Yeasts associated with the curculionid beetle *Xyloterinus politus*: *Candida xyloterini* sp. nov., *Candida palmyrensis* sp. nov. and three common ambrosia yeasts

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Seven yeast strains were isolated from the body surface and galleries of *Xyloterinus politus*, the ambrosia beetle that attacks black oak trees. Based on rDNA sequence comparisons and other taxonomic characteristics, five of the strains were identified as members of the species *Saccharomycopsis microspora*, *Wickerhamomyces hampshirensis* and *Candida mycetangii*, which have been reported previously as being associated with insects. The remaining two yeast strains were proposed as representatives of two novel species, *Candida xyloterini* sp. nov. (type strain ATCC 62898^T=CBS 11547^T) and *Candida palmyrensis* sp. nov. (type strain ATCC 62899^T=CBS 11546^T). *C. xyloterini* sp. nov. is a close sister taxon to *Ogataea dorogensis* and assimilates methanol as a sole carbon source but lacks ascospores. On the other hand, *C. palmyrensis* sp. nov. is phylogenetically distinct from any other ambrosia yeast reported so far. The species was placed near *Candida sophiae-reginae* and *Candida beechii* based on DNA sequence analyses, but neither of these were close sister taxa to *C. palmyrensis* sp. nov.

As lignified cellulose, the major component of wood, is not easily digested by most insects, fungi that colonize wood serve as ambrosia for certain beetle groups (Batra, 1966; Harrington, 2005). Several phylogenetically distinct yeasts and filamentous fungi have been reported as ambrosia fungi. For example, it is well known that species of the genus *Ambrosiozyma* can be isolated from sources associated with bark or ambrosia beetles (van der Walt, 1972; Smith, 1998). Several mitosporic yeasts, such as *Candida mycetangii*, *Candida kashinagacola* and *Candida pseudovanderkliftii*, have also been isolated directly from beetles or their galleries (Kurtzman, 2000; Endoh *et al.*, 2008b).

In the 1980s, the ATCC Mycology Collection acquired seven unidentified yeast strains that were associated with *Xyloterinus politus* (Coleoptera: Curculionidae), the ambrosia beetle that attacks black oak (Haanstad & Norris, 1985). These strains were recently characterized phenotypically and genotypically; their taxonomy and molecular phylogeny are reported here and their ecological roles compared with those of other previously discovered ambrosia yeasts are discussed.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the rDNA sequences from this study are as follows: FJ381686–FJ381692 for the SSU rDNA; FJ381693–FJ381699 for ITS regions and 5.8S rDNA; and FJ381700–FJ381706 for D1/D2 regions of the LSU rDNA.

Yeast strains and identification

All the yeast strains used in this study were isolated from both the adult females of *Xyloterinus politus* (Coleoptera: Curculionidae) and their galleries in black oak collected in Palmyra, Wisconsin, USA, by Haanstad & Norris (1985). Seven yeast strains were deposited at the ATCC in 1987 and were assigned the accession numbers ATCC 62895–ATCC 62901. Morphological observations and metabolic tests were performed according to established methods (Yarrow, 1998; Barnett *et al.*, 2000). Assimilation tests for carbon and nitrogen compounds were performed in liquid media. The yeasts were observed on YM agar, 2 % malt agar, cornmeal agar and V8 juice agar at 15, 25 and 30 °C for up to 6 weeks to determine ascospore formation.

DNA sequencing and sequence analyses

Nucleic acids were extracted and purified following the procedures of Lee & Taylor (1990). The primer sets NS1/NS8, ITS5/ITS4 and LR0R/LR5 were used for PCR amplification of nuclear rRNA gene (rDNA) repeats (White *et al.*, 1990; Hausner *et al.*, 1993). PCR products were purified using Quantum Prep PCR Kleen spin columns (Bio-Rad Laboratories) and the purified PCR products were used as templates for sequencing with an ABI PRISM BigDye Terminator cycle sequencing kit v.3.1

(PE Applied Biosystems). The complete sequences of SSU rDNA, internal transcribed spacer (ITS) regions including 5.8S rDNA and the D1/D2 region of LSU rDNA were obtained with primers NS1, NS2, NS4, NS8, ITS1, ITS4, LR0R and LR3 using an ABI 3130xl automated DNA sequencer. Sequences from newly isolated yeasts were compared with those of other yeasts in GenBank by using the BLAST search program (Altschul *et al.*, 1990). Selected DNA sequences were aligned with the multialignment program CLUSTAL_X (Thompson *et al.*, 1997) and optimized visually. Maximum-parsimony analyses were performed using PAUP 4.0b10 (Swofford, 2002). 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 were counted using BLAST2 sequences (Tatusova & Madden, 1999) or from the manually aligned sequence database.

Yeast identification and novel species

Based on phenetic characteristics from more than 80 physiological and morphological tests and phylogenetic analyses from sequences of rDNA repeats, all the yeast isolates associated with the beetle *X. politus* were identified as members of the subphylum Saccharomycotina. Ascospores were observed only from strains ATCC 62895, ATCC 62896 and ATCC 62897 on YM agar or cornmeal agar at 25 °C within 1 month (data not shown).

Strain ATCC 62895 was convincingly identified as *Saccharomycopsis microspora*; similarly, strains ATCC 62896 and ATCC 62897 were identified as *Wickerhamomyces* (= *Pichia*) *hampshirensis*, and strains ATCC 62900 and ATCC 62901 were identified as *Candida mycetangii*. Sequences in the D1/D2 region of the LSU rDNA were identical or had a maximum of 1 bp difference between the isolates and the type strains of each species, which indicates that these isolates are conspecific to the three species. Taxonomic placement of those isolates was also well supported by high similarities between the isolates and the three yeast species in the SSU rDNA and ITS regions, including the 5.8S rDNA, as well as in other taxonomic characteristics such as ascospore formation and results of physiological tests. However, these isolates can be distinguished from other strains in the same species published previously by some carbon and nitrogen utilization tests: strain ATCC 62895 by L-sorbose (delayed positive), ethanol (–) and D-tryptophan (+) utilization, and growth on 50 % glucose (+); strains ATCC 62896 and ATCC 62897 by citrate (–) and D-tryptophan (–) utilization; and strain ATCC 62900 by *myo*-inositol (+) utilization (Kurtzman, 1987, 1999, 2000).

Strains ATCC 62898^T and ATCC 62899^T, on the other hand, were identified as representatives of two novel species of the genus *Candida* according to molecular, physiological and morphological taxonomic criteria. rDNA sequence similar-

ity searches in GenBank retrieved *Ogataea* (= *Pichia*) *dorogensis* strains as the closest matches to strain ATCC 62898^T, but strain ATCC 62898^T could be distinguished from this species by 6 nt substitutions in the D1/D2 region of the LSU rDNA and by 7 nt substitutions and 1 gap in the

Table 1. Physiological characteristics of *Candida xyloterini* sp. nov. and *Candida palmyrensis* sp. nov.

Strains: 1, *C. xyloterini* ATCC 62898^T; 2, *C. palmyrensis* ATCC 62899^T. The following characteristics are invariable in both strains: fermentation of maltose (–), sucrose (–), methyl α -D-glucoside (–), melibiose (–), lactose (–), cellobiose (–), melezitose (–), inulin (–), starch (–), raffinose (–) and D-xylose (–); assimilation of D-glucose (+), D-glucosamine (–), L-arabinose (–), D-arabinose (–), L-rhamnose (–), sucrose (–), maltose (–), trehalose (+), methyl α -D-glucoside (–), melibiose (–), lactose (–), inulin (–), raffinose (–), melezitose (–), soluble starch (–), ribitol (+), glycerol (+), *myo*-inositol (–), D-glucitol (+), D-mannitol (+), galactitol (–), citrate (–), ethanol (+), D-gluconate (–), D-glucuronate (–), D-galacturonic acid (–), propane-1,2-diol (–), butane-2,3-diol (–), quinic acid (–), nitrate (–), nitrite (–), ethylamine (+), L-lysine (+), cadaverine (+), D-tryptophan (–), creatine (–), creatinine (–), D-glucosamine (as nitrogen) (–) and imidazole (–); vitamin requirements, growth in the absence of vitamins (–), *myo*-inositol (+), pantothenate (+), biotin (+), thiamin (–), biotin and thiamin (–), pyridoxine (+), pyridoxine and thiamin (–), niacin (+) and *p*-aminobenzoic acid (+); growth at 25 °C (+), 30 °C (+) and 40 °C (–); growth in 0.01 % cycloheximide (+); growth in 1 % acetic acid (–), 50 % D-glucose (–), 60 % D-glucose (–), 10 % NaCl (–), 16 % NaCl (–); additional tests, starch formation (–), urea hydrolysis (–), Diazonium Blue B reaction (–). +, Positive reaction; –, negative reaction; d, delayed positive reaction; w, weak positive reaction.

Characteristic	1	2
Fermentation of carbon compounds		
D-Glucose	d	+
D-Galactose	–	+
α,α -Trehalose	d	+
Assimilation of carbon compounds		
L-Arabinitol	d	–
Arbutin	+	–
Cellobiose	+	–
Erythritol	+	–
D-Galactose	–	+
D-Glucono-1,5-lactone	–	+
2-Keto-D-gluconate	–	+
DL-Lactate	–	+
Methanol	d	–
D-Ribose	+	–
Salicin	+	–
L-Sorbose	+	–
Succinate	–	+
Xylitol	+	–
D-Xylose	+	–
Growth at 37 °C	+	w

Fig. 1. Novel yeasts from ambrosia beetles. Vegetative cells of *Candida xylosterini* sp. nov. ATCC 62898^T (a) and *Candida palmyrensis* sp. nov. ATCC 62899^T (b) after 7 days on YM agar at 25 °C. Bars, 5 µm.

SSU rDNA. Physiologically, strain ATCC 62898^T and *O. dorogensis* differed in their assimilation of L-arabinose, D-arabinose, D-glucono-1,5-lactone, succinate and citrate (Table 1; Péter *et al.*, 2003). Strain ATCC 62898^T showed several unique characteristics of the genus *Ogataea* such as assimilation of methanol as sole carbon source and growth at high temperatures (Table 1; Yamada *et al.*, 1994; Nagatsuka *et al.*, 2008), but no ascospores were observed from the strain grown for up to 6 weeks on several test media at 15–30 °C. Using similar characterizations, strain ATCC 62899^T was found to be significantly different from any other yeast species. The D1/D2 sequence similarity search found that strains of *Candida sophiae-reginae*, *Debaryomyces castellii* and *Candida lignicola* were among the highest matches in GenBank, but similarities in the D1/D2 region between strain ATCC 62899^T and these species were less than 90 % and these organisms are not closely related to each other phylogenetically (Kurtzman & Robnett, 1998; Jindamorakot *et al.*, 2007). Strain ATCC 62899^T could also be clearly distinguished from the three species mentioned above based on physiological characteristics (Table 1). Ascospores were not observed from the strain grown for up to 6 weeks. Based on the molecular and other taxonomic characteristics mentioned above, two novel species, *Candida xylosterini* sp. nov. and *Candida palmyrensis* sp. nov., are proposed to accommodate strains ATCC 62898^T and ATCC 62899^T, respectively.

Latin diagnosis of *Candida xylosterini* Suh et Zhou sp. nov.

Cultura in agar extramalti et faecis continente post 7 dies ad 25 °C, albida, hebes et teres. Cellulae vegetativae globosae aut subglobosae (2.0–3.0 × 2.0–3.0 µm), singulae vel binae; pseudohyphae non fiunt. Cultura in agar cum glucose et extracto fermento et extracto malto et peptone post 10 dies ad 25 °C albida et mucoideae. Pseudohyphae et hyphae verae non fiunt. Glucosum (lente) et trehalosum (lente) fermentantur. Galactosum, maltosum, methyl α-D-glucosidum, sucrosum, melibiosum, lactosum, cellobiosum, melezitium, raffinatum, inulinum, amyllum solubile et D-xylosum non fermentantur. Assimilantur glucosum, L-sorbosum, D-ribosum, D-xylosum, trehalosum, cellobiosum, salicinum, arbutinum, glycerolum, erythritolum, ribitolium, xylitolium, L-arabinitolium (lente), D-glucitolium, D-mannitolium, methanolium (lente) et ethanolium. Non assimilantur galactosum, D-glucosaminum, L-arabiosum, D-arabiosum, L-rhamnosum, sucrosum, maltosum, methyl α-D-glucosidum, melibiosum, lactosum, raffinatum, melezitium, inulinum, amyllum solubile, galactitolium, inositolium, gluconolactonum, 2-keto-D-gluconatum, D-gluconatum, D-glucuronatum, D-galacturonatum, DL-acidum lacticum, acidum succinicum, acidum citricum, propane-1,2-diolum, butano-2,3-diolum et acidum quinicum. Assimilantur ethylaminum, L-lysinum et cadaverinum. Non assimilantur kalium nitricum, natrium nitrosum, creatinum, creatininum, glucosaminum, imidazolium et D-tryptophanum. Amyllum non formatur. Thiaminum externum ad crescentiam necessarium

Fig. 2. Consensus of 64 most-parsimonious trees obtained from combined SSU rDNA and the D1/D2 region of LSU rDNA sequence data of selected yeast taxa. *Neurospora crassa* NRRL 13141 and *Hypocrea lutea* IFO 9061 (SSU rDNA) and GJS-89-129 (LSU rDNA) were chosen as the outgroup taxa. The species names in the tree follow the nomenclature of Kurtzman & Fell (1998) except for some names that have been updated by several recent taxonomic studies, e.g. Kurtzman *et al.* (2008), Limtong *et al.* (2008) and Nagatsuka *et al.* (2008). GenBank accession numbers after the names of yeast species are for SSU and LSU rDNA sequences, respectively. Strain NRRL Y-2214^T is the type strain of *Pichia angusta*, not *Ogataea polymorpha*. A to D on the branches indicate the major clades that include the ambrosia yeasts described in this study: A=*Saccharomycopsis* clade; B=*Ogataea* clade; C=*Wickerhamomyces/Lindnera* clade; D=*Debaryomyces/Lodderomyces* clade. Tree length=3190; consistency index=0.3448; homoplasy index=0.6552; retention index=0.7301; rescaled consistency index=0.2518. Numbers on tree branches indicate the percentages of bootstrap samplings derived from 1000 samples that supported the internal branches by 50 % or higher. Bar, 10 changes.

*est. Augmentum fiunt in temperatura 37 °C. In medio 0.01 % cycloheximido addito crescit. Typus: ATCC 62898^T (=CBS 11547^T) designat stirpem typicam. Isolata coleopterorum (*Xyloterinus politus*, Curculionidae), Palmyra, Wisconsin, USA, depositata in Collectione Culturarum ATCC (American Type Culture Collection), Manassas, Virginia, USA.*

Description of *Candida xyloterini* Suh & Zhou sp. nov.

Candida xyloterini (xyl.o.te.ri'ni. N.L. gen. n. *xyloterini* of *Xyloterinus*, referring to the genus name of the host beetle *Xyloterinus politus*).

After 7 days growth on YM agar at 25 °C, cells are globose to subglobose (2.0–3.0 × 2.0–3.0 µm) and occur singly or in pairs (Fig. 1a); pseudohyphae are not present and colonies are white to cream coloured, smooth, shiny, flat and slightly mucoid. After 10 days growth on malt extract agar at 25 °C, colonies are white and mucoid. Pseudohyphae and septate hyphae are absent. No ascospores are observed after 6 weeks at 25 °C on YM agar, 2 % malt extract agar, cornmeal agar or V8 juice agar. A summary of physiological and other characteristics is presented in Table 1.

The type strain, ATCC 62898^T (=CBS 11547^T), was isolated from *Xyloterinus politus* (Coleoptera: Curculionidae), Palmyra, Wisconsin, USA.

Latin diagnosis of *Candida palmyrensis* Suh et Zhou sp. nov.

*Cultura in agaro extramalti et faecis continente post 7 dies ad 25 °C, albida, hebes, teres et margine ciliata. Cellulae vegetativae ellipsoideae aut fusiformes (2.0–2.5 × 3.0–5.0 µm), singulae vel binae; pseudohyphae fiunt. In agaro farina Zeae maydis confecto post 10 dies ad 25 °C, pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum, galactosum et trehalosum fermentantur. Maltosum, methyl α-D-glucosidum, sucrosus, melibiosum, lactosum, cellobiosum, melezitium, raffinatum, inulinum, amyllum solubile et D-xylosum non fermentantur. Assimilantur glucosum, galactosum, trehalosum, glycerolum, ribitolium, D-glucitolium, D-mannitolium, gluconolactonum, 2-keto-D-gluconatum, DL-acidum lacticum, acidum succinicum et ethanolum. Non assimilantur L-sorbosum, D-glucosaminum, D-ribosum, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosus, maltosum, methyl α-D-glucosidum, cellobiosum, salicinum, arbutinum, melibiosum, lactosum, raffinatum, melezitium, inulinum, amyllum solubile, erythritolum, xylitolium, L-arabinitolium, galactitolium, inositolium, D-gluconatum, D-glucuronatum, D-galacturonatum, acidum citricum, methanolium, propane-1,2-diolium, butano-2,3-diolium et acidum quinicum. Assimilantur ethylaminum, L-lysinum et cadaverinum. Non assimilantur kalium nitricum, natrium nitrosum, creatinum, creatininum, glucosaminum, imidazolium et D-tryptophanum. Amyllum non formatur. Thiaminum externum ad crescentiam necessarium est. Augmentum fiunt in temperatura 37 °C (infirme). In medio 0.01 % cycloheximido addito crescit. Typus: ATCC 62899^T (=CBS 11546^T) designat stirpem typicum. Isolata coleopterorum (*Xyloterinus politus*, Curculionidae), Palmyra, Wisconsin, USA, depositata in Collectione Culturarum ATCC, Manassas, Virginia, USA.*

Description of *Candida palmyrensis* Suh & Zhou sp. nov.

Candida palmyrensis (pal.my.ren'sis. N.L. fem. adj. *palmyrensis* referring to Palmyra, Wisconsin, USA, the area where the type strain was collected).

After 7 days growth on YM agar at 25 °C, cells are ellipsoidal to fusiform (2.0–2.5 × 3.0–5.0 µm) and occur

singly, in pairs or in short chains (Fig. 1b); pseudohyphae are present and colonies are white to cream coloured, smooth on the top and wrinkled on the edge with irregular margins. After 10 days growth on cornmeal agar at 25 °C, pseudohyphae are present and septate hyphae are absent; colonies are white, shiny and smooth with filamentous margins. No ascospores are observed after 6 weeks at 25 °C on YM agar, 2 % malt extract agar, cornmeal agar or V8 juice agar. See Table 1 for a summary of physiological and other characteristics.

The type strain, ATCC 62899^T (=CBS 11546^T), was isolated from *Xyloterinus politus* (Coleoptera: Curculionidae), Palmyra, Wisconsin, USA.

Molecular phylogeny and ecology of ambrosia yeasts

A phylogenetic tree was constructed from the combined SSU and LSU rDNA (about 2400 bp) sequence data of 88 yeast taxa, including the strains reported in this study (Fig. 2). The seven ambrosia yeasts were placed into four different phylogenetic clades, indicated by A to D in Fig. 2.

W. hampshirensis ATCC 62896 and ATCC 62897, and *C. mycetangii* ATCC 62900 and ATCC 62901 were located in the *Wickerhamomyces/Lindnera* clade (clade C in Fig. 2; Kurtzman *et al.*, 2008) and showed close relationships to their type strains, *W. hampshirensis* NRRL YB-4128^T and *C. mycetangii* NRRL Y-6843^T, respectively. Strains of *C. mycetangii* were first isolated from mycetangia, the fungus-inhabiting organ of ambrosia beetles (Kurtzman, 2000). The presence of additional strains in the species from the ambrosia beetle *X. politus* in this study provides further evidence that *C. mycetangii* is a common yeast that is widely associated with ambrosia beetles. On the other hand, only one strain of *Pichia hampshirensis* has been described; this strain, NRRL YB-4128^T (=CBS 7208^T), was isolated from frass on dead cut oak in 1956 and was reclassified recently as a member of the genus *Wickerhamomyces* along with many other related *Pichia* species (Kurtzman, 1987, 1998; Kurtzman *et al.*, 2008). Strains ATCC 62896 and 62897 are the first additional strains of *W. hampshirensis* to be described since its initial description (Kurtzman, 1987) and were also isolated from beetle galleries on oak trees. Phylogenetically, *W. hampshirensis* and *C. mycetangii* were placed in a clade with many other insect-associated taxa (Fig. 2, clade C). Species near *C. mycetangii*, e.g. *Lindnera* (= *Pichia*) *fabianii*, *Lindnera* (= *Pichia*) *mississippiensis*, *Lindnera* (= *Pichia*) *americana*, *Lindnera* (= *Pichia*) *bimundalis* and *Candida maritima* have often been isolated either directly from insects or from their associated sources (Kurtzman, 1998; Meyer *et al.*, 1998). The two undescribed *Candida* strains in the clade, JW01-7-11-1-2-y3 and BG01-7-26-007A-3-1, were also isolated from a bark beetle and a passalid, respectively (Suh *et al.*, 2005).

Strain ATCC 62895 was identified as a member of the ambrosia yeast species *Saccharomycopsis microspora*, although it showed some minor variations from the type strain of the species in SSU and LSU rDNA sequences and in some physiological characteristics (Table 1; Fig. 2). The species was originally described as a member of *Pichia* for the yeasts isolated from a bark beetle, *Crossotarsus wollastoni* (Curculionidae: Scolytinae), but was later reclassified in the genus *Saccharomycopsis* (Batra, 1971; Kurtzman, 1999). Interestingly, its close sister *Saccharomycopsis synnaedendra* and two undescribed *Candida* strains in the same clade, JW01-7-11-1-4-y3 and JW01-7-11-1-2-y1, were also isolated directly from bark beetles or from their tunnels in wood materials (clade A in Fig. 2; van der Walt & Scott, 1971; Suh *et al.*, 2005).

The novel species *C. xylosterini* sp. nov., described here to accommodate strain ATCC 62898^T, was a close sister taxon of *O. dorogensis* and *Ogataea* (= *Pichia*) *kodamae*, and was placed in a clade with many other species of the genus *Ogataea* and related *Candida* species that can be characterized by their ability to assimilate methanol as sole carbon source (Fig. 2, clade B). Strain ATCC 62898^T was the only methanol-assimilating strain among the ambrosia yeasts reported here (Table 1). Many methylotrophic yeasts, e.g. *O. dorogensis*, *O. kodamae*, *Ogataea* (= *Pichia*) *trehaloabstinens* and *Ogataea* (= *Pichia*) *zsoltii*, have often been found in rotten wood material or insect associated plant sources. This association may correspond to the fact that methanol can be derived from the methoxy groups present in wood lignin (De Koning & Harder, 1992; Péter *et al.*, 2003). Very recently, several additional species in the genus *Ogataea* have been described, i.e. *Ogataea ganodermae*, *Ogataea paradorogensis*, *Ogataea neopini* and *Ogataea corticis* (Ji & Bai, 2008; Nagatsuka *et al.*, 2008; Nakase *et al.*, 2008). Among these species, *O. paradorogensis*, isolated from galleries of ambrosia beetles in Japan, showed a close phylogenetic relationship with *C. xylosterini* sp. nov. (data not shown). However, the two species can be clearly distinguished by more than 1 % variation in the sequences of the ITS and D1/D2 region of the LSU rDNA and by several physiological characteristics (Nakase *et al.*, 2008). Species of the genus *Ambrosiozyma* are well known yeasts associated with the ambrosia beetles (e.g. van der Walt, 1972; Smith, 1998). Several novel species of the genera *Ambrosiozyma* and *Candida*, i.e. *Ambrosiozyma kamigamensis*, *Ambrosiozyma neoplatypodis*, *C. kashinagacola*, *C. pseudovanderkliftii* and *Candida vanderkliftii*, have been reported recently from galleries of the ambrosia beetle, *Platypus quercivorus* (Coleoptera: Platypodidae), on oak trees (Endoh *et al.*, 2008a, b). However, those *Ambrosiozyma* species and related mitosporic yeasts could be distinguished phylogenetically from *C. xylosterini* sp. nov. and other species in the *Ogataea* clade, and formed a sister group to the clade (Fig. 2). The diversity of yeasts associated with ambrosia beetles indicates that such a partnership is probably functionally based, not taxonomically determined.

C. palmyrensis sp. nov., described here to accommodate strain ATCC 62899^T, was phylogenetically distinct from

any other ambrosia yeasts studied (Fig. 2). The species constituted an independent branch with *C. sophiae-reginae* and was placed in a clade that included species of *Debaryomyces* and related *Candida* spp. (Fig. 2, clade D). Although *C. sophiae-reginae* was the closest taxon to *C. palmyrensis* sp. nov. among the yeast species currently recognized, phylogenetically, the two were not close sisters based on the evolutionary rate, as shown by branch length (Fig. 2). Many gut yeasts isolated from a variety of beetles were placed in this *Debaryomyces/Lodderomyces* clade near *Candida albicans*, *Pichia guilliermondii* and *Pichia stipitis* (Suh & Blackwell, 2004; Suh *et al.*, 2003, 2008); however, those yeasts were not significantly related to *C. palmyrensis* sp. nov. Finding additional taxa near *C. palmyrensis* sp. nov. will help in the understanding of the phylogeny and ecology of this mitosporic yeast.

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