBS 8244 ^T	DQ442697	DQ898168	DQ442776	DQ443104
<i>Blastobotrys arbuscula</i> NRRL Y-17585 ^T = CBS 227.83 ^T	DQ442689		DQ442768	DQ443096
<i>Blastobotrys aristata</i> NRRL Y-17579 ^T = CBS 521.75 ^T	DQ442686		DQ442765	DQ443093
<i>Blastobotrys capitulata</i> NRRL Y-17573 ^T = CBS 287.82 ^T	DQ442688		DQ442767	DQ443095
<i>Blastobotrys chiropterorum</i> NRRL Y-17071 ^T = CBS 6064 ^T	DQ442682		DQ442761	DQ443089
<i>Blastobotrys elegans</i> NRRL Y-17572 ^T = CBS 530.83 ^T	DQ442687		DQ442766	DQ443094
<i>Blastobotrys indianensis</i> NRRL YB-1950 ^T = CBS 9600 ^{T*}	DQ442692		DQ442771	DQ443099
<i>Blastobotrys mokoenaui</i> NRRL Y-27120 ^T = CBS 8435 ^T	DQ442694	DQ898171	DQ442773	DQ443101
<i>Blastobotrys nivea</i> NRRL Y-17581 ^T = CBS 163.67 ^T	DQ442690		DQ442769	DQ443097
<i>Blastobotrys parvus</i> NRRL Y-10004 ^T = CBS 6147 ^T	DQ442693		DQ442772	DQ443100
<i>Blastobotrys proliferans</i> NRRL Y-17577 ^T = CBS 522.75 ^T	DQ442684		DQ442763	DQ443091
<i>Blastobotrys terrestris</i> NRRL Y-17704 ^T = CBS 7376 ^T	DQ442683		DQ442762	DQ443090
<i>Sporopachydermia lactativora</i> NRRL Y-11591 ^T = CBS 6192 ^T	DQ438185		DQ442714	DQ443042
<i>Sugiyamaella smithiae</i> NRRL Y-17850 ^I = CBS 7522.2 ^I	DQ438218		DQ442734	DQ443062
<i>Trichomonascus ciferrii</i> NRRL Y-10943 ^I = CBS 5295 ^I	DQ442681		DQ442760	DQ443088
<i>Trichomonascus farinosus</i> NRRL Y-17593 ^T = CBS 140.71 ^T	DQ442685		DQ442764	DQ443092
<i>Trichomonascus petasosporus</i> NRRL YB-2092 ^T = CBS 9602 ^T	DQ442691		DQ442770	DQ443098
<i>Trigonopsis variabilis</i> NRRL Y-1579 ^T = CBS 1040 ^T	DQ442707		DQ442785	DQ443114
<i>Wickerhamiella domercqiae</i> NRRL Y-6692 ^T = CBS 4351 ^T	DQ438240		DQ442756	DQ443084
<i>Zygoascus hellenicus</i> NRRL Y-7136 ^T = CBS 5839 ^T	DQ438216		DQ442719	DQ443047

*The original spelling of the species epithet, *indianaensis*, has been treated as an orthographical error.

(1998, 2003, 2007). Both strands of the DNAs analysed were sequenced with the ABI BigDye Terminator Cycle Sequencing kit (Applied Biosystems) using either an ABI 3100 or an ABI 3730 automated DNA sequencer according to the manufacturer's instructions. For phylogenetic analysis, sequences were aligned visually and regions of uncertain alignment were removed. Estimates of phylogenetic relatedness among species were determined using the maximum-parsimony and neighbour-joining programs of PAUP* 4.063a (Swofford, 1998). Bootstrap support for phylogenetic trees was determined from 1000 replications.

Maximum-parsimony analysis of concatenated nucleotide sequences from the nearly entire LSU rDNA, mitochondrial SSU rDNA and COXII gene demonstrated genetic separation of the six proposed novel species from previously described species in the *Trichomonascus/Blastobotrys* clade (Fig. 1). Results were the same when the dataset was analysed by neighbour-joining with Kimura's two-parameter correction. Two of the proposed novel species are closely related to each other and to *Blastobotrys mokoenaui*, and one of the proposed novel species is closely related to

Blastobotrys adeninivorans. Genetic separation of these species was further examined from ITS1–5.8S–ITS2 sequence analysis as discussed in the species descriptions. Sequences for LSU rDNA, mitochondrial SSU rDNA and the COXII gene were not determined for the recently described *Sympodiomyces attinorum* (Carreiro *et al.*, 2004) in the present study but, because D1/D2 LSU rDNA sequence analysis showed it to be a sister species of *Blastobotrys* (*Sympodiomyces*) *parvus*, Kurtzman & Robnett (2007) transferred it to *Blastobotrys* along with other species of *Sympodiomyces*.

Each of the proposed novel species is represented by just one strain. While it is desirable to base novel species descriptions on more than one isolate, type strains for most of the proposed novel species were obtained 30–50 years ago, and the probability of finding additional strains in the near future may be enhanced by these descriptions. Furthermore, awareness of these species brings additional perspective to the diversity within the genus *Blastobotrys*. The novel species *Blastobotrys illinoisensis* and *Blastobotrys malaysiensis* are closely related to each other and to *B. mokoenaui* on the basis of D1/D2 LSU divergence, and this is also seen for the species

Fig. 1. Phylogenetic placement of the six proposed novel species of *Blastobotrys* among related ascomycetous yeasts as represented by the single most-parsimonious tree determined from maximum-parsimony analysis of nucleotide sequences from the nearly entire LSU rDNA, mitochondrial SSU rDNA and the COXII gene. Tree length = 2640 steps, consistency index = 0.556, retention index = 0.572, rescaled consistency index = 0.318, homoplasy index = 0.444. Bootstrap values (1000 replicates) ≥ 50% are given at nodes. *Trigonopsis variabilis* NRRL Y-1579^T was the designated outgroup for the analysis. ARS Culture Collection strain numbers follow the names of taxa; GenBank accession numbers for the gene sequences are given in Table 1. *Blastobotrys attinorum* was not included in this multigene analysis, but D1/D2 LSU rDNA sequence analysis showed it to be a sister species of *Blastobotrys parvus* (Carreiro *et al.*, 2004).

pair *Blastobotrys raffinosifermentans* and *B. adeninivorans*. Nucleotide divergence in ITS, mitochondrial SSU and the COXII gene is concordant with D1/D2 LSU divergence (Table 2) and further suggests that these taxa are separate species, because the divergence is greater than that seen between strains of a species in closely related clades (Kurtzman & Robnett, 2007). Noteworthy in comparisons of these particular species pairs is that many of the sequence differences in ITS and mitochondrial SSU rDNA result from

indels, whereas differences in D1/D2 LSU rDNA and the COXII gene are exclusively from nucleotide substitutions.

The genus *Blastobotrys* is unusual among yeasts because of the presence of setae, which are long, tapered, tail-like extensions of cell wall material arising from the ends of cells. In their treatment of *Blastobotrys*, de Hoog & Smith (1998) reported that three of seven known species formed setae, i.e. *Blastobotrys aristata*, *Blastobotrys capitulata* and *Blastobotrys*

Table 2. Nucleotide divergence between closely related species of *Blastobotrys*

D1/D2, Domains 1 and 2 of LSU (26S) rDNA; ITS, ITS1–5.8S–ITS2 rDNA; MtSm, mitochondrial SSU rDNA; COXII, cytochrome oxidase II. s, Nucleotide substitution; i, indel.

Strain pair	Nucleotide differences between strains			
	D1/D2	ITS	MtSm	COXII
<i>B. adeninivorans</i> NRRL Y-17692 ^T – <i>B. raffinosifermentans</i> NRRL Y-27150 ^T	4s	3 (2s, 1i)	28 (1s, 27i)	17s
<i>B. mokenaii</i> NRRL Y-27120 ^T – <i>B. malaysiensis</i> NRRL Y-6417 ^T	4s	6 (4s, 2i)	8 (6s, 2i)	19s
<i>B. mokenaii</i> NRRL Y-27120 ^T – <i>B. illinoisensis</i> NRRL YB-1343 ^T	2s	20 (14s, 6i)	2 (1s, 1i)	13s
<i>B. malaysiensis</i> NRRL Y-6417 ^T – <i>B. illinoisensis</i> NRRL YB-1343 ^T	2s	16 (10s, 6i)	10 (7s, 3i)	20s

proliferans. In the present study, two of the six novel species, *B. illinoisensis* and *B. malaysiensis*, were found to produce setae, as did the recently described *B. mokoensis* (Mokwena *et al.*, 2000). Seta-forming species are not distributed uniformly within *Blastobotrys* and occur in only two subclades, but not all members of the *B. proliferans* subclade produce setae. All presently known ascosporeogenous species of the *Trichomonascus/Blastobotrys* clade are heterothallic (Kurtzman, 2004; Smith & de Hoog, 1998), which raises the possibility that anamorphic species in the clade may represent mating types.

Latin diagnosis of *Blastobotrys americana* Kurtzman sp. nov.

In agar *malti* post dies 3 ad 25 °C, *cellulae vegetativae globosae* (2.3–4.1 µm) *aut elongatae* (1.3–3.5 × 2.0–12.0 µm), *singulae, binae et in racemis brevibus*. In agar *morphologico* post dies 7 ad 25 °C, *incrementum fuscum pallidum, hebes, filamentosum; margo glabra vel undulata. Pseudohyphae et hyphae verae fiunt. Ascosporae non fiunt. Glucosum et trehalosum (infirme) fermentantur. Sucrosum, raffinose, galactosum, lactosum et maltosum non fermentantur. Assimilantur glucosum, raffinose, melibiosum, galactosum, trehalosum, methyl-α-D-glucosidum, cellobiosum, salicinum, L-sorbose, D-xylose, L-arabinose, D-arabinose, D-ribose, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, inositol, DL-acidum lacticum (infirme), acidum succinicum, D-glucosaminum, D-glucosaminum, N-acetyl-D-glucosaminum, hexadecanum, 5-keto-D-glucosaminum et cadaverinum. Non assimilantur inulinum, sucrosum, lactosum, maltosum, melezitiosum, amylinum solubile, L-rhamnosum, methanol, acidum citricum, potassii nitras, 2-keto-D-glucosaminum et saccharatum. Amylinum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 µg cycloheximido ml⁻¹ addito, et in medio 10 % sodii chloridum/5 % glucosum. Augmentum non fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleus submonas largus rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-6844^T (CBS 10337^T) designat stirpem typicam. Isolata e substrato incognito, Kansas, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.*

Description of *Blastobotrys americana* Kurtzman sp. nov.

Blastobotrys americana (a.me.ri.ca'na. N.L. nom. fem. adj. *americana* from America, i.e. the USA).

After 3 days growth on 5 % malt extract (ME) agar at 25 °C, budding is multilateral and cells are occasionally spherical, 2.3–4.1 µm, but are generally elongate, 1.3–3.5 × 2.0–12.0 µm, single, in pairs and in short chains (Fig. 2a). Colony growth is restricted, dull, tannish-white in colour and mycelial. Growth under the cover glass of a Dalmau plate culture with yeast morphology (YMo) agar is quite

restricted and is composed of pseudohyphae bearing infrequent blastoconidia after 7 days at 25 °C (Fig. 2b). Aerobic growth on this medium is also restricted, dull, mycelial and tannish-white. The colony margin is entire to lobed. After 7–10 days, growth begins to show true hyphae, often with tip cells that are inflated and globose. However, these inflated hyphal cells do not appear to be asci. Occasionally, intercalary hyphal cells become enlarged and spherical. Cultures were examined weekly for ascospore formation on yeast extract, malt extract, peptone, glucose (YM), 5 % ME, restricted growth (RG) and McClary's acetate agar media incubated at 15 and 25 °C. Ascospores were not detected after 3 months. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Dry, climbing pellicles are formed on stationary liquid media.

NRRL Y-6844^T (= CBS 10337^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was received by the ARS Culture Collection in 1965, as an unidentified isolate from Lekh Batra, then at the University of Kansas, Lawrence, KS, USA. The substrate from which the strain was isolated in Kansas was not described.

Latin diagnosis of *Blastobotrys illinoisensis* Kurtzman sp. nov.

In agar *malti* post dies 3 ad 25 °C, *cellulae vegetativae globosae* (2.0–4.0 µm) *aut elongatae* (2.0–3.0 × 2.5–8.0 µm) *et ovoideae cellulae cum saetis* (1.0–1.3 × 18–125 µm), *singulae et binae*. In agar *morphologico* post dies 7 ad 25 °C, *incrementum fuscum pallidum, hebes, filamentosum; margo glabra vel undulata. Pseudohyphae et hyphae verae fiunt. Ascosporae non fiunt. Glucosum, sucrosum (infirme), galactosum, trehalosum et maltosum fermentantur. Raffinose et lactosum non fermentantur. Assimilantur glucosum, sucrosum, galactosum, maltosum, methyl-α-D-glucosidum, amylinum solubile, cellobiosum, salicinum, L-sorbose, L-rhamnosum, D-xylose, L-arabinose, D-arabinose, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, inositol, acidum succinicum, acidum citricum, D-glucosaminum, D-glucosaminum, N-acetyl-D-glucosaminum, hexadecanum, 2-keto-D-glucosaminum, 5-keto-D-glucosaminum et cadaverinum. Non assimilantur inulinum, raffinose, melibiosum, lactosum, trehalosum, melezitiosum, D-ribose, methanol, DL-acidum lacticum, potassii nitras et saccharatum. Amylinum non formatur. Vitamina externa ad crescentiam necessaria non sunt. Gelatinum liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 µg cycloheximido ml⁻¹ addito, et in medio 10 % sodii chloridum/5 % glucosum. Augmentum fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleus submonas largus rRNA gene, ITS1–5.8S–ITS2 rRNA genes, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL YB-1343^T (CBS 10339^T) designat stirpem typicam. Isolata detruta a arbor mortua, Marion, IL, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.*

Fig. 2. Cellular morphology of the six novel species of *Blastobotrys*. (a–b) *Blastobotrys americana* NRRL Y-6844^T: budding cells (a); pseudohyphae (b). (c–d) *Blastobotrys illinoisensis* NRRL YB-1343^T: cells, some budded, with seta-like cell-wall extensions (c) (bar, 5 µm); denticulate hypha with a blastoconidium (d). (e–g) *Blastobotrys malaysiensis* NRRL Y-6417^T: budding cells (e); inflated hyphal tip cell with a bud (f); denticulate hypha with blastoconidia (g). (h–i) *Blastobotrys muscicola* NRRL Y-7993^T: spindle-shaped yeast cells and an elongated cell with terminal denticles (h); large, spherical cells on a denticulate hypha (i). (j–k) *Blastobotrys peoriensis* NRRL YB-2290^T: budding cells and a pseudohyphal cell with a rachis-like tip (j); budding cells, some with tapered extensions that form blastoconidia (k). (l–m) *Blastobotrys raffinofermentans* NRRL Y-27150^T: budding cells (l); pseudohypha with blastoconidia (m). Cultures were grown on 5% ME agar for 3 days at 25 °C (a, c, e, f, h–j, l) or on YMo agar for 7 days at 25 °C (b, d, g, k, m). The bar in (c) applies to all panels.

Description of *Blastobotrys illinoisensis* Kurtzman sp. nov.

Blastobotrys illinoisensis (il.lin.ois.en'sis. N.L. nom. fem. adj. *illinoisensis* from the State of Illinois, USA).

After 3 days growth on 5% ME agar at 25 °C, cells are spherical, 2.0–4.0 µm, to elongate, 2.0–3.0 × 2.5–8.0 µm, and often teardrop-shaped. Cells form by multilateral budding and may be single or in pairs. Not infrequently, cells 4–5 × 6–8 µm have a tapered seta-like extension that has a basal diameter of 1.0–1.3 µm and a length of 18–125 µm

(Fig. 2c). Seta-bearing cells often produce buds. Colony growth on 5% ME agar is white with an almost powdery appearance and a prominent mycelial fringe. After 7 days at 25 °C, growth under the cover glass of a Dalmau plate culture with YMo agar shows pseudohyphae and abundant true hyphae with side branches having highly denticulate tips bearing blastoconidia. Hyphal cells also form denticles with blastoconidia (Fig. 2d). Aerobic colonies are white, mycelial and somewhat powdery with an entire to lobate margin. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Moderately thick,

Table 3. Comparative physiological characteristics of the six novel *Blastobotrys* species

Strains: 1, *B. americana* NRRL Y-6844^T; 2, *B. illinoisensis* NRRL YB-1343^T; 3, *B. malaysiensis* NRRL Y-6417^T; 4, *B. muscicola* NRRL Y-7993^T; 5, *B. peoriensis* NRRL YB-2290^T; 6, *B. raffinosisfermentans* NRRL Y-27150^T. None of the strains fermented lactose. All assimilated glucose, galactose, cellobiose, glycerol, D-mannitol, succinate, N-acetyl-D-glucosamine and cadaverine. Strains were not inhibited by cycloheximide at 100 µg ml⁻¹. None of the strains assimilated methanol or saccharate and none formed extracellular starch-like compounds. –, Negative; +, positive; w, weak; v, strain variable (positive or negative). Variable reactions are based on a repeat using the same strain and resulted from a delayed positive reaction in some of the tests.

Physiological test	1	2	3	4	5	6
Fermentation of:						
Glucose	+	+	+	–	+	+
Sucrose	–	w	w	–	–	w
Raffinose	–	–	–	–	–	+
Galactose	–	+	+	–	+	+
Trehalose	w	+	+	–	+	+
Maltose	–	+	+	–	–	+
Assimilation of:						
Inulin	–	–	–	–	–	+
Sucrose	–	+	+	+	+	+
Raffinose	+	–	–	v	+	+
Melibiose	+	–	–	v	+	+
Lactose	–	–	+	+	+	–
Trehalose	+	–	+	v	–	–
Maltose	–	+	+	+	+	+
Melezitose	–	–	w	+	+	+
Methyl α-D-glucoside	+	+	+	v	+	+
Soluble starch	–	+	+	+	w	+
Salicin	+	+	+	v	+	+
L-Sorbose	+	+	+	–	+	+
L-Rhamnose	–	+	+	–	–	–
D-Xylose	+	+	+	–	+	+
L-Arabinose	+	+	+	–	+	+
D-Arabinose	+	+	+	–	+	+
D-Ribose	+	–	+	–	+	+
Ethanol	+	+	+	–	+	+
Erythritol	+	+	+	–	+	+
Ribitol	+	+	+	–	+	+
Galactitol	+	+	–	–	+	+
D-Glucitol	+	+	+	–	+	+
Inositol	+	+	+	–	+	+
DL-Lactate	w	–	+	–	–	+
Citrate	–	+	+	+	–	+
D-Gluconate	+	+	–	–	+	+
D-Glucosamine	+	+	+	v	+	+
Hexadecane	+	+	–	–	+	+
Nitrate	–	–	–	–	+	+
2-Keto-D-gluconate	–	+	+	–	+	+
5-Keto-D-gluconate	+	+	+	–	+	+
Additional growth tests						
Growth in vitamin-free medium	–	+	+	v	–	–
Growth in 10 % NaCl/5 % glucose	+	+	+	–	+	+
Gelatin liquefaction	–	+	+	+	–	–
Growth at 37 °C	–	+	+	+	–	+

climbing pellicles are formed on stationary liquid media. Cultures grown on YM, 5 % ME and RG agar media at 15 and 25 °C produced no ascospores after 6 weeks. The type strain differs from *B. mokoensis* NRRL Y-27120^T and *B. malaysiensis* sp. nov. NRRL Y-6417^T by just two nucleotide substitutions in the D1/D2 domain of the LSU rDNA. This small difference raised the possibility that the taxa may be conspecific. However, greater divergence was found in the nucleotide sequences of ITS1–5.8S–ITS2 rDNA, mitochondrial SSU rDNA and the COXII gene, providing additional evidence that the three taxa are closely related but separate species (Table 2). In further tests, cultures of the three species were mixed on YM, 5 % ME and RG media, incubated at 25 °C and observed over a 6-week period. Neither conjugation between cells nor ascospores was detected. Cultures of *B. mokoensis*, especially on 5 % ME agar, occasionally formed spherical endospores in hyphal cells.

NRRL YB-1343^T (= CBS 10339^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated by L. J. Wickerham in 1949, from debris surrounding the base of a standing, dead deciduous tree near Marion, IL, USA.

Latin diagnosis of *Blastobotrys malaysiensis* Kurtzman sp. nov.

In agar malti post dies 3 ad 25 °C, cellulae vegetativae globosae (3.0–5.5 µm) aut ellipsoideae (2.5–4.0 × 3.5–6.0 µm) et ovoideae cellulae cum saetis, singulae et binae. In agar morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes et filamentosum; margo glabra vel undulata. Pseudohyphae et hyphae verae fiunt. Ascospores non fiunt. Glucosum, sucrosum (infirme), galactosum, trehalosum et maltosum fermentantur. Raffinosum et lactosum non fermentantur. Assimilantur glucosum, sucrosum, galactosum, lactosum, trehalosum, maltosum, melezitolum (infirme), methyl-α-D-glucosidum, amyllum solubile, cellobiosum, salicinum, L-sorbosum, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum, erythritolum, ribitolum, D-mannitolum, D-glucitolum, inositolum, DL-acidum lacticum, acidum succinicum, acidum citricum, D-glucosaminum, N-acetyl-D-glucosaminum, 2-keto-D-gluconatum, 5-keto-D-gluconatum et cadaverinum. Non assimilantur inulinum, raffinolum, melibiosum, methanolum, galactitolum, D-gluconatum, hexadecanum, potassii nitras et saccharatum. Amyllum non formatur. Vitamina externa ad crescentiam necessaria non sunt. Gelatinum liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 µg cycloheximido ml⁻¹ addito, et in medio 10 % sodii chloridum/5 % glucosum. Augmentum fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleus submonas largus rRNA gene, ITS1–5.8S–ITS2 rDNA genes, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-6417^T (CBS 10336^T) designat stirpem typicam. Isolata soli, Malaysia. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Blastobotrys malaysiensis* Kurtzman sp. nov.

Blastobotrys malaysiensis (ma.lay.si.en'sis. N.L. nom. fem. adj. *malaysiensis* from the country of Malaysia).

After 3 days growth on 5 % ME agar at 25 °C, yeast cells are spherical, 3.0–5.5 µm, to ellipsoidal, 2.5–4.0 × 3.5–6.0 µm, form by multilateral budding and occur singly or in pairs (Fig. 2e). In addition, seta-bearing cells are also present, as seen for *B. illinoisensis* and *B. mokoensis* (observation from this study), but lengths of setae seldom exceed 25 µm. Growth on 5 % ME agar is tannish-white, dull, somewhat butyrous and with a prominent mycelial fringe. Growth under the cover glass of a Dalmau plate culture with YMO agar shows mostly true hyphae with a few interspersed pseudohyphae after 7 days at 25 °C. Aerobic growth is white, mycelial and almost powdery, with radial furrows and with margins that are broadly lobate. Hyphae on various media often show a spherical, inflated terminal cell that may produce buds (Fig. 2f). Blastoconidia often form on short denticles on the hyphal cells (Fig. 2g). Cultures grown on YM, 5 % ME and RG agar media incubated at 15 and 25 °C produced no ascospores after 6 weeks and, as noted above, there was no mating reaction or ascoporation in mixtures with the closely related species *B. illinoisensis* and *B. mokoensis*. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Dry, climbing pellicles are formed on stationary liquid media.

NRRL Y-6417^T (= CBS 10336^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was received by the ARS Culture Collection in 1963 from Chester W. Emmons, who reported that the strain had been isolated from soil in a cave in Malaysia.

Latin diagnosis of *Blastobotrys muscicola* Kurtzman sp. nov.

In agar malti post dies 3 ad 25 °C, cellulae vegetativae globosae (2.0–4.1 µm) aut elongatae et fusiformae (1.5–2.5 × 8.0–22.0 µm), singulae et binae. In agar morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes, filamentosum; margo undulata. Pseudohyphae et hyphae verae fiunt. Ascospores non fiunt. Saccharas non fermentantur. Assimilantur glucosum, sucrosum, raffinolum (variabile), melibiosum (variabile), galactosum, lactosum, trehalosum (variabile), maltosum, melezitolum, methyl-α-D-glucosidum (variabile), amyllum solubile, cellobiosum, salicinum (variabile), glycerolum, D-mannitolum, acidum succinicum, acidum citricum, D-glucosaminum (variabile), N-acetyl-D-glucosaminum et cadaverinum. Non assimilantur inulinum, L-sorbosum, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, ethanolum, erythritolum, ribitolum, galactitolum, D-glucitolum, inositolum, DL-acidum lacticum, D-gluconatum, hexadecanum, potassii nitras, 2-keto-D-gluconatum, 5-keto-D-gluconatum et saccharatum. Amyllum non formatur. Vitamina externa ad crescentiam

necessaria non sunt (variabile). Gelatinum liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 µg cycloheximido ml⁻¹ addito. Non crescit in medio 10 % sodii chloridum/5 % glucosum. Augmentum fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleus submonas largus rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-7993^T (CBS 10338^T) designat stirpem typicam. Isolata musca, propinqua New Orleans, LA, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Blastobotrys muscicola* Kurtzman sp. nov.

Blastobotrys muscicola (mus.ci'co.la. N.L. nom. fem. n. *muscicola* moss-dweller).

After 3 days growth on 5 % ME agar at 25 °C, budding is multilateral and cells are single or in pairs, infrequently spherical, 2.0–4.1 µm, but the majority are elongate and often spindle-shaped, 1.5–2.5 × 8.0–22.0 µm (Fig. 2h). Larger elongated cells often form terminal denticles that produce blastoconidia. Colony growth is dull, white and mycelial. Growth under the cover glass of a Dalmau plate culture with YMo agar shows both true hyphae and pseudohyphae after 7 days at 25 °C. Aerobic growth on this medium is dull, white and mycelial with a lobate margin. Cultures were examined weekly for ascospore formation on YM, 5 % ME, RG and McClary's acetate agar media incubated at 15 and 25 °C, but ascospores were not detected after 3 months. Hyphae on various media develop inflated spherical cells borne on short denticles (Fig. 2i), but these cells do not form ascospores and do not appear to be asci. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Dry, climbing pellicles are formed on stationary liquid media.

NRRL Y-7993^T (= CBS 10338^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated by the author in 1976 from an unidentified moss growing on a fallen log in a wilderness area in St Tammany Parish, near New Orleans, LA, USA.

Latin diagnosis of *Blastobotrys peoriensis* Kurtzman sp. nov.

In agaro malti post dies 3 ad 25 °C, cellulae vegetativae globosae (2.0–5.0 µm) aut elongatae (2.0–4.0 × 2.5–12.0 µm), singulae et binae. In agaro morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes, butyrosus; margo glabra vel serrata. Pseudohyphae et hyphae verae fiunt. Ascosporae non fiunt. Glucosum, galactosum et trehalosum fermentantur. Sucrosum, raffinose, lactosum et maltosum non fermentantur. Assimilantur glucosum, sucrosum, raffinose, melibiosum, galactosum, lactosum, maltosum, melizitosum, methyl-α-D-glucosidum, amyllum solubile (infirme), cellobiosum, salicinum, L-sorbose, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum,

erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, inositolum, acidum succinicum, D-gluconatum, D-glucosaminum, N-acetyl-D-glucosaminum, hexadecanum, potassii nitras, 2-keto-D-gluconatum, 5-keto-D-gluconatum et cadaverinum. Non assimilantur inulinum, trehalosum, L-rhamnosum, methanolum, DL-acidum lacticum, acidum citricum et saccharatum. Amyllum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 µg cycloheximido ml⁻¹ addito, et in medio 10 % sodii chloridum/5 % glucosum. Augmentum non fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleus submonas largus rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL YB-2290^T (CBS 10340^T) designat stirpem typicam. Isolatus ex fungus incognitus, Peoria, IL, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Blastobotrys peoriensis* Kurtzman sp. nov.

Blastobotrys peoriensis (pe.o.ri.en'sis. N.L. nom. fem. adj. *peoriensis* from Peoria, IL, USA, the collection site of the type strain).

After 3 days growth on 5 % ME agar at 25 °C, cells are spherical, 2.0–5.0 µm, ovoid and elongate, 2.0–4.0 × 2.5–12.0 µm, occur singly or in pairs and divide by multilateral budding (Fig. 2j, k). Colony growth is tannish-white and dull and ranges from butyrous to mycelial. Growth under the cover glass of a Dalmau plate culture with YMo agar shows abundant pseudohyphae with clusters of blastoconidia after 7 days at 25 °C. True hyphae are infrequent but become more common in older cultures. Both hyphae and individual cells form rachis-like extensions that produce blastoconidia (Fig. 2j, k). Aerobic colony growth on the Dalmau plate is white to tannish-white, dull with a convoluted surface and butyrous in texture. Ascospore formation was not observed in cultures grown on YM, 5 % ME, RG and McClary's acetate agar media incubated at 15 and 25 °C for 3 months. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Thick pellicles are formed on stationary liquid media.

NRRL YB-2290^T (= CBS 10340^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated in 1950 by L. J. Wickerham from unidentified fungal growth on a rotted log, Peoria, IL, USA.

Latin diagnosis of *Blastobotrys* *raffinosefermentans* Kurtzman sp. nov.

In agaro malti post dies 3 ad 25 °C, cellulae vegetativae globosae (2.0–4.0 µm) aut elongatae (2.0–3.5 × 3.0–13.0 µm), singulae, binae et fasciculatae. In agaro morphologico post dies 7 ad 25 °C, incrementum fuscum pallidum, hebes et butyrosus; margo glabra vel undulata. Pseudohyphae fiunt; hyphae

verae non fiunt. Ascosporae non fiunt. Glucosum, sucrosum (infirme), raffinoseum, galactosum, trehalosum et maltosum fermentantur. Lactosum non fermentatur. Assimilantur glucosum, inulinum, sucrosum, raffinoseum, melibiosum, galactosum, maltosum, melezitosum, methyl- α -D-glucosidum, amyllum solubile, cellobiosum, salicinum, L-sorbosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum, erythritolum, ribitololum, galactitololum, D-mannitololum, D-glucitololum, inositololum, DL-acidum lacticum, acidum succinicum, acidum citricum, D-gluconatum, D-glucosaminum, N-acetyl-D-glucosaminum, hexadecanum, potassii nitras, 2-keto-D-gluconatum, 5-keto-D-gluconatum et cada-verinum. Non assimilantur lactosum, trehalosum, L-rhamnosum, methanolum et saccharatum. Amyllum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esteres non fiunt; pellicula fiunt. Crescit in medio 100 μ g cycloheximido ml⁻¹ addito, et in medio 10 % sodii chloridum/5 % glucosum. Augmentum fiunt in temperatura 37 °C. Species nova a speciebus aliis sequentibus nucleotiditis nucleis submonas largus rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-27150^T (CBS 6800^T) designat stirpem typicam. Isolata e substrato incognito. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Blastobotrys raffinosifermentans* Kurtzman sp. nov.

Blastobotrys raffinosifermentans (raf'fi.no.si.fer.men'tans. N.L. part. adj. *raffinose* fermentans fermenting raffinose, a property rare among species of the *Trichomonascus/Blastobotrys* clade).

After 3 days growth on 5 % ME agar at 25 °C, cells are spherical, 2.0–4.0 μ m, to elongate, 2.0–3.5 $\times$ 3.0–13.0 μ m, and occur singly, in pairs and in small clusters (Fig. 2l). Budding is multilateral. Some cells form short denticles that produce blastoconidia. Colony growth is white, dull and butyrous. Growth under the cover glass of a Dalmau plate culture with YMo agar shows abundant well-branched pseudohyphae bearing blastoconidia after 7 days at 25 °C (Fig. 2m), but true hyphae are not detected. Aerobic colony growth is tannish-white, smooth, dull, with a cupulate centre and margins with infrequent small lobes. Ascosporeulation was not observed in cultures grown on YM, 5 % ME and RG agar media at 15 and 25 °C for 2 months. Reactions for fermentation, assimilation and other diagnostic tests are given in Table 3. Moderately thick, climbing pellicles form on stationary liquid media.

NRRL Y-27150^T (= CBS 6800^T) is the designated type strain and is preserved as a lyophilized preparation in the ARS Culture Collection (NRRL), Peoria, IL, USA. The strain was received at the CBS Culture Collection from J. Shennan, but the source is unknown.

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