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Three new beetle-associated yeast species in the *Pichia guilliermondii* clade

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Abstract

New yeasts in the *Pichia guilliermondii* clade were isolated from the digestive tract of basidiocarp-feeding members of seven families of Coleoptera. A molecular phylogeny and unique traits placed eight isolates in *Candida fermentati* and three undescribed taxa in the genus *Candida*. The new species and type strains are *C. smithsonii* (type strain NRRL Y-27642^T), *C. athensensis* (type strain NRRL Y-27644^T), and *C. elateridarum* (type strain NRRL Y-27647^T). Based on comparison of small-and large-subunit rDNA sequences, *C. smithsonii* and *C. athensensis* form a statistically well-supported subclade with *P. guilliermondii*, *C. xestobii*, and *C. fermentati*; *C. elateridarum* is basal to this subclade.

Keywords: New *Candida* species; Beetle gut; Molecular systematics; Ribosomal RNA gene

1. Introduction

Certain yeasts can be isolated consistently from the digestive tract of beetles [1]. For example anobiid and cerambycid beetles possess gut yeasts that are transmitted when the offspring hatch by eating through the contaminated egg shell [2,3]. Beetle-associated yeasts do not form a monophyletic group, and closely related yeasts are not necessarily associated with related beetle hosts [2,4,5]. For example, two related yeasts that once were considered to be conspecific based on similar D1/D2 sequences of the large-subunit ribosomal DNA (LSU rDNA) are known from relatively distantly related beetles [6]: *Candida xestobi*, was isolated from *Xestobium plumbeum* (Anobiidae), but *C. guilliermondii* (anamorph of *Pichia guilliermondii*) is known from a host in a different superfamily, *Phoracantha semipunctata* (Cerambycidae).

During a study of fungi from the gut of basidiocarp-feeding beetles, we repeatedly isolated previously unknown yeasts some of which are closely related to *P. guilliermondii*. Here we discuss the relationships of the yeasts and their insect host associations. We describe three new species in the Saccharomycetes based on their molecular phylogeny and unique taxonomic traits.

2. Materials and methods

2.1. Yeast isolation and identification

The beetles were usually placed in Petri dishes for 1–3 days without food prior to dissection. Withholding food helps to eliminate some contaminating organisms that might be isolated from the gut. Surface disinfection with 95% ethanol was followed by a rinse in sterile 0.7% saline; the rinse liquid was plated on acidified YM agar (Difco YM broth, 2% plain agar, adjusted to pH 3.5 with HCl) as a negative control. The beetle gut was removed aseptically under a dissecting microscope, and gut segments were streaked on acidified YM agar plates. Voucher specimens of the beetles have been placed in the

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Table 1
Three new species of *Candida* and *C. fermentati* isolated in this study

Species	Strain designation[a]			GenBank No./bp differences[b]			Source[c]
	NRRL	CBS	LSU	SSU rDNA	LSU rDNA	ITS & 5.8S rDNA	
Candida smithsonii	Y-27642^T	9839	BG 02-7-13-007B-1-2	AY518520	AY518525	AY553844	Gut of unidentified endomychid larva, BCI, Panama.
			BG 02-7-13-007B-1-3	–	AY518526/0		
	Y-27643		BG 02-7-13-007E-3-1	AY518521/1	AY518527/0	AY553845/0	Gut of *Iphiclus* (*Megaprotus*) *pulcher* (Erotylidae), BCI, Panama.
Candida athensensis	Y-27644^T	9840	BG 02-5-23-003I-4	AY518522	AY518528	AY553846	Gut of unidentified cucujoid, Athens, GA, USA.
	Y-27645		BG 02-7-13-014-3-1	AY518523/0	AY518529/0	AY553847/0	Gut of unidentified curculionid, BCI, Panama.
	Y-27646	9841	BG 99-8-11-1-C1	AY242261/1	AY242152/3	AY553848/0	Surface of *Megalodacne fasciata* (Erotylidae), Athens, GA, USA.
Candida elateridarum	Y-27647^T	9842	BG 02-7-21-004G-1-2-2	AY518524	AY518530	AY553849	Gut of unidentified elaterid, BCI, Panama.
Candida fermentati	Y-27648		BG 98-8-14-1-2-1	AY242189/2	AY242299/0	AY553850/0	Gut of *Carpophilus* sp. (Nitidulidae) ex *Pisolithus* tinctorius, Baton Rouge, Louisiana, USA.
			BG 98-8-14-1-3-3	–	AY518531/0		
			BG 98-8-14-1-4-3	–	AY518532/0		
	Y-27649		BG 98-8-5-1-1	AY242177/0	AY242287/0	AY553851/0	Unidentified scarabaeid ex unidentified bolete, Baton Rouge, LA, USA.

[a] T, type strain; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; LSU, Mycology Laboratory, Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA.

[b] DNA base pair differences were calculated by comparison with the sequences of type strains. The sequences of *Candida fermentati* were compared with AY553853 (SSU rDNA), AY187283 (LSU rDNA), and AB032175 (ITS & 5.8S rDNA).

[c] BCI, Barro Colorado Island.

University of Georgia Collection of Arthropods, Athens, Georgia; beetle specimen numbers correspond to those of the yeast isolate so that individual yeast hosts can be traced. The plates were incubated at 25 °C, and single colonies were streaked for purification. Yeast strains isolated in this study and their host beetles are listed in Table 1. Selected isolates including type strains of each new species have been deposited at the Agricultural Research Service Culture Collection (NRRL) and Centraalbureau voor Schimmelcultures (CBS) (Table 1). The morphological observations and metabolic tests comprising the yeast standard description were performed according to established methods [7,8].

2.2. DNA sequencing and phylogenetic analysis

Nucleic acids were extracted and purified following the procedures of Lee and Taylor [9]. The primer sets NS1-NS8, LS1-LR5, and ITS5-ITS4 were used for PCR amplification of the rDNA gene repeat [10,11]. The purified double-stranded PCR products were used as templates for sequencing with an ABI PRISM™ BigDye Terminator cycle sequencing kit. The complete sequence of the SSU rDNA and the D1/D2 region of the LSU rDNA were obtained with the primers NS1, NS2, NS4, NS5, NS8, LS1, LR3, ITS1, and ITS4 using an ABI PRISM 377 Automated DNA sequencer (PE Biosystems, Inc., Foster City, CA, USA). The GenBank Accession Nos. for DNA sequences from this study are listed in Table 1. The sequences for SSU rDNA of *C. fermentati* NRRL Y-17903^T and ITS and 5.8S rDNA of *C. xestobii* ATCC 24001^T were determined to compare with other isolates from beetles (GenBank AY553853 and AY553852, respectively). DNA sequences initially were aligned with the multialignment program Clustal X [12] and then optimized visually. Ambiguous regions were excluded from the analyses. The sequences from newly isolated yeasts were compared to lsu and ssu rDNA sequences of other yeasts and fungi obtained from GenBank. Maximum parsimony analyses were performed using PAUP 4.0b10 [13]. Heuristic tree searches were executed using the tree bisection-reconnection branch-swapping algorithm with random sequence analysis. Bootstrap values of the most parsimonious tree were obtained from 1000 replications. Base pair differences (Table 2) were counted using BLAST2 sequences [14] or from the manually aligned sequence database.

3. Results and discussion

3.1. Yeast isolation and description of the new species

About 650 yeast isolates were cultured from 27 families of beetles, and all yeasts were initially screened and grouped by LSU rDNA D1/D2 loop genotypes and host beetle family [15]. Based on this information, eleven yeast isolates from beetle species in seven families were placed within a clade with *P. guilliermondii* and its related anamorphs (Table 1). Except for the yeast associated with an unidentified elaterid, the yeasts reported here were not the sole yeast isolated from the gut of the host insect, and one to four other species of yeast also were present. A consensus of nine parsimonious trees from the combined dataset of SSU and LSU rDNA sequences (about 2400 bp; Fig. 1) indicated that the *P. guilliermondii* clade is composed of four subclades; (1) *P. guilliermondii* and two previously known *Candida* species, including isolates from *Carpophilus* sp. (Nitidulidae) and scarabaeid beetles; (2) yeasts from an endomychid beetle and *Iphiclus* (*Megaprotus*) *pulcher* (Erotylidae); (3) yeasts isolated from cucujoid, curculionid, and *Megalodacne fasciata* (Erotylidae); and (4) a yeast from an elaterid beetle. Among the isolates, yeasts from nitidulid and scarabaeid beetles were identified as *C. fermentati* based on their identical sequences of D1/D2, ITS, and 5.8S rDNA, and morphological and physiological similarity to this species, although there are minor sequence variations in SSU rDNA among the taxa (Tables 1, 3; [8,16]). On the other hand, members of the other three subclades are proposed as undescribed yeasts, with the subclades easily separated at the level of species on the basis of molecular and other taxonomic characters. Base pair variation of the D1/D2 loop is less than 3 within each subclade, and more than 6 between the four subclades (Fig. 1 and Tables 1, 2). The results of sequence comparisons of ITS and 5.8S-rDNA, physiological and other taxonomic tests also served to characterize members of the three subclades as three new species in the genus *Candida* (Tables 1–3).

Table 2
Base pair differences of LSU rDNA (D1/D2 region; bold type) and ITS and 5.8S rDNA among the new species and other related taxa

Species[a]	1	2	3	4	5	6
1. *Pichia guilliermondii*	–	6	4	11	21	44
2. *Candida xestobii*	**2**	–	2	11	23	42
3. *Candida fermentati*	**5**	**3**	–	9	20	42
4. *Candida smithsonii*	**8**	**6**	**7**	–	15	35
5. *Candida athensensis*	**13**	**11**	**14**	**11**	–	42
6. *Candida elateridarum*	**45**	**46**	**45**	**43**	**40**	–

[a] The sequences from type strains of each species were compared.

3.1.1. Latin diagnosis of Candida smithsonii Suh et Blackwell, sp. nov.

In medio liquido dextrosum et peptonum et extractum levidinis continente post 5 dies ad 25 °C cellulae vegetativae globosae aut ellipsoidae (2–5 × 2–6 μm), plerumque subglobosae, singulae vel binae; pseudohyphae fiunt. Cultura in agaro extramalti et faecis continente post 7 dies ad 25 °C, Candida, butyrosa et teres. In agaro

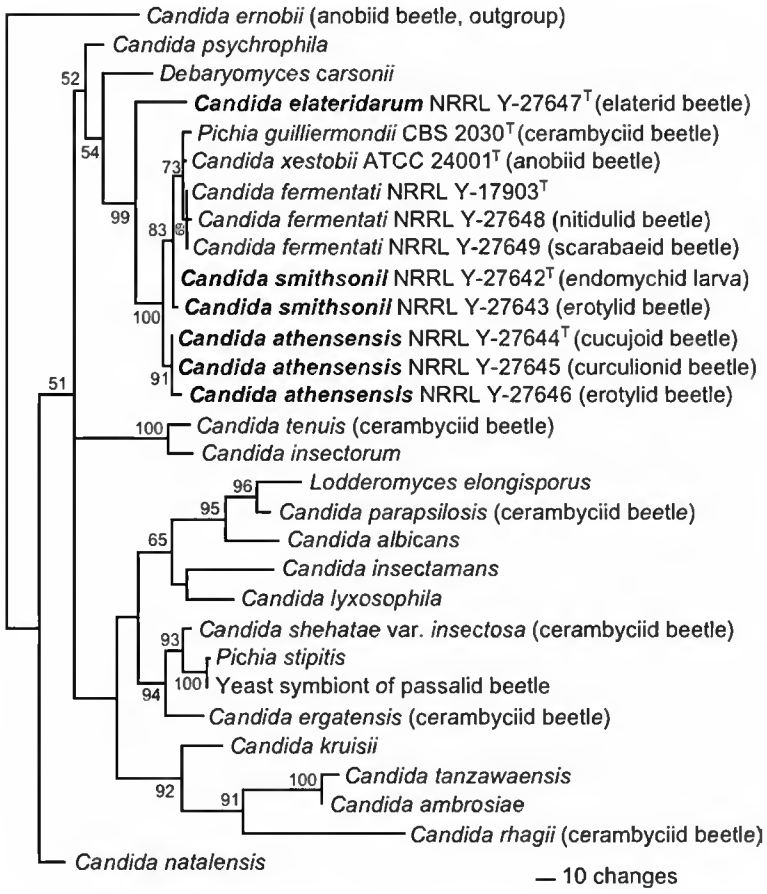

Fig. 1. Consensus of six most-parsimonious trees obtained from combined SSU and LSU rDNA sequence data. *C. ernobii* was used as outgroup taxon. The *Candida* species with boldface type are newly described in this paper. Host names are listed after the names of yeast species that have been isolated from insects. Tree length = 913; consistency index = 0.5860; homoplasy index = 0.4140; retention index = 0.6919; rescaled consistency index = 0.4055. Numbers on tree branches indicate the percentages of bootstrap samplings derived from 1000 samples that supported the internal branches by 50% or higher.

farina Zeae maydis confecto post 10 dies ad 25 °C, pseudohyphae fiunt. Ascosporae non fiunt. Glucosum, galactosum, α-methyl-D-glucosidum (variabiliter), trehalosum et cellobiosum (lente) fermentantur. Maltosum, sucrosum, melibiosum, lactosum, melezitosum, raffinosum, inulinum, amylum solubile et D-xylosum non fermentantur. Assimilantur glucosum, galactosum, D-glucosaminum, D-ribosum (infirme, variabiliter), D-xylosum, sucrosum, maltosum, trehalosum, α-methyl-D-glucosidum, cellobiosum, salicinum, arbutinum, melezitosum, glycerolum, ribitolum, xylitolum, L-arabinitolum (infirme, variabiliter), D-glucitolum, D-mannitolum, gluconolactonum, 2-keto-D-gluconatum, D-gluconatum (infirme, variabiliter), DL-acidum lacticum (lente, infirme), acidum succinicum, acidum citricum, ethanolum (lente, variabiliter) et propane-1, 2-diolum (infirme, variabiliter). Non assimilantur L-sorbosum, L-arabinosum, D-arabinosum, L-rhamnosum, melibiosum, lactosum, raffinosum, inulinum, amylum solubile, erythritolum, galactitolum, inositolum, D-glucuronatum, methanolum, butano-2, 3-diolum, acidum quinicum, D-glucaratum et D-galactonatum. Assimilantur ethylaminum, L-lysinum, cadaverinum et glucosaminum (infirme). Non assimilantur kali nitratum, sodii nitritum, creatinum, creatininum, imidazolum et D-tryptophanum. Amylum non formatur. Biotinum externum ad crescentiam necessarium est. Augmentum non fiunt in temperatura 35 °C. Variabile in medio 10 $\mu g\,ml^{-1}$ cycloheximido addito. Holotypus: NRRL Y-27642^T (= CBS 9839), designat stirpem typicum. Isolata a ile coleopterorum (Endomychidae), Barro Colorado Island, Panama, depositata in Collectione Culturarum (NRRL), Peoria, Illinois, USA.

3.1.2. Description of Candida smithsonii *Suh et Blackwell, sp. nov.*

After 5 days at 25 °C in YM broth, yeast cells are globose to ellipsoidal, (2–5) × (2–6) μm, mostly subglobose; occurring singly, in pairs, or in short chains (Figs. 2(a) and (b)); pseudohyphae may be present. After 7 days at 25 °C on YM agar, colonies are white, butyrous, and smooth on surface. On Dalmau plate culture on

Table 3
Physiological traits of three new *Candida* species and *C. fermentati* isolates from beetles

Physiological test[a]	*Candida smithsonii*[b]	*Candida athensensis*[b]	*Candida elateridarum*	*C. fermentati (from beetles)*[b]
Fermentation				
D- Glucose	+	+/D	D	+/D
D-Galactose	+	D	D	+/D
Maltose	–	D/W	–	–
α-Methyl-D-glucoside	D	–	–	–
Sucrose	–	D	D	+
α, α-Trehalose	+	D/W	D	+/D
Cellobiose	D	–	–	V
Raffinose	–	–	–	+/D
Inulin	–	–	–	+/D
Carbon assimilation				
L-Sorbose	–	–	–	+/D
D-Glucosamine	+/D	+/D	+	+/D
D-Ribose	W/–	+/D	+	+/D
D-Xylose	+	+/D	+	+/D
L-Arabinose	–	W	W	+/D
D-Arabinose	–	+/D/W	–	+
Arbutin	+	+/D	+	+
Melibiose	–	–	+	+
Raffinose	–	–	+	+
Melezitose	+	–	+	+
Inulin	–	–	–	+
Erythritol	–	W/–	+	–
L-Arabinitol	W/–	D	W	+
D-Mannitol	+	+	+	+/D
Galactitol	–	–	–	+
D-Glucono-1,5-lactone	+	+/W	D	+/W
D-Gluconate	W/–	+/W	D	+/D/W
DL-Lactate	S/W	+/D	W	+/D
Succinate	+	+	+	+/D
Ethanol	–	D/–	+	+
Propane 1,2 diol	W/–	D	D	+/D/W
Nitrogen assimilation				
Ethylamine	+	+/D	+	+
D-Glucosamine	W	W/–	W	+/W
D-Tryptophan	–	–	–	W/–
Vitamin requirement				
Without Thiamin	+	+	–	+
Without Pyridoxine & Thiamin	+	+	–	+
Growth temperature				
Growth at 35 °C	–	D/W/–	+	+
Growth at 40 °C	–	–	–	+
Growth in/on				
0.1% Cycloheximide	+	+	–	+
50% D-Glucose	+	+/D	W	+/D
60% D-Glucose	–	–	–	D/W
10% NaCl	+/W	V	W	+
16% NaCl	W	W/–	–	W

[a] The following characteristics were invariable in all species compared; *Fermentation*, Melibiose (–), Lactose (–), Melezitose (–), Starch (–), D-Xylose (–); *Carbon assimilation*, D-Glucose (+), D-Galactose(+), L-Rhamnose (–), Sucrose (+), Maltose (+), α, α-Trehalose (+), α-Methyl-D-glucoside (+), Cellobiose (+), Salicin (+), Lactose (–), Soluble starch (–), Glycerol (+), Ribitol (+), Xylitol (+), D-Glucitol (+), *myo* -Inositol (–), 2-Keto-D-gluconate (+), D-Glucuronate (–), Citrate (+), Methanol (–), Butane 2, 3 diol (–), Quinic acid (–), D-Glucarate (–), D-Galactonate (–); *Nitrogen assimilation*, Nitrate (–), Nitrite (–), L-Lysine (+), Cadaverine (+), Creatine (–), Creatinine (–), Imidazole (–); *Vitamin requirement*, growth without (w/o) all vitamins (–), w/o *myo* -Inositol (+), w/o Pantothenate (+), w/o Biotin (–), w/o Biotin & Thiamin (–), w/o Pyridoxine (+), w/o Niacin (+), w/o PABA (+); *Growth at* 30 °C (+), at 45 °C (–); *Growth in/on* 0.01% Cycloheximide (+), 1% Acetic acid (–); *Other additional tests*, Starch formation (–), Acetic acid production (–), Urea hydrolysis (–), DBB reaction (–). +, positive; –, negative; D, delayed positive; W, weak; V, strain variable.

[b] Reactions are from at least two strains of each species, including type strain.

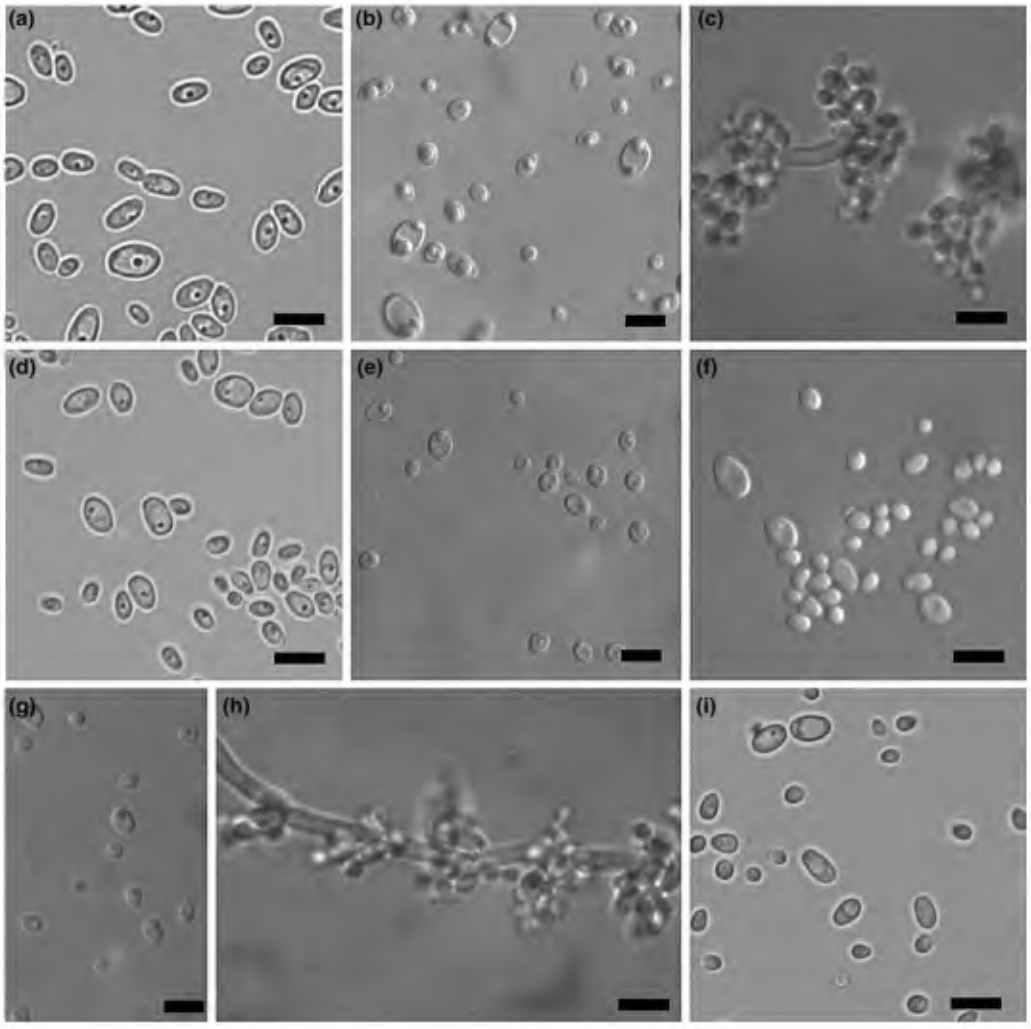

Fig. 2. New species of *Candida* and *C. fermentati* isolated from beetles. *Candida smithsonii* NRRL Y-27642^T. (a) Budding cells on cornmeal agar, 4 days, 25 °C. (b) Yeast cells on 2% malt extract agar, 10 days, 25 °C. (c) Pseudohyphae with blastoconidia on cornmeal agar, 7 days, 25 °C. Budding cells of *C. athensensis* on cornmeal agar, 7 days, 25 °C. (d) NRRL Y-27644^T. (e) NRRL Y-27646. (f) NRRL Y-27645. *Candida elateridarum* NRRL Y-27647^T. (g) Budding cells on cornmeal agar, 4 days, 25 °C. (h) Pseudohyphae with blastoconidia on cornmeal agar, 7 days, 25 °C. *Candida fermentati* NRRL Y-27649. (i) Budding cells on cornmeal agar, 7 d, 25 °C. Bar = 5 μm.

cornmeal agar pseudohyphae with blastoconidia are present after 10 days at 25 °C (Fig. 2(c)); aerobic growth, white, shiny, and smooth. No ascospores are produced after two months at 17 °C from individual strains or strains crossed in all combinations on half-strength cornmeal agar. The results of fermentation, assimilation, and other physiological tests are listed in Table 3. Type: NRRL Y-27642^T (= CBS 9839) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated from gut of an endomychid larva at Barro Colorado Island, Panama. Etymology: The species name *smithsonii* (N.L. gen. n.) honors James Smithson, F.R.S., to aid in promoting his name more widely than that of his father Percy and to acknowledge the significant tropical research fostered by the institution he founded.

3.1.3. Latin diagnosis of Candida athensensis *Suh et Blackwell, sp. nov.*

In medio liquido dextrosum et peptonum et extractum levidinis continente post 5 dies ad 25 °C cellulae vegetativae globosae aut ovoidae (1.25–5 × 1.25–5 μm), plerumque subglobosae, singulae vel binae; pseudohyphae non fiunt. Cultura in agaro extramalti et faecis continente post 7 dies ad 25 °C, Candida, butyrosa et teres. In agaro farina Zeae maydis confecto post 10 dies ad 25 °C, pseudohyphae et hyphae verae non fiunt. Ascosporae non fiunt. Glucosum, galactosum, maltosum, sucrosum et trehalosum fermentantur. α-Methyl-D-glucosidum, melibiosum, lactosum, cellobiosum, melezitosum, raffinosum, inulinum, amylum solubile et D-xylosum non fermentantur. Assimilantur glucosum, galactosum, D-glucosaminum, D-ribosum, D-xylosum, L-arabinosum (lente, variabiliter), D-arabinosum, su-

crosum, maltosum, trehalosum, α-methyl-D-glucosidum, cellobiosum, salicinum, arbutinum, glycerolum, erythritolum (infirme, variabiliter), ribitolum, xylitolum, L-arabinitolum (lente), D-glucitolum, D-mannitolum, gluconolactonum, D-gluconatum, DL-acidum lacticum, acidum succinicum, acidum citricum, ethanolum (variabiliter) et propane-1, 2-diolum (lente). Non assimilantur L-sorbosum, L-rhamnosum, melibiosum, lactosum, raffinosum, melezitosum, inulinum, amylum solubile, galactitolum, inositolum, 2-keto-D-gluconatum, D-glucuronatum, methanolum, butano-2, 3-diolum, acidum quinicum, D-glucaratum et D-galactonatum. Assimilantur ethylaminum, L-lysinum, cadaverinum et glucosaminum (infirme, variabiliter). Non assimilantur kali nitratum, sodii nitritum, creatinum, creatininum, imidazolum et D-tryptophanum. Amylum non formatur. Biotinum externum ad crescentiam necessarium est. Augmentum non fiunt in temperatura 40 °C. Crescit in medio 100 $\mu g\,ml^{-1}$ cycloheximido addito. Holotypus: NRRL Y-27644^T (= CBS 9840), designat stirpem typicum. Isolata a ile coleopterorum (Cucujoidae), Athens, GA, USA, depositata in Collectione Culturarum (NRRL), Peoria, IL, USA.

3.1.4. Description of Candida athensensis *Suh et Blackwell, sp. nov.*

After 5 days at 25 °C in YM broth, yeast cells are globose to oval, (1.25–5) × (1.25–5) μm, mostly subglobose; occurring singly, in pairs, or in short chains (Figs. 2(d)–(f)); pseudohyphae not present. After 7 days at 25 °C on YM agar, colonies are white, butyrous, and smooth on surface. On Dalmau plate culture on cornmeal agar pseudohyphae are not present after 10 days at 25 °C; aerobic growth, white, shiny, and smooth. No ascospores produced after two months at 17 °C from individual strains or strains crossed in all combinations on half-strength cornmeal agar. The results of fermentation, assimilation, and other physiological tests are listed in Table 3. Type: NRRL Y-27644^T (= CBS 9840) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated from the gut of an unidentified cucujoid beetle from Athens, GA, USA. Etymology: The species name *athensensis* (N.L. fem. adj.) referring to Athens, GA, USA, collection locality of the type stain.

3.1.5. Latin diagnosis of Candida elateridarum *Suh et Blackwell, sp. nov.*

In medio liquido dextrosum et peptonum et extractum levidinis continente post 5 dies ad 25 °C cellulae vegetativae globosae aut subglobosae (2–4 × 2–4 μm), plerumque globosae, singulae vel binae; pseudohyphae fiunt. Cultura in agaro extramalti et faecis continente post 7 dies ad 25 °C, Candida aut cremea, butyrosa et teres. In agaro farina Zeae maydis confecto post 10 dies ad 25 °C, pseudohyphae et hyphae verae fiunt. Ascosporae non fiunt. Glucosum (lente), galactosum (lente), sucrosum (lente) et trehalosum (lente) fermentantur. Maltosum, α-methyl-D-glucosidum, melibiosum, lactosum, cellobiosum melezitosum, raffinosum, inulinum, amylum solubile et D-xylosum non fermentantur. Assimilantur glucosum, galactosum, D-glucosaminum, D-ribosum, D-xylosum, L-arabinosum (infirme), sucrosum, maltosum, trehalosum, α-methyl-D-glucosidum, cellobiosum, salicinum, arbutinum, melibiosum, raffinosum, melezitosum, glycerolum, erythritolum, ribitolum, xylitolum, L-arabinitolum, D-glucitolum, D-mannitolum, gluconolactonum (lente), D-gluconatum (lente), DL-acidum lacticum (infirme), acidum succinicum, acidum citricum, ethanolum et propane-1, 2-diolum (lente). Non assimilantur L-sorbosum, D-arabinosum, L-rhamnosum, lactosum, inulinum, amylum solubile, galactitolum, inositolum, D-glucuronatum, methanolum, butano-2, 3-diolum, acidum quinicum et D-glucaratum. Assimilantur ethylaminum, L-lysinum, cadaverinum et glucosaminum (infirme). Non assimilantur kali nitratum, sodii nitritum, creatinum, creatininum, imidazolum et D-tryptophanum. Amylum non formatur. Biotinum et thiaminum externum ad crescentiam necessarium est. Augmentum fiunt in temperatura 35 °C. Crescit in medio 10$\mu g\,ml^{-1}$ cycloheximido addito, non crescit in medio 100 $\mu g\,ml^{-1}$. Holotypus: NRRL Y-27647^T (= CBS 9842), designat stirpem typicum. Isolata a ile coleopterorum (Elateridae), Barro Colorado Island, Panama, depositata in Collectione Culturarum (NRRL), Peoria, IL, USA.

3.1.6. Description of Candida elateridarum *Suh et Blackwell, sp. nov.*

After 5 days at 25 °C in YM broth, yeast cells are globose to subglobose, (2–4) × (2–4) μm, mostly globose; occurring singly, in pairs, or in short chains (Fig. 2(g)); pseudohyphae present. After 7 days at 25 °C on YM agar, colonies are white to cream colored, butyrous, and smooth on surface. On Dalmau plate culture on cornmeal agar pseudohyphae with blastoconidia and septate hyphae are present after 10 days at 25 °C (Fig. 2(h)); aerobic growth, white and smooth. No ascospores are produced after two months at 17 °C on half-strength cornmeal agar. The results of fermentation, assimilation, and other physiological tests are listed in Table 3. Type: NRRL Y-27647^T (= CBS 9842) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, IL, USA. The strain was isolated from the gut of an unidentified elaterid beetle in Barro Colorado Island, Panama. Etymology: The species name *elateridarum* (N.L. gen. pl. n.) refers to the insect host family from which the type strain was isolated.

3.1.7. Candida fermentati *isolates from the gut of beetle*

After 5 days at 25 °C in YM broth, yeast cells are globose to oval, (1.25–3.75) × (2.5–3.75) μm; occurring singly, in pairs, or in short chains (Fig. 2(i)); pseudohyphae may be present. After 7 days at 25 °C on YM agar, colonies are white to cream colored, and smooth and glossy on the surface. On Dalmau plate culture on cornmeal agar pseudohyphae may be present after 10 days at 25 °C; aerobic growth white, shiny, and smooth. No ascospores are produced after two months at 17 °C from individual strains or strains crossed in all combinations on half-strength cornmeal agar. Yeasts were isolated from the gut of *Carpophilus* sp. (Nitidulidae) and an unidentified scarabaeid beetle. The results of fermentation, assimilation and other physiological tests matched the published data of *C. fermentati* with only minor variations between the new isolates and previously known members of the species (Table 3; [8]).

3.2. Phylogeny of the yeasts in the P. guilliermondii *clade including the new species*

P. guilliermondii is a common species in both natural and clinical environments [8]. This yeast also has received additional attention due to reports that it suppress the growth of other fungi and eliminates toxic heavy metals from the environment [17–20]. Taxonomy of the species and related taxa, however, is problematic due to the strong possibility that LSU rDNA D1/D2 sequences and other traits may be too conservative to characterize species within the clade. Phylogenetic analysis of rDNA sequences indicated that species of the clade are closely related to each other, and this is reflected in the physiological characters that do not consistently delimit the species (Fig. 1; Table 3; [8,16]). For example, Kurtzman and Robnett [6] have suggested that *C. fukuyamaensis* and *C. xestobii* were anamorphs of *P. guilliermondii* on the basis of their similar or identical D1/D2 sequence of LSU rDNA, but Bai et al. [21] concluded that *C. xestobii* could be readily distinguished from *P. guilliermondii* by electrophoretic karyotyping. *C. fermentati*, another common species closely related to *P. guilliermondii*, also was differentiated by electrophoretic karyotyping [21]. Additional taxonomic studies on the *P. guilliermondii* complex using less conservative markers will be necessary to clarify the taxonomy. The three new species, *C. smithsonii*, *C. athensensis*, and *C. elateridarum*, however, were clearly distinguished from each other and from other known species in the clade based on molecular and physiological characters (Fig. 1 Tables 2, 3). *C. smithsonii* and *C. athensensis* were sister taxa of other known species in the clade (Fig. 1), and were distinguished from each other based on results of assimilation tests and sequence comparison of the D1/D2 and ITS regions, although the two species had identical SSU rDNA sequences (Tables 2, 3). The D1/D2 sequence of strain NRRL Y-27646 differed by 3 bp from that of the other two strains in *C. athensensis*, but morphological and physiological traits were similar to those of other strains of the species (Fig. 2; Table 3). *C. elateridarum* was phylogenetically distinct from all other species and was basal to other members of the clade. The relatively large differences in sequence comparisons supported the basal position and indicated that *C. elateridarum* is an outlier in the *P. guilliermondii* clade, although it was grouped within the clade with high statistical support (Fig. 1 and Table 2).

3.3. Yeasts and associated beetles

Yeasts in the *P. guilliermondii* clade often have been isolated from insects or insect associated frass. *P. guilliermondii* was isolated from cerambycid beetles and other insects [22,23], and *C. xestobii* has been found only with the anobiid beetle *Xestobium plumbeum*. Some isolates of *C. fermentati* have come from ants, as well as from soil and other habitats [8]. The yeasts from *Carpophilus* sp., a tiny nitidulid beetle living in the bolete relative, *Pisolithus tinctorius*, and from a scarabaeid beetle in a bolete were identified as *C. fermentati* based on molecular and other traits. The new species did not show strict host specificity and were isolated from several species or families of beetles. As we mentioned in the introduction, beetle-associated yeasts do not form a monophyletic group. Such a scattered distribution in the tree is exemplified by the yeasts isolated from cerambycid beetles (Fig. 1). It is not unusual for yeasts associated with unrelated beetles and other insects, however, to occur together in distinct clusters, as seen here among the *P. guilliermondii* clade members [15].

The function of yeasts associated with many of the beetles remains unclear, but it is important to note that several of the yeasts reported here were not the sole yeast obtained from the gut of an individual host. Our continuing studies on the mycota of the insect gut are resulting in the discovery of a large number of undescribed species. This work also provides research resources for studies that will help to elucidate the role of these yeasts [15,24]. For example, the consistent finding of xylose-fermenting yeasts from gut of wood-ingesting passalid beetles (yeast symbiont of passalid beetle in Fig. 1) indicates a possible role for the yeast. In addition the discovery of such functions could provide useful genetic resources, such as the potential use of the xylose-fermenting and assimilating yeasts in certain industrial processes [24,25].

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