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Wickerhamomyces spegazzinii sp. nov., an ascomycetous yeast isolated from the fungus garden of *Acromyrmex lundii* nest (Hymenoptera: Formicidae)

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A novel ascomycetous yeast species in the genus *Wickerhamomyces* was isolated from the fungus garden of an attine ant nest, *Acromyrmex lundii* (Hymenoptera: Formicidae), from Santa Fe province, Argentina. Pairwise sequence alignment of D1/D2 sequences in the GenBank (http://www.ncbi.nlm.nih.gov) database revealed that the novel species is related most closely to *Wickerhamomyces subpelliculosus*, *Wickerhamomyces linferdii*, *Wickerhamomyces anomalus*, *Wickerhamomyces siamensis* and *Wickerhamomyces ciferrii* with 96% similarity to the first four. The species name *Wickerhamomyces spegazzinii* sp. nov. is proposed to accommodate this novel strain, which differs from the above species in melibiose, 5-keto-D-gluconate, succinate, and DL-lactate assimilation among others. The type strain is JLU025^T (=CBS 12756^T=CBMAI 1619^T).

Kurtzman *et al.* (2008) proposed the genus *Wickerhamomyces* based on phylogenetic analysis using a concatenated dataset of gene sequences from the SSU rRNA, the LSU rRNA and the translation elongation factor-1α (EF-1α). The genus *Wickerhamomyces* consists of a group of species that were earlier nested in the polyphyletic group *Pichia* (Kurtzman & Robnett, 1998). According to their study, the *Wickerhamomyces* clade is the most divergent between the clades *Barnettozyma* and *Lindnera* [currently *Cyberlindnera* (Minter, 2009)]. Some characteristics of the genus *Wickerhamomyces* are the formation of hat-shaped ascospores or spherical ascospores with an equatorial ledge; cell division is by multilateral budding; some species form pseudohyphae and true hyphae; asci are globose to ellipsoid, and unconjugated; some species ferment glucose and they do not assimilate methanol or hexadecane; and the diazonium blue B reaction is negative (Kurtzman *et al.*, 2008, 2011a). Species currently accepted in the genus have been isolated from soil (Limtong *et al.*, 2009; Kurtzman *et al.*, 2011a; Shin *et al.*, 2011; Limtong *et al.*, 2012), plant material (Groenewald *et al.*, 2011; Kaewwichian *et al.*, 2013; James *et al.*, 2014), tree exudates (Kurtzman *et al.*, 2011a; de García *et al.*, 2010), flowers (Nakase *et al.*, 2012; James *et al.*, 2014), digestive tract of insects (Hui *et al.*, 2013; James *et al.*, 2014), insect frass (Kurtzman *et al.*, 2011a), larvae of diptera (Rosa *et al.*, 2009), birds (Francesca *et al.*, 2013), natural fermentation of coffee cherries (Silva *et al.*, 2000) and brined vegetables (Etchells & Bell, 1950). *Acromyrmex lundii* is an ant species in the tribe Attini (Hymenoptera: Formicidae), which have a mutualistic relationship with basidiomycetous fungi in the order Agaricales (Bonetto, 1959). They cut different parts of the plants such as flowers, leaves and fruits that they use as substrate for the mutualistic fungus that is the main food source of the colony (Weber, 1972; Hölldobler & Wilson, 1990). This substrate is carefully processed and the fungus inoculated on it, giving arise to delicate structures called 'fungus garden' that are found inside the nests (Möller, 1893). In the sampling area *A. lundii* builds nest with a single fungus garden which has usually a volume of 20–30 litres. Nests in the sampling area are associated with roots of trees (Bonetto, 1959). In this work, we describe a novel species of *Wickerhamomyces* isolated from the fungus garden of a leaf-cutter ant, *A. lundii* from Argentina.

The fungus garden of the leaf-cutting ant *A. lundii* was collected from a field nest in September 2009 in Santurce, Santa Fe province, Argentina (30° 10′ 52.40″ S 61° 10′ 05.15″ W). Three samples collected from different parts of the fungus garden each of 0.5 g were separately vortexed in 4.5 ml sterile yeast extract/malt extract/ peptone/glucose broth (YMB) supplemented with 150 mg chloramphenicol l^{-1} and pH adjusted to 4 to suppress bacterial growth. One millilitre of each suspension was inoculated in 9 ml of the same medium

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 region of the LSU rRNA gene and ITS sequences of JLU025^T are KJ832071 and KJ832072, respectively. The MycoBank number for *Wickerhamomyces spegazzinii* sp. nov. JLU25^T is MB814527.

Abbreviation: ITS, internal transcribed spacer.

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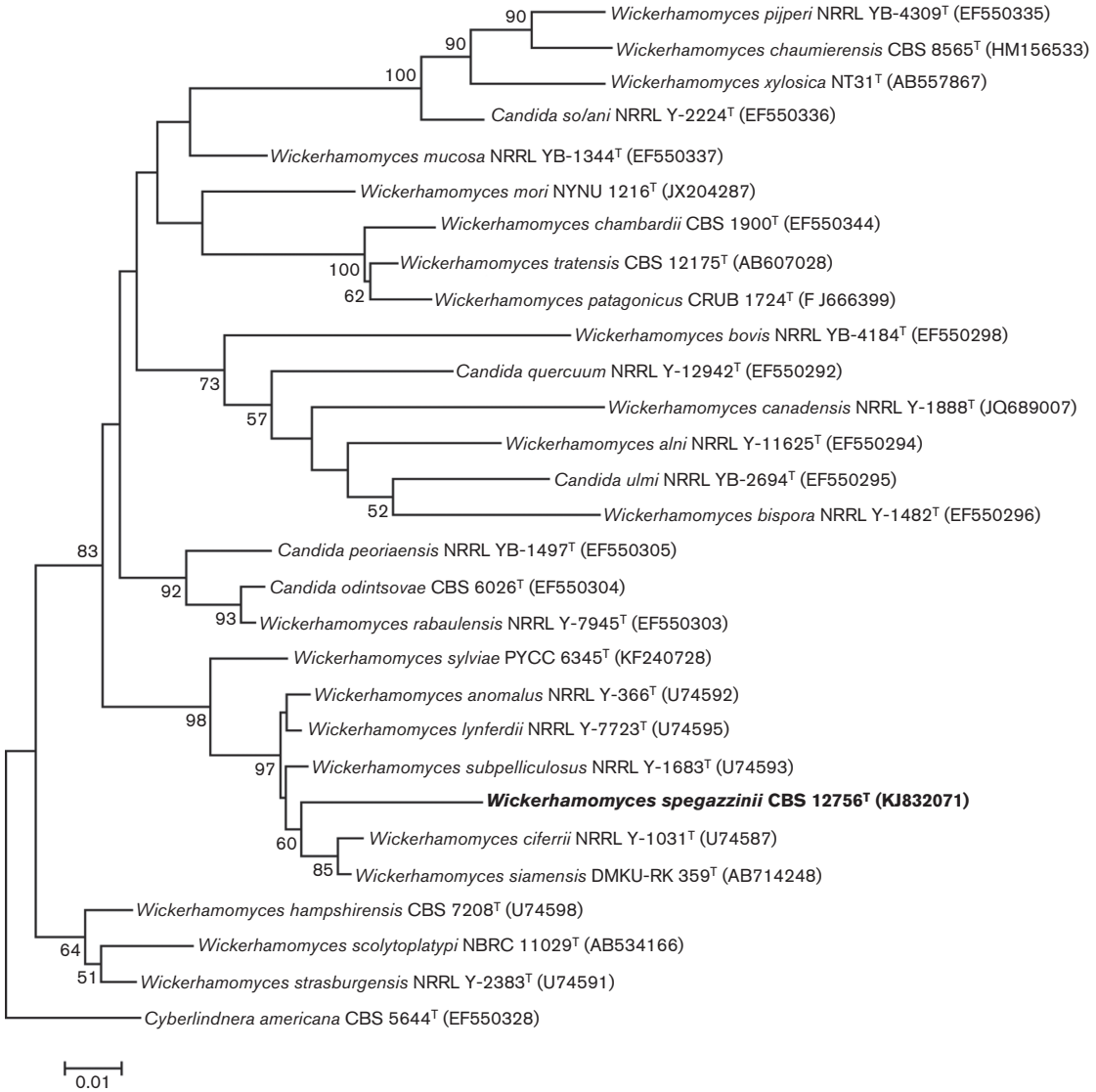

Fig. 1. Phylogenetic analysis of the D1/D2 domain of the LSU rRNA gene from strain JLU025^T(=CBS12756^T =CBMAI 1619^T) and related species. Evolutionary distances were calculated according to the Kimura's two-parameter correction (Kimura, 1980) by the neighbour-joining method using the MEGA version 6 software package. Bootstrap values $\geq$50 % based on 1000 replications are given at nodes. Numbers in parentheses are GenBank accession numbers. Bar, 0.01 substitutions per nucleotide position.

and after 4 days of incubation at 20 °C aliquots of 150 µl were spread on Sabouraud dextrose agar (Acumedia) supplemented with 150 mg chloramphenicol l^{-1} and of pH 4. Analyses were conducted in ten replicates per sample. All the colonies growing after 4 days of incubation were stored in GYMP medium (glucose/malt extract/yeast extract/NaH_2PO_4) at 6–8 °C and in 15 % glycerol at –80 °C.

The isolated strains were phenotypically characterized according to the methods described by Kurtzman *et al.* (2011b). Genomic DNA was extracted following the protocol described by Sampaio *et al.* (2001). The internal transcribed spacer (ITS) region was amplified with the primers forward ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.*, 1990) and the D1/D2 domain of the LSU rRNA gene was amplified using the pair primers forward NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and reverse NL4 (5′GGTCCGTGTTTCAAGACGG-3′) (O'Donnell, 1993). PCR amplification products were purified with illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare) and the sequencing reaction was performed with the same primers used during the amplification and with ABI Prism Big Dye Terminator v3.1 Cycle Sequencing

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Table 1. Differential physiological characteristics between JLU025^T (CBS 12756^T) and closely related species

Strains: 1, JLU025^T; 2, *W. subpelliculosus* NRRL Y-1683^T; 3, *W. lynferdii* NRRL Y-7723^T; 4, *W. anomalus* NRRL Y-366^T; 5, *W. siamensis* DMKU-RK359^T; 6, *W. ciferrii* NRRL Y-1031^T. +, Positive; –, negative; W, weak; S, positive but slow; V, variable; N, no data available. Data for reference species were taken from Kurtzman *et al.* (2011b) and Kaewwichian *et al.* (2013)

Characteristic	1	2	3	4	5	6
Assimilation of:						
Melibiose	+	–	–	–	–	–
Soluble starch	V	S		+	N	+
L-Rhamnose	+	–	–	–	–	+/W
D-Xylose	+	V	–	V	N	+/W
L-Arabinose	–	V	–	V	–	+/W
D-Arabinose	–	V	–	–	–	–
D-Ribose	–	V	+	V	–	+
Ribitol	–	V	+	V	–	+
DL-Lactate	–	+	+	+	N	+
Succinate	–	+	+	+	N	+
5-Keto-D-gluconate	+	–	–	–	N	–
Growth on/at:						
Vitamin-free medium	+	–	+	+	–	+
37 °C	+	V	–	V	+	W/–

Kits (Applied Biosystems). The products of the sequencing reaction were purified using 125 mM EDTA, 3 M sodium acetate and ethanol. The strands were sequenced in a 3130 Genetic Analyzer (Applied Biosystems). Sequences were assembled and edited manually with the software BioEdit Sequence Aligment Editor v. 7.0.5.3 (Hall, 1999) and were compared with those deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/) and MycoBank (http://www.mycobank.org/).

Multiple sequence alignment was performed using MAFFT v. 6 (Katoh & Toh, 2008), and the software package MEGA v. 6 (Tamura *et al.*, 2013) was used for the data analysis. Phylogenetic trees were reconstructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980) by the neighbour-joining method (Saitou & Nei, 1987). *Cyberlindnera americana* EF550328 was used as an outgroup. Bootstrap analysis (1000 replicates) was performed to assess the confidence limits of the branching (Felsenstein, 1985).

A total of 27 colonies were recovered as follows: *Candida* sp. (three), *Galactomyces geotrichum* (five), *Geotrichum* sp. (two), *Torulaspora delbruekii* (12) and *Wickerhamomyces* sp. (five). During the characterization procedures, including methylation-specific PCR (data not shown), the five isolates of *Wickerhamomyces* sp. were showed to represent a putative novel species. Comparing the results of molecular characterization from the D1/D2 domains of the LSU rRNA gene between the type strain (JLU025^T) and the four additional isolates obtained from different plates confirmed that they were conspecific and belonged to the genus *Wickerhamomyces*. The length of strain JLU025^T sequences was 559 bp for the D1/D2 domain (GenBank accession no. KJ832071) and 543 bp for the ITS region (KJ832072).

Phylogenetic analysis indicated that strain JLU025^T was related most closely to a subclade containing the following species: *Wickerhamomyces subpelliculosus* (NRRL Y-1683^T/U74593), *Wickerhamomyces lynferdii* (NRRL Y-7723^T/U74595), *Wickerhamomyces anomalus* (NRRL Y-366^T/U74592), *Wickerhamomyces siamensis* (DMKU-RK 359^T/AB714248) and *Wickerhamomyces ciferrii* (NRRL Y-1031^T/U74587) (Fig. 1). Divergence of strain JLU025^T in the D1/D2 domain was 3.3 and 3.5% when compared with *W. subpelicullosus* (19 nt substitutions and two indels) and *W. ciferrii* (20 nt substitutions and sixindels), in 559 nt, respectively. Divergence of JLU025^T with *W. siamensis, W. lynferdii* and *W. anomalus* iwas s 3.7% (21 nt and two indels in 559 nt), 4.1% (23 nt and two indels) and 4.3 % (24 nt and four indels), respectively.

Phenotype-based separation of the novel species proposed herein, *W. spegazzinii* sp. nov., from its closest relatives is detailed in Table 1. The assimilation of melibiose, L-rhamnose, DL-lactate, succinate and 5-keto-D-gluconate could be used as good markers for *W. spegazzinii* JLU025^T (Table 1). Ascospores were formed by all the strains including JLU025^T on acetate agar and cornmeal agar after 3–10 days at 20 °C and asci were unconjugated and produced one to four hat-shaped ascospores (Fig. 2).

Four new yeast species associated with the fungus garden of Attini ants have been described: *Cryptococcus haglerorum* (Middelhoven *et al.*, 2003), *Blastobotrys attinorum* (Carreiro *et al.*, 2004), *Trichosporon chiarellii* (Pagnocca *et al.*, 2010) and *Starmerella aceti* (Melo *et al.*, 2014) and this is the first report for *Wickerhamomyces* in this substrate.

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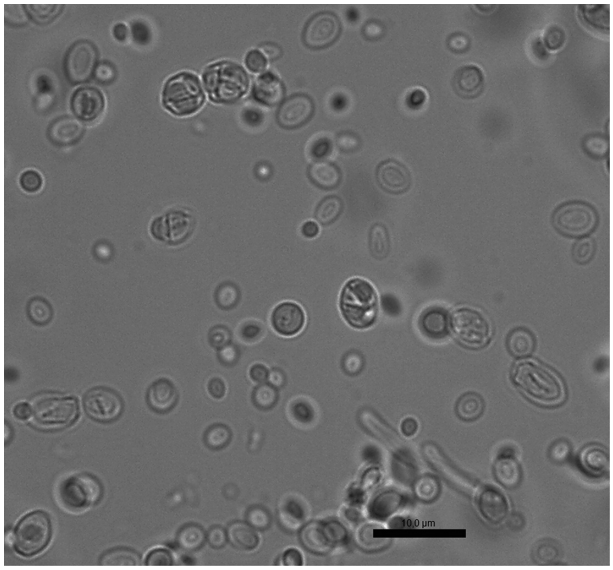

Fig. 2. *Wickerhamomyces spegazzinii* sp. nov. (JLU025^T). Ascospores produced on cornmeal agar after 10 days at 20 °C.

Considering the analysis of the D1/D2 domain of the LSU rRNA, and the morphological, biochemical and physiological properties, we inferred that strain JLU025^T isolated from the fungus garden of *Acromyrmex lundii* represents a novel ascomycetous yeast species for which the name *Whickerhamomyces spegazzinii* sp. nov. is proposed. The origin of the yeast strain was likely to be the plant material foraged by the ants.

Description of *Wickerhamomyces spegazzinii* sp. nov.

Wickerhamomyces spegazzinii [spe.ga.zzi'ni.i. N.L. gen. n. *spegazzinii* named in memory of Dr Carlos Spegazzini (1858–1926), one of the most prestigious mycologists of Argentina].

After 3 days of growth on 5 % malt extract agar at 25 °C, cells are ellipsoidal and globose (2–3 × 5–7.5 µm), ovoid and occur singly or in pairs. Budding is multilateral. Pseudohyphae are formed. The streak culture is cream-coloured, butyrous, with a smooth surface and has an entire margin. Asci are unconjugated and form one to four hat-shaped ascospores. Ascospores are abundant on cornmeal agar and acetate agar after 3–10 days at 20 °C (Fig. 2). The carbon compounds assimilated are: glucose, sucrose, raffinose, melibiose, galactose, maltose, melezitose, methyl α-D-glucoside, soluble starch (slow), cellobiose, salicin, L-rhamnose, D-xylose, ethanol, glycerol, erythritol (slow), D-mannitol, D-glucitol, D-gluconate, citrate (weak), 5-keto-D-gluconate, xylitol, propane 1,2 diol (slow) and butane 2,3 diol (weak). Inulin, lactose, L-sorbose, L-arabinose, D-arabinose, D-ribose, methanol, ribitol, galactitol, *myo*-inositol, DL-lactate, succinate, D-glucosamine, *N*-acetyl-D-glucosamine, 2-keto-D-gluconate, saccharate, D-glucuronate and L-arabinitol are not assimilated. Growth on nitrate, nitrite, L-lysine, cadaverine and vitamin-free medium is positive. No growth occurs on creatinine, ethylamine, 50 % glucose, 10 % NaCl/5 % glucose, or 0.01 or 0.1 % cycloheximide. Production of starch-like compounds is negative. Fermentation is positive with glucose, sucrose and raffinose, but negative with galactose, maltose, lactose and trehalose. Urease and diazonium blue B are negative. Growth is observed at 25 and 37 °C.

The type strain, JLU025^T (=CBS 12756^T =CBMAI 1619^T), was isolated in September 2009 from the fungus garden of an ant nest of *Acromyrmex lundii* (Hymenoptera: Formicidae) from Santurce, Santa Fe province, Argentina. The MycoBank accession number is MB814527.

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References

Bonetto, A. A. (1959). *Las Hormigas "Cortadoras" De La Provincia De Santa Fe (Géneros: Atta Y Acromyrmex)*. Santa Fe: Dirección General de Reservas Naturales, Ministerio de Agricultura y Ganadería.

Carreiro, S. C., Pagnocca, F. C., Bacci, M., Lachance, M. A., Bueno, O. C., Hebling, M. J., Ruivo, C. C. & Rosa, C. A. (2004). *Sympodiomyces attinorum* sp. nov., a yeast species associated with nests of the leaf-cutting ant *Atta sexdens*. *Int J Syst Evol Microbiol* **54**, 1891–1894.

de García, V., Brizzio, S., Libkind, D., Rosa, C. A. & van Broock, M. (2010). *Wickerhamomyces patagonicus* sp. nov., an ascomycetous yeast species from Patagonia, Argentina. *Int J Syst Evol Microbiol* **60**, 1693–1696.

Etchells, J. L. & Bell, T. A. (1950). Film yeasts on commercial cucumber brines. *Food Technol* **4**, 77–83.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Francesca, N., Carvalho, C., Almeida, P. M., Sannino, C., Settani, L., Sampaio, J. P. & Moschetti, G. (2013). *Wickerhamomyces sylviae* f. a., sp. nov., an ascomycetous yeast species isolated from migratory birds. *Int J Syst Evol Microbiol* **63**, 4824–4830.

Groenewald, M., Robert, V. & Smith, M. T. (2011). Five novel *Wickerhamomyces* and *Metschnikowia* related yeast species, *Wickerhamomyces chaumierensis* sp. nov., *Candida pseudofloscullorum* sp. nov., *Candida danieliae* sp. nov., and *Candida eppingiae* sp. nov., isolated from plants. *Int J Syst Evol Microbiol* **61**, 2015–2022.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

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Hui, F.-L., Chen, L., Chu, X.-Y., Niu, Q.-H. & Ke, T. (2013). *Wickerhamomyces mori* sp. nov., an anamorphic yeast species found in the guts of wood-boring insect larvae. *Int J Syst Evol Microbiol* **63**, 1174–1178.

Hölldobler, B. & Wilson, E. O. (1990). *The Ants*. Cambridge, MA: Harvard University Press.

James, S. A., Barriga, E. J., Barahona, P. P., Harrington, T. C., Lee, C. F., Bond, C. J. & Roberts, I. N. (2014). *Wickerhamomyces arborarius* f.a sp. nov., an ascomycetous yeast species found in arboreal habitats on three different continents. *Int J Syst Evol Microbiol* **64**, 1057–1061.

Kaewwichian, R., Kawasaki, H. & Limtong, S. (2013). *Wickerhamomyces siamensis* sp. nov., a novel yeast species isolated from the phylloplane in Thailand. *Int J Syst Evol Microbiol* **63**, 1568–1573.

Katoh, K. & Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* **9**, 286–298.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.

Kurtzman, C. P., Robnett, C. J. & Basehoar-Powers, E. (2008). Phylogenetic relationships among species of *Pichia*, *Issatchenkia* and *Williopsis* determined from multigene phylogenetic analysis, and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov and *Wickerhamomyces* gen. nov. *FEMS Yeast Res* **8**, 939–954.

Kurtzman, C. P. (2011a). *Wickerhamomyces* Kurtzman, Robnett & Basehoar-Powers (2008). In *The Yeasts, a Taxonomic Study*, pp. 87-110. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Oxford, UK: Elsevier

Kurtzman, C. P., Fell, J. W., Boekhout, T. & Robert, V. (2011b). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The Yeasts, a Taxonomic Study*, pp. 87-110. Edited by C. P Kurtzman, J. W Fell & T. Boekhout. Oxford, UK: Elsevier.

Limtong, S., Yongmanitchai, W., Kawasaki, H. & Fujiyama, K. (2009). *Wickerhamomyces edaphicus* sp. nov. and *Pichia jaroonii* sp. nov.: two ascomycetous yeast species isolated from forest soil in Thailand. *FEMS Yeast Res* **9**, 504–510.

Limtong, S., Nitiyon, S., Kaewwichian, R., Jindamorakot, S., Am-In, S. & Yongmanitchai, W. (2012). *Wickerhamomyces xylosica* sp. nov. and *Candida phayaonensis* sp. nov.: two xylose-assimilating yeast species from soil. *Int J Syst Evol Microbiol* **62**, 2786–2792.

Melo, W. G. P., Arcuri, S. L., Rodrigues, A., Morais, P. B., Meirelles, L. A. & Pagnocca, F. C. (2014). *Starmerella aceti* f.a., sp. nov., an ascomycetous yeast species isolated from fungus garden of leafcutter ant *Acromyrmex balzani*. *Int J Syst Evol Microbiol* **64**, 1428–1433.

Middelhoven, W. J., Fonseca, A., Carreiro, S. C., Pagnocca, F. C. & Bueno, O. C. (2003). *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. *Antonie van Leeuwenhoek* **83**, 167–174.

Minter, D. W. (2009). *Cyberlindnera*, a replacement name for *Lindnera* Kurtzman, et al., nom. Illegit. *Mycotaxon* **11**, 473–476.

Möller, A. (1893). Die Pilzgärten einiger Südamerikanischer Ameisen. *Bot Mitt Trop* **6**, 1–127.

Nakase, T., Jindamorakot, S., Am-In, S., Ninomiya, S. & Kawasaki, H. (2012). *Wickerhamomyces tratensis* sp. nov. and *Candida namnaoensis* sp. nov., two novel ascomycetous yeast species in the *Wickerhamomyces* clade found in Thailand. *J Gen Appl Microbiol* **58**, 145–152.

O'Donnell, K. (1993). *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*. pp. 225–233. Edited by D. R. Reynolds & J. W. Taylor. Wallingford, UK: CAB International.

Pagnocca, F. C., Legaspe, M. F., Rodrigues, A., Ruivo, C. C., Nagamoto, N. S., Bacci, M. & Forti, L. C. (2010). Yeasts isolated from a fungus-growing ant nest, including the description of *Trichosporon chiarellii* sp. nov., an anamorphic basidiomycetous yeast. *Int J Syst Evol Microbiol* **60**, 1454–1459.

Rosa, C. A., Morais, P. B., Lachance, M. A., Santos, R. O., Melo, W. G. P., Viana, R. H. O., Braganc, M. A. L. & Pimenta, R. S. (2009). *Wickerhamomyces queroliae* sp. nov. and *Candida jalapaonensis* sp. nov., two yeast species isolated from Cerrado ecosystem in North Brazil. *Int J Syst Evol Microbiol* **59**, 1232–1236.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sampaio, J. P., Gadanho, M., Santos, S., Duarte, F. L., Pais, C., Fonseca, A. & Fell, J. W. (2001). Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *Rhodosporidium kratochvilovae* and related anamorphic species. *Int J Syst Evol Microbiol* **51**, 687–697.

Shin, K. S., Bae, K. S., Lee, K. H., Park, D. S., Kwon, G. S. & Lee, J. B. (2011). *Wickerhamomyces ochangensis* sp. nov., an ascomycetous yeast isolated from the soil of a potato field. *Int J Syst Evol Microbiol* **61**, 2543–2546.

Silva, C. F., Schwan, R. F., Sousa Dias, Ë. & Wheals, A. E. (2000). Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. *Int J Food Microbiol* **60**, 251–260.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Weber, N. A. (1972). *Gardening Ants: The Attines*. Philadelphia, PA: American Philosophical Society.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322. Edited by M. A Innis, D. H. Gelfand, J. J. Sninsky & T. J. White. New York: Academic Press.

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