Sugiyamaella mastotermitis sp. nov. and *Papiliotrema odontotermitis* f.a., sp. nov. from the gut of the termites *Mastotermes darwiniensis* and *Odontotermes obesus*

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Two novel yeast species were isolated from the guts of two different termite species. A new member of the genus *Sugiyamaella* was isolated from the hindgut and nest material of the lower Australian termite *Mastotermes darwiniensis*. The second novel yeast species, isolated from the higher termite *Odontotermes obesus*, was identified as a member of the genus *Papiliotrema*. Both yeast species were able to hydrolyse xylan, methylumbelliferyl β-xylobiose and methylumbelliferyl β-xylotriose. The ability to debranch different hemicellulose side chains and growth without the addition of external vitamins was observed. A symbiotic role of the novel yeast species is indicated, especially in respect to xylan degradation and the production of vitamins. Here, we describe these species as *Sugiyamaella mastotermitis* sp. nov., MycoBank 816574 (type strain MD39V^T=DSM 100793^T=CBS 14182^T), and *Papiliotrema odontotermitis* f.a., sp. nov., MycoBank 816575 (type strain OO5^T=DSM 100791^T=CBS 14181^T). Additionally, we transfer *Candida qingdaonensis* to the genus *Sugiyamaella* and propose the following combination: *Sugiyamaella qingdaonensis* f.a., comb. nov., MycoBank 816576.

Termites are social insects which are differentiated into castes, such as workers, soldiers or reproductives. All known termites feed on lignocellulosic materials. Lower termites primarily consume wood (Mastotermitidae, Kalotermitidae, Termopsidae, Rhinotermitidae) or grass (Hodotermitidae). Higher termites (Termitidae) do not feed exclusively on wood or dry grass, but also on soil or dung (Brune, 2014; König & Varma, 2006; König *et al.*, 2013; Ni & Tokuda, 2013). Members of the Macrotermitinae, a Termitidae subfamily, cultivate lignocellulolytic fungi (*Termitomyces* spp.), which provide them with preprocessed lignocellulolytic material, lignocellulolytic enzymes and fungal biomass. Consumed wood is ground down by the termite's mandibles and its chitin-coated gizzard. The micrometre-sized food particles are 10–30 μm or 100-300 μm small, depending on the termite species. The food particles are initially mixed with cellulases of the termite. The termite's cellulases are secreted by the salivary glands and, in the case of some higher termites, also by the midgut epithelium. Glucose derived from cleaved cellulose is resorbed in the midgut. The hindgut contains a complex microbiome which is pooled mostly in the paunch. It consists of bacteria, archaea, yeasts and, in the case of lower termites, flagellates in addition (Brune, 2014; König *et al.*, 2007, 2013; Ni & Tokuda, 2013; Prillinger *et al.*, 1996; Schäfer *et al.*, 1996). Some members of the endosymbiotic groups (bacteria, archaea, yeasts, flagellates) are able to produce their own enzymes for carbohydrate degradation (e.g. cellulases, xylanases) and convert mobilized sugar monomers to volatile fatty acids. Acetate is the most important volatile fatty acid and it is the main nutrient for the termite host. Volatile fatty acids are taken up by the hindgut epithelium. Another important symbiotic activity of the microbiota is the recycling of uric acid and the fixation of N_2. This is necessary due to the low nitrogen content of the lignocellulosic materials (Brune, 2014; König *et al.*, 2013; Ni & Tokuda, 2013). A possible impact of symbiotic yeasts in the provision of essential vitamins or amino acids for their termite hosts has been hypothesized, but our knowledge about the physiological role of yeasts found in association with termites is still

Abbreviations: 4-MUF, 4-methylumbelliferyl; ITS, internal transcribed spacer; ML, maximum-likelihood.

GenBank/EMBL/DDBJ accession numbers for the sequences gathered in this study are given in Tables S2-S4.

Supplementary methods, two supplementary figures and four supplementary tables are available with the online Supplementary Material.

limited (Ganter, 2006; Handel & König, 2016; Houseknecht *et al.*, 2011; Molnar *et al.*, 2004; Prillinger *et al.*, 1996; Vega & Dowd, 2005).

In the present study, we report on the isolation of two novel yeast species from the termites *Mastotermes darwiniensis* and *Odontotermes obesus*.

M. darwiniensis is the only living representative of the termite family Mastotermitidae. Fossils from the Caribbean, Europe and Central America date the genus back to the Eocene and Miocene. Thus, *M. darwiniensis* is the most primordial species still extant. It can be found in northern Australia, and it was spread to New Guinea at the end of World War II. Its ability to feed on wood classifies this insect as vermin for human constructions and agriculture (König *et al.*, 2013; Krishna & Weesner, 1970). This termite is closely related to the wood-feeding cockroach *Cryptocercus punctulatus*, which lives in the Appalachian Mountains (PA, VA, WV in the USA; Kambhampati & Peterson, 2007).

O. obesus is a termite which is distributed in India, Pakistan and Bangladesh. Similar to *M. darwiniensis*, it feeds on wood and is a pest in urban areas and villages (Akhtar & Rashid, 2001; Manzoor & Akhtar, 2006; Nageswara Rao *et al.*, 2012).

The purpose of this study was to describe two novel lignocellulolytic yeast species on the basis of yeast isolates from the guts of *M. darwiniensis* and *O. obesus*.

Mastotermes darwiniensis (Froggatt) specimens were obtained from the Federal Institute for Materials Research and Testing (BAM), Berlin, in 2014. *Odontotermes obesus* (Rambur) was collected at the Jawaharlal Nehru University (New Delhi, India) in 1994 in cooperation with Professor Dr Ajit Varma (School of Life Sciences). The termites were dissected as described previously, and nest material was suspended in 0.9 % NaCl before enrichment (50 %, w/v; Kuhnigk *et al.*, 1994). Enrichment cultures were inoculated at 1 % (v/v). Cultivations were performed aerobically at 30 °C. Novel species of the genus *Sugiyamaella* were isolated from the gut of *M. darwiniensis* with modified Vogel's minimal salt medium (MV medium), GYP medium, malt extract medium or Sabouraud (SAB) medium (Prillinger *et al.*, 1996). MV medium consisted of Vogel's salts without trace elements and biotin solution or chloroform, 0.1 % (w/v) Tween 80, 0.67 % yeast nitrogen base and 0.5 % CM-cellulose (medium viscosity; Vogel, 1956). The novel species of the genus *Papiliotrema* was isolated from the gut of *O. obesus* using GYP medium. Purification was performed by streaking on agar plate medium and finally by micromanipulation (membrane method; Fröhlich & König, 2008).

For DNA extraction, a washed yeast cell pellet of 1 mm in diameter was suspended in 100 µl InstaGene matrix (with glass beads; Biorad) and 10 µl lyticase (3000 U ml^{-1}; Sigma-Aldrich). This suspension was incubated for 1 h at 37 °C with continuous shaking at 400 r.p.m. in a thermomixer. These parameters were then increased to 56 °C and 1400 r.p.m. for 30 min. All succeeding steps were performed according to the manufacturer's protocol.

Amplification and sequencing of the internal transcribed spacers 1 and 2 and the 5.8S rRNA gene (ITS region) were performed with *Taq* polymerase (Peqlab) and the primers ITS5 and ITS4 (White *et al.*, 1990). Other PCR reactions were performed with *Pfu* polymerase (Thermo Scientific). RFLP analysis of ITS regions was performed as described previously (Christ *et al.*, 2015). Sanger and Illumina sequencing was performed by Seqlab (Germany). Primers used for the amplification of the 18S rRNA gene (SSU) were modified versions of the standard primers NS-1 and NS-8 (Kurtzman & Robnett, 2003; White *et al.*, 1990). The modified versions were NS-1 mod (5′-CTGCCAGTAGTCATATGCTTG-3′) as the forward primer and NS-8 mod (5′-TCCGCAGGTTCACCTAC-3′) as the reverse primer. Amplified PCR products were sequenced with 18-NS-1 mod and 18S_SeqPrimer_intern1fwd (5′-GTTGGTTTCTAGGACCGTCG-3′) primers. Amplification and sequencing of the 26S/28S rRNA gene (D1 and D2 domains; LSU) were performed with NL1 and NL4 primers (Kurtzman & Robnett, 1998; O'Donnel, 1993). Nucleotide sequences corresponding to the genes encoding subunit 2 of the cytochrome C oxidase (*COXII*) and the mitochondrial small subunit rRNA (MtSSU) were obtained from the whole-genome sequence of the novel species of the genus *Sugiyamaella*. Gene regions were mapped in contigs by alignment of homologous gene sequences. Due to a TGA repeat, two separated contigs of *COXII* had to be assembled by PCR and Sanger sequencing with the primers COXII_F1 (5′-CCAGCTATGACAATTAAAGC-3′) and COXII_F2 (5′-GATAGTGGTGAAACAGTTC-3′) as forward primers and COXII_R1 (5′-CAACTGGTATTACAACACG-3′) as the reverse primer.

Independent alignments and phylogenetic analyses were performed for each locus. Multiple sequence alignments were performed with the genomic sequences using an online version of the MAFFT algorithm (E-INS-i option) with the default parameters (Katoh & Standley, 2013). Alignments for the *Sugiyamaella* clade were additionally curated with Gblocks (Castresana, 2000). Phylogenetic relationships were determined by the maximum-likelihood (ML) method based on the general time reversible (GTR) model with RaxML (version 7.4.2), using raxmlGUI 1.3.1 and the GTRCAT option with 1000 rounds of bootstrap replicates (Silvestro & Michalak, 2012; Stamatakis *et al.*, 2008).

The fermentation tests were performed with Durham tubes in YP medium (0.3 % yeast extract, 0.5 % meat peptone) which contained 2 % of the respective carbon source. Gas formation and pH shifts were documented after 18 days of growth at 30 °C. Additionally, ethanol production was determined by HPLC (Pfeiffer & Radler, 1985). Assimilation profiles were obtained with the API 32C and API 50 CH tests (bioMérieux), according to the manufacturer's instructions and were done in triplicate and duplicate, respectively. API C medium was used for cultivation (bioMérieux). Other physiological tests were performed aerobically at 30 °C in SAB medium or in yeast nitrogen base medium with 0.5 % glucose (alkali-ethanol-DBB method;

Hagler & Ahearn, 1981). Vitamin-free growth tests were performed in MV medium with various vitamins and 0.9 % glucose instead of CM-cellulose.

The morphology of yeast colonies was determined on GYP agar plates (1.5 % agarose). The duplicate plates were incubated at 25 or 30 °C for 7 days. Cell morphologies were determined after 3 days of aerobic incubation at 30 °C in liquid GYP medium. Formation of pseudohyphae was detected by incubation at 25 and 30 °C for 7 days on cornmeal agar (Dalmau technique; Wickerham, 1951). Light and phase-contrast microscopy were performed with a Keyence BZ-8000K and a Zeiss Axioskop 40, respectively.

A few additional assays such as hydrolysis of polysaccharides (e.g. xylan, CM-cellulose), degradation of nitrophenol and 4-methylumbelliferyl (4-MUF)-linked sugars and the API *Candida* test (bioMérieux,) were performed (Methods S1, available in the online Supplementary Material).

Species delineation

A total of 20 yeast strains were isolated from the gut of *M. darwiniensis* and a single strain was isolated from the nest samples (Table S1). One yeast strain was isolated from the gut of *O. obesus*. All strains described in this study belonged to two novel species. Analysis of the ITS regions by RFLP was employed to group and differentiate the strains down to the species level (Methods S1, Fig. S1). Sequencing of the ITS region of representative strains and identification with the aid of the NCBI GenBank and MycoBank databases showed that the yeast community of *M. darwiniensis* contained a novel species of the genus *Sugiyamaella*. It also comprised *Apiotrichum* (*Trichosporon*) *mycotoxinivorans* (Molnar *et al.*, 2004). Analyses of yeast communities of *O. obesus* yielded three basidiomycetous yeasts belonging to the Tremellomycetes, namely *Naganishia albida* (*Cryptococcus albidus*), *Saitozyma flava* (*Cryptococcus flavus*) and the novel species of the genus *Papiliotrema* described here. Most termite and yeast species are reported as mutualistic, such as *Neotermes jouteli* and *Sugiyamaella smithiae* (Handel & König, 2016).

The available strains of the novel species of the genus *Sugiyamaella* except MDE6G and HA167 were crossed in all possible combinations (see also Strains studied). Crossed strains were incubated for 5 weeks at 25 °C on 2 % malt extract medium plus 2 % agar. Tubular asci containing two spores were observed in crosses of the strains MD17G×MD16S, MD17G×MD18S, MD17G×MD74V, MD17G×MD75V, MD17G×MD88V, MD17M×MD72V, MD17S×MD75V, MD39V^T×MD16S and MD39V^T ×MD18M. Tubular asci containing four spores were observed in crosses for the combinations of the strains MD17G×MD72V, MD17S×MD18G, MD19S×MD16S and MD39V^T×MD18S.

The novel species of the genus *Sugiyamaella* was placed in the genus *Sugiyamaella* with good support (ML, 94 %) in the multilocus phylogenetic analysis based on concatenated alignments of ITS, LSU, MtSSU and *COXII* sequences (Fig. 1). The species clustered together with *Sugiyamaella smithiae*, *Sugiyamaella marilandica* and *Sugiyamaella chiloensis* with good statistical support (ML, 86 %). This cluster was also present in the phylogenetic analysis based on the LSU sequence and *Candida qingdaonensis*, which appeared to also be a member of this subclade (Fig. S2). Isolates of the novel species of the genus *Sugiyamaella* (strains MD39V^T, MD15M, MD17G) clustered together with *Candida* sp. HA167 (NCBI Taxonomy ID: 78167) in the LSU-based analysis. These strains showed 99 % identity in LSU and SSU sequences. The strain *Candida* sp. HA167 is designated (in sequence features) as *Galactocandida mastotermitis*, but the names of neither this genus nor the species have been validly published to date (Schweigkofler *et al.*, 2000). Our results show that these isolates represent a novel species of the genus *Sugiyamaella* (see Taxonomy). Additionally, we provide a new combination for *Candida qingdaonensis* to transfer it into the genus *Sugiyamaella* as *Sugiyamaella qingdaonensis* comb. nov. (see Taxonomy; Wang *et al.*, 2010).

The sexual genus *Papiliotrema* (type species *Papiliotrema bandonii*) has been reclassified recently to accommodate phylogenetically related asexual species which were previously classified in the genus *Cryptococcus*, for example, *Cryptococcus aureus* clade, *Cryptococcus laurentii* clade, etc. (Liu *et al.*, 2015; Yurkov *et al.*, 2015). The novel species of the genus *Papiliotrema* clustered with *Papiliotrema laurentii*, *Papiliotrema rajasthanensis* and *Papiliotrema aspenensis* with strong statistical support (ML, 97 %) in the phylogenetic analysis of concatenated alignments of ITS and LSU sequences (Fig. 2).

Nucleotide sequences of LSU regions of the novel species differed in 13 (98 % similarity) and 15 (97 % similarity) nucleotide positions from those of *P. aspenensis* and *P. rajasthanensis*, respectively. Here, we describe the yeast isolated from the termite *O. obesus* as a representative of a novel species of the genus *Papiliotrema* (see Taxonomy). The nucleotide sequence of *Cryptococcus* sp. BC-2011 (NCBI Taxonomy ID: 1081692) could be conspecific to the novel species of the genus *Papiliotrema*, as suggested by a rather high 99 % similarity (five nucleotide substitutions and two gaps) of the respective ITS sequence (JN635412). Interestingly, this strain originates from the gut of an unspecified termite species, as provided in the GenBank entry.

The results of the physiological tests are provided in Tables 1 and S4. The ability of the novel species of the genus *Sugiyamaella* to grow at elevated temperatures up to 40 °C is a rare feature among yeasts (Deák, 2008; Raspor & Zupan, 2006). Similarly, the novel species of the genus *Papiliotrema* exhibited a relatively high maximum growth temperature of 35 °C, which is unusual for basidiomycetous yeasts. Our results show that the novel species of the genus *Sugiyamaella* can be distinguished from *Sugiyamaella chiloensis*, the most closely related species, in the fermentation of D-glucose, D-galactose, maltose and sucrose, and in the assimilation of D-arabinose, D-ribose, glycerol and ribitol (adonitol). Additionally, *Sugiyamaella chiloensis* was not able to grow at 37 °C (Kurtzman, 2007). On the contrary, the novel species of the genus *Papiliotrema* cannot

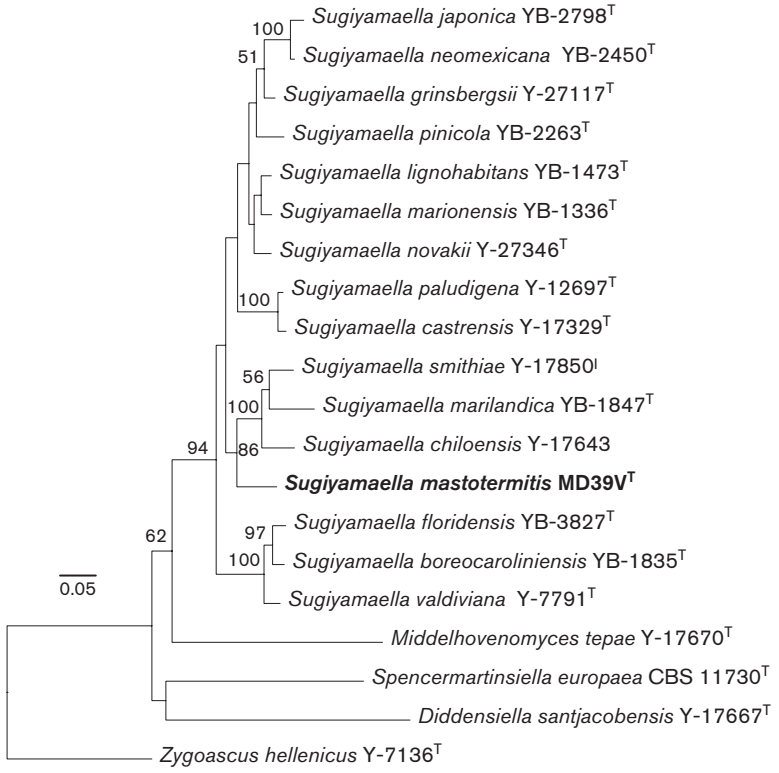

Fig. 1. Phylogenetic assignment of *Sugiyamaella mastotermitis* sp. nov. inferred from the ML analysis of ITS, LSU, MtSSU and *COXII* nucleotide sequences. The numbers given at the branching points are frequencies (>50%) with which a given branch appeared in 1000 bootstrap replications. Bar, number of expected substitutions accumulated per site. Accession numbers of nucleotide sequences are provided in Table S2.

be distinguished from *Papiliotrema aspenensis* based on phenotype (Ferreira-Paim *et al.*, 2014). The novel species differs from *Papiliotrema rajasthanensis* in the assimilation of D-glucosamine, L-sorbose and soluble starch (Saluja & Prasad, 2007). The highest growth temperature among the three species of the genus *Papiliotrema* was recorded for *P. aspenensis* (37 °C), followed by the novel species of the genus *Papiliotrema* (35 °C) and *P. rajasthanensis* (30 °C).

Taxonomy

Description of *Sugiyamaella mastotermitis* Handel, Wang, Yurkov & König sp. nov. (MB 816574)

Sugiyamaella mastotermitis (mas.to.ter′mi.tis. N.L. gen. n. *mastotermitis* of the termite genus *Mastotermes*).

Standard description: Member of the genus *Sugiyamaella* in the family Trichomonascaceae of Saccharomycetales. Tubular asci with 2 or 4 spores are observed. No sexual structures have been observed for unmated strains, indicating that the species is heterothallic. The strains MD17G, MD17M, MD17S, MD19S and MD39V^T are associated to the mating type 'a', and the strains MD16S, MD18G, MD18M, MD18S, MD72V, MD74V, MD75V and MD88V to the 'alpha' mating type. The texture is butyrous, and the margin is entire. Yeast cells are spherical to ovoid (2.5–6.2×2.3–6.0 μm) and proliferate by multilateral budding. Cells occur singly or in pairs (Fig. 3). After 1 week at 25 °C and 30 °C, colonies on GYP agar plates are tannish-white, glistering, smooth and raised. After 5 weeks on corn meal agar, a hyphal fringe is observed (Fig. 4b). Pseudohyphae bearing blastoconidia on short denticles (Fig. 4a, c, d) are observed in Dalmau plate culture on corn meal agar. Physiological characteristics are listed in Tables 1 and S4. Maximum growth temperature is 40 °C.

Unambiguous identification and phylogenetic placement is based on DNA sequences of the following nuclear loci (type strain): LSU (KU883286), ITS (KU883293), *COXII* (KU883279), MtSSU (KU883282).

Deposits: The holotype strain, MD39V^T, was isolated from gut contents of the lower termite *Mastotermes darwiniensis*, which was obtained from the Federal Institute for Materials Research and Testing (BAM), Berlin, Germany. It is preserved in a metabolically inactive state at the Institute of Microbiology and Wine Research, Johannes Gutenberg University, Mainz, Germany. Ex-type cultures are deposited at the Leibniz Institute DSMZ – German Culture Collection of Microorganisms and Cell

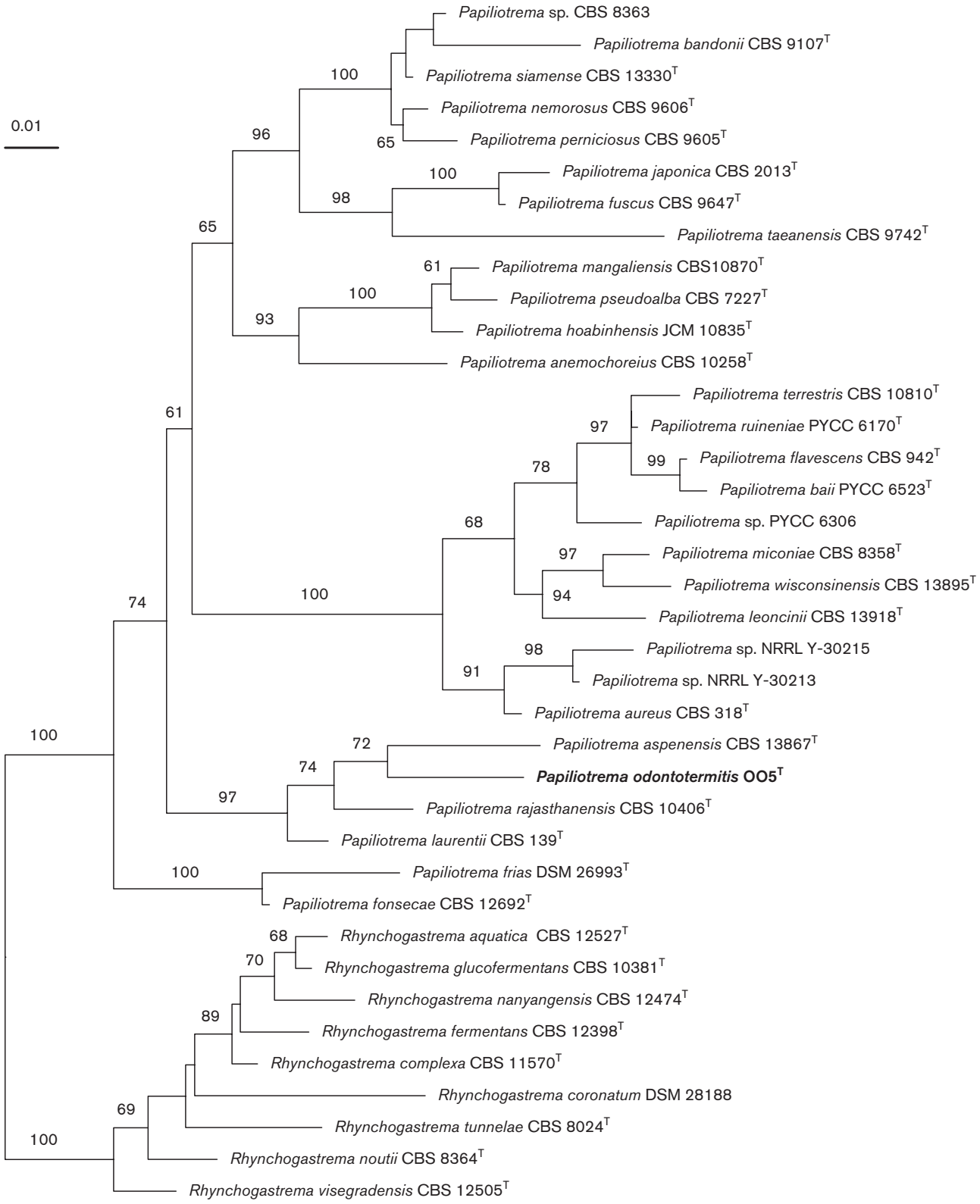

Fig. 2. Unrooted tree showing the phylogenetic placement of *Papiliotrema odontotermitis* f.a., sp. nov. inferred from the MLa-nalysis of the ITS and LSU nucleotide sequences. The numbers given on branches are frequencies (>50%) with which a given branch appeared in 1000 bootstrap replications. Bar, number of expected substitutions accumulated per site. Accession num-bers of nucleotide sequences are provided in Table S3.

Table 1. Assimilation and fermentation profile of *Sugiyamaella mastotermitis* sp. nov. and *Papiliotrema odontotermitis* f.a., sp. nov., observed after 48 h unless mentioned otherwise

All species tested assimilated L-arabinose, D-arabitol, arbutin, cellobiose, erythritol, aesculin ferric citrate, D-fructose, D-galactose, gentiobiose, D-glucose, inositol (W), maltose, D-mannitol (72 h), D-mannose, melezitose, *N*-acetylglucosamine, palatinose, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, raffinose, L-rhamnose, sucrose, salicin, sodium glucoronate, D-sorbitol, trehalose, turanose, xylitol and D-xylose. Both yeast species were negative regarding the fermentation of lactose, raffinose, trehalose and D-xylose as well as the assimilation of inulin, levulinic acid and methyl α-D-mannopyranoside. Growth was positive at 25 °C, 30 °C and 32 °C or without addition of biotin and/or thiamin or vitamins. Growth was negative at 42 °C and 45 °C and with 50 % or 60 % glucose. Starch was not produced. +, Positive. −, negative. W, weakly positive. (x %), 100−x % of the reactions were negative.

Characteristic	*Sugiyamaella mastotermitis* sp. nov.	*Papiliotrema odontotermitis* f.a., sp. nov.	Characteristic	*Sugiyamaella mastotermitis* sp. nov.	*Papiliotrema odontotermitis* f.a., sp. nov.
Fermentation			Assimilation		
D-Galactose	+	−	Lactose (bovine origin)	−	+
D-Glucose	+	−	D-Lyxose	w	−
Maltose	+	−	Melibiose	+	w (83 %)
Sucrose	+	−	Methyl α-D-glucopyranoside	+	−
			Methyl β-D-xylopyranoside	+ (96 h)	−
Assimilation			D-Ribose	−	+
D-Adonitol	−	+	L-Sorbose	+	−
Starch	w (96 h)	+	D-Tagatose	−	w
Amygdalin	−	+	L-Xylose	−	w (72 h)
D-Arabinose	−	+			
L-Arabitol	+	+ (72 h)	Other tests		
Cycloheximide (actidione)	+	−	Diazonium blue B reaction	−	+
Dulcitol	W (96 h)	w	Ethanol tolerance	5 % (v/v)	1 % (v/v)
D-Fucose	−	w	pH optimum	4.0	5.5
L-Fucose	−	+ (72 h)	Growth at 35 °C	+	w
Glucosamine	+	−	Growth at 37 °C	+	−
Glycerol	−	w	Growth at 40 °C	+	−
Glycogen	−	w	Growth with 10 % NaCl and 5 % glucose	−	+
Lactic acid	−	w	Growth with 0.1 % cycloheximide	+	−

Cultures, Braunschweig, Germany (DSM 100793^T), and at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 14182^T).

Strains studied: A total of 21 strains were studied. Strain *Sugiyamaella mastotermitis* MDE6G was isolated from the nest material of *Mastotermes darwiniensis* (Table S1). Strains HA167 and HA616 were isolated in former studies of our working group and are reported by Prillinger *et al.* (1996). Both strains are deposited with the ACBR strain collection of the University of Natural Resources and Life Sciences, Vienna (BOKU).

Description of *Sugiyamaella qingdaonensis* (F.-L. Li & S.-A. Wang) Handel, Wang, Yurkov & König comb. nov. (MB 816576)

Basionym: *Candida qingdaonensis* F.-L. Li & S.-A. Wang, *Int J Syst Evol Microbiol* 60, 1697–1701 (2010). MB 514463.

Member of the genus *Sugiyamaella* as suggested by Daniel *et al.* (2014) and shown in the phylogenetic analyses in the present study (Figs 1 and S2). Urbina *et al.* (2013) reclassified 13 *Candida* species in this clade and transferred them to the genus *Sugiyamaella*. However, *C. qingdaonensis* was not included in this study. The species belongs to the subclade comprised by *Sugiyamaella smithiae*, *Sugiyamaella marilandica*, *Sugiyamaella chiloensis* and *Sugiyamaella mastotermitis*. This species is apparently anamorphic as described by Wang *et al.* (2010).

Description of *Papiliotrema odontotermitis* Handel, Wang, Yurkov & König sp. nov. (MB 816575)

Papiliotrema odontotermitis. (o.don.to.ter′mi.tis. N.L. gen. n. *odontotermitis* of the termite genus *Odontotermes*).

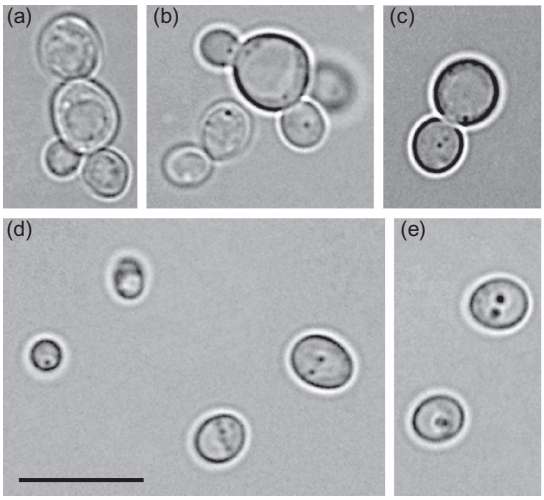

Fig. 3. Light microscopic images of cells of *Sugiyamaella mastotermitis* after 3 days in liquid GYP medium at 30 °C; Bar, 10 μm. (a–c) Yeast cells reproducing by budding. (d–e) Single yeast cells.

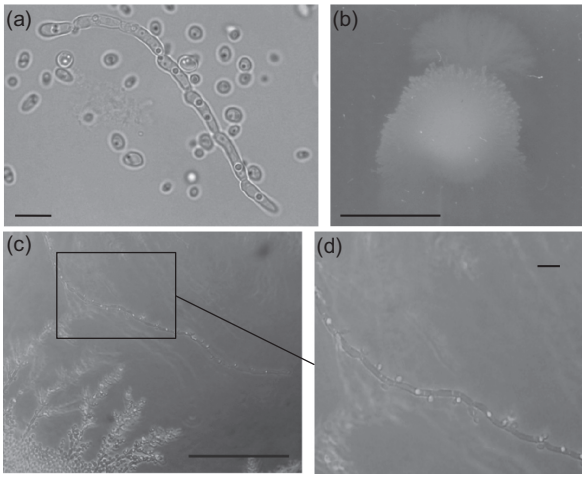

Fig. 4. Images showing development of pseudohyphae of *Sugiyamaella mastotermitis* in Dalmau plate culture on corn meal agar at 25 °C. (a) A fragment of pseudhypha and single cells after 7 days of incubation; light microscopy; bar, 10 μm. (b) Colony morphology after 5 weeks of incubation showing hyphal fringe and filaments growing into the agar; scan of plate; bar, 5 mm. (c, d) Phase contrast micrographs after 7 days of incubations showing pseudohyphae with blastospores; bar, 100 μm (c), bar, 10 μm (d).

Standard description: The species belongs to the genus *Papiliotrema* (sensu Liu *et al.*, 2015) in the Tremellales. The species is apparently anamorphic since no sexual structures have been observed. After 1 week at 25 °C, colonies on YM and GYP agar plates are circular, white, smooth, glistening and raised. The texture is butyrous and the margins are entire. Yeast cells are ovoid, ellipsoid to globose (2.4–11.8×2.4–11.9 μm), form by multilateral budding and occur singly, in pairs or in short chains (Fig. 5). Short chains of cells (up to six in a row) are observed in Dalmau plate culture on corn meal agar (Fig. 6). In Dalmau plate culture, cells become elongated, 3.8×11.7 μm on average. Physiological characteristics are listed in Tables 1 and S4. The maximum growth temperature is 35 °C.

Unambiguous identification and phylogenetic placement is based on DNA sequences of the following nuclear loci (type strain): LSU (KU883278) and ITS (KU883277).

Deposits: The holotype strain, OO5^T, was isolated from the gut of the higher termite *Odontotermes obesus* which was collected at the Jawaharlal Nehru University, New Delhi, India. It is preserved in a metabolically inactive state at the Institute of Microbiology and Wine Research, Johannes Gutenberg University, Mainz, Germany. Ex-type cultures are deposited at the Leibniz Institute DSMZ – German Culture Collection of Microorganisms and Cell Cultures, Braunschweig, Germany (DSM 100791^T), and at the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands (CBS 14181^T).

Ecology

A total of 21 species in the genus *Sugiyamaella* are described to date, including the novel species *Sugiyamaella mastotermitis*. According to the reports available, 17 species were isolated from habitats which are enriched with lignocellulose, such as rotten wood, peat, wood-feeding insects or insect frass (Houseknecht *et al.*, 2011; Kurtzman, 2007; Morais *et al.*, 2013; Urbina *et al.*, 2013; van der Walt & Nel, 1968; Wang *et al.*, 2010). A few studies have addressed the potential of lignocellulose degradation by these yeasts, suggesting the need for further research in this field (e.g. Morais *et al.*, 2013). Frequent occurrence of *Sugiyamaella mastotermitis* in the gut and nest of *M. darwiniensis*, and physiological adaption of the yeast to the conditions characteristic to the termite's gut (e.g. vitamin-free growth, xylanase activity) suggest symbiotic relationships between these organisms.

One of the close relatives of *P. odontotermitis*, *P. rajasthanensis*, was isolated from inflorescences of false amaranth (*Digera* sp.) and false water willow (*Andrographis echioides*) in the province of Rajasthan, India (Saluja & Prasad, 2007). Interestingly, *O. obesus*, the source of *P. odontotermitis*, is a common termite species in Rajasthan (Roonwal & Bose, 1978). The nucleotide sequence of strain BC-2011 (GenBank JN635412), which is conspecific or closely related to *P. odontotermitis*, was also obtained from the gut of an unknown termite from India. Thus, the historic origin of yeasts comprising this *Papiliotrema* subclade in association with plant- or wood-feeding insects is feasible. Mutualistic relationships of *P. odontotermitis* as an endosymbiont of the termite host are likely if we consider xylanase activity and a few further physiological adaptions of the yeast (e.g. vitamin-free growth).

Since the two novel yeast species were isolated from a habitat which is enriched with lignocellulose, it is not surprising that

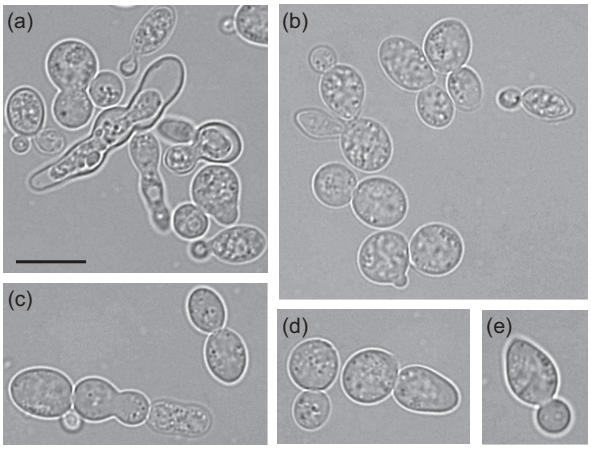

Fig. 5. Light microscopic images of cells of *Papiliotrema odontotermitis*. (a–e) Yeast cells reproducing by budding after 3 days in liquid GYP medium at 30 °C, bar, 10 μm.

they share physiological properties which enable them to take part in lignocellulose digestion. Both species showed xylanase activity. Furthermore, they assimilated several hemicellulose-related substrates (e.g. D-xylose, L-arabinose, sodium glucuronate, D-mannose and cellobiose), which can also be a common trait for gut-dwellers of plant- and wood-feeders. Additionally, *P. odontotermitis* was able to grow on L-fucose. Further properties which could point to the yeasts' adaption to termite guts are enzymic activities of 4-MUF α-D-xylobiosidase, 4-MUF α-D-xylotriosidase, 4-MUF α-D-glucopyranosidase, 4-MUF α-L-arabinofuranosidase (*P. odontotermitis* only) and 4-MUF α-D-mannopyranosidase (*Sugiyamaella mastotermitis* only).

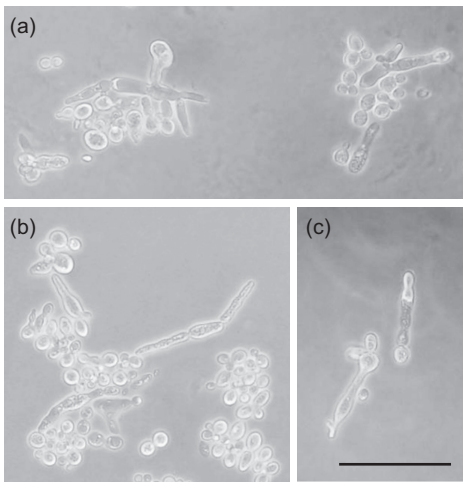

Fig. 6. Phase contrast microscopic images of cells of *Papiliotrema odontotermitis* in Dalmau plate culture on corn meal agar after 7 days of incubation at 25 °C. (a–c) Elongated cells and budding cells in short chains, bar, 50 μm.

Thirteen species of the genus *Sugiyamaella* have been tested for growth without external vitamins (Houseknecht *et al.*, 2011; Kurtzman, 2007; Wang *et al.*, 2010). Only four species, *Sugiyamaella chiloensis*, *Sugiyamaella qingdaonensis*, *Sugiyamaella marilandica* (weak) and *Sugiyamaella grinsbergsii* (variable), showed some growth. The first three species belong to the same subclade as *Sugiyamaella mastotermitis* (Figs 1 and S2). Therefore, it would appear that vitamin independence is a feature of this subclade. When *Sugiyamaella mastotermitis* and *P. odontotermitis* grow in an environment lacking externally available vitamins, they are able to synthesize their own. This may allow them to supplement the diet of host termites with vitamins (Houseknecht *et al.*, 2011; Vega & Dowd, 2005) in addition to monosaccharides and oligosaccharides from the hydrolysis of xylan.

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