Wickerhamomyces sylviae f.a., sp. nov., an ascomycetous yeast species isolated from migratory birds

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In the present work, we investigated the phylogenetic position and phenotypic characteristics of eight yeast isolates collected from migratory birds on the island of Ustica, Italy. A phylogenetic analysis based on the D1/D2 region of the large-subunit rRNA gene showed that all isolates clustered as a single separate lineage within the *Wickerhamomyces* clade. They exhibited distinct morphological and physiological characteristics and were clearly separated from their closest relatives, *Wickerhamomyces lynferdii*, *Wickerhamomyces anomalus* and *Wickerhamomyces subpelliculosus*, in BLASTN searches. On the basis of the isolation source, physiological features and molecular strain typing carried out with randomly amplified polymorphic DNA (RAPD)-PCR and minisatellite-primed (MSP)-PCR analysis, the isolates were identified as strains of the same species. The name *Wickerhamomyces sylviae* f.a., sp. nov. is proposed to accommodate these novel strains; the type strain is U88A2^T (=PYCC 6345^T=CBS 12888^T). The MycoBank number is MB 804762.

The number of species of the genus *Wickerhamomyces* is increasing rapidly. The genus was proposed by Kurtzman *et al.* (2008) and is part of a large clade that includes the genera *Barnettozyma*, *Lindnera* and *Starmera*, together with the 17 species transferred from the genera *Pichia*, *Williopsis* and *Hansenula* (Kurtzman *et al.*, 2008; Kurtzman, 2011a). *Candida solani* (Kurtzman, 2011b) has been also included in the genus *Wickerhamomyces*. The species most recently described in the genus *Wickerhamomyces* are *Wickerhamomyces queroliae* (Rosa *et al.*, 2009), *W. edaphicus* (Limtong *et al.*, 2009), *W. patagonicus* (de García *et al.*, 2010), *W. chaumierensis* (Groenewald *et al.*, 2011), *W. ochangensis* (Shin *et al.*, 2011), *W. tratensis* (Nakase *et al.*, 2012), *W. xylosica* (Limtong *et al.*, 2012), *W. mori* (Hui *et al.*, 2013) and *W. scolytoplatypi* (Ninomiya *et al.*, 2013).

The diversity of ascomycetous yeasts associated with migratory birds has not been fully explored. Recently, cultivable yeasts have been found in the cloacae of migratory birds (Cafarchia *et al.*, 2006a; Francesca *et al.*, 2012), vineyard-inhabiting birds (Francesca *et al.*, 2010) and birds of prey (Cafarchia *et al.*, 2006b), suggesting that birds are long-distance vectors of living yeasts across the Mediterranean area (Francesca *et al.*, 2012).

The small islands that surround Sicily represent important resting sites (stop-over points) where birds find food and regain fat lost during their flights to and from North Africa and Europe (Goymann *et al.*, 2010). These resting sites are strategic for analysis of the microbial communities transported by migratory birds (Francesca *et al.*, 2012; Alfonzo *et al.*, 2013). During a survey of the yeasts transported by migratory birds, eight yeasts collected from the cloacae of five birds caught on the island of Ustica, an important stop-over point, were found to be closely related to species belonging to the genus *Wickerhamomyces*, but could not be identified as any of the presently described species. This study presents phenotypic and genotypic evidence to support the description of these isolates as members of a novel species of the genus *Wickerhamomyces*, for which the name *Wickerhamomyces sylviae* f.a., sp. nov. is proposed.

Birds were caught during the 2012 autumnal migration on the island of Ustica (38° 42′ 31.66″ N 13° 10′ 46.62″ E)

Abbreviations: f.a., *forma asexualis* (asexual form); MSP, minisatellite-primed; RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain sequences of the 26S rRNA genes of strains U13B1, U78B1, U78B2, U80B1, U80B2, U88A1, U88A2^T and U92A2 are KF240722–KF240729, respectively.

following the procedure reported by Francesca *et al.* (2012). All birds were treated following the official regulations of the Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA, Italy) to avoid injuries or stress.

Cloacae were plugged with sterile cotton swabs and streaked onto malt extract agar (Oxoid) supplemented with chloramphenicol (0.5 g l^{-1}) and biphenyl (0.1 g l^{-1}) to prevent growth of bacteria and moulds, respectively. Petri dishes were kept at room temperature (22–25 °C) during transport and incubated for 48–72 h at 28±2 °C once they had arrived in the laboratory. After growth, several colonies were picked from agar plates and purified to homogeneity after several subculturing steps on malt extract agar and resuspended in malt extract broth.

DNA was extracted by cell lysis using the InstaGene Matrix kit (Bio-Rad Laboratories) according to the manufacturer's instructions. The D1/D2 region of the 26S rRNA gene was sequenced following the protocol of Settanni *et al.* (2012). Chromatograms obtained from sequencing were inspected and converted by using BioEdit version 7.0.9. Sequences were aligned using CLUSTAL W and curated manually in BioEdit version 7.0.9. Alignments were analysed using Best model test by MEGA version 5.10 (Tamura *et al.*, 2011) to choose the most realistic evolutionary model and optimal values of parameters used for the phylogenetic analysis. For phylogenetic reconstruction, the neighbour-joining algorithm (Saitou & Nei, 1987) and Kimura's two-parameter model (Kimura, 1980) were used with complete deletion of positions containing gaps or missing data and 1000 bootstrap replications (Felsenstein, 1985). Phylogenetic analyses were carried out in MEGA version 5.10.

Strain differentiation was first approached phenotypically. Colony and cell morphology were examined after growth for 3 days at 25 °C on malt extract (5 %, w/v) agar. Pseudohyphae formation was observed after 7 days at 25 °C on Dalmau plates on cornmeal agar (Yarrow, 1998). Formation of ascospores was tested on 5 % malt extract agar, V8 juice agar, V8 juice agar diluted 1 : 9, V8 juice agar diluted 1 : 19, glucose yeast extract, cornmeal agar, acetate agar and yeast carbon base supplemented with 0.01 % ammonium sulphate (YCBAS) (Yarrow, 1998). All media were incubated at 25 and 15 °C with the exception of glucose yeast extract agar, which was incubated at 25 and 18 °C (de García *et al.*, 2010). The cultures were inspected at intervals of 3 and 7 days for 2 months. All isolates were tested singly, in pairs and by mixing all eight strains together (Kurtzman *et al.*, 2011). Fermentation tests were carried out in liquid media (Yarrow, 1998); assimilation of carbon and nitrogen compounds was determined in microplates (Robert *et al.*, 1997; Robert, 2003; Kurtzman *et al.*, 2011). Additional tests such as starch formation, gelatin liquefaction, growth in the presence of 10 % NaCl and 5 % glucose, 2-keto-D-gluconate and at 37 °C were also conducted (Kurtzman *et al.*, 2011). Furthermore, all isolates were also tested for their ability to grow in glucose-nitrogen base broth at 40 and 42 °C after 3 days.

Genetic fingerprinting was performed by randomly amplified polymorphic DNA (RAPD)-PCR analysis using primer M13 (Stenlid *et al.*, 1994) according to Valmorri *et al.* (2010) and by the minisatellite-primed (MSP)-PCR technique using primer $(GTG)_5$ as reported by Sampaio *et al.* (2001). Strains analysed in the present study are listed in Table 1.

All eight sequences of the D1/D2 domain of the 26S rRNA gene were identical and were clearly related to the genus *Wickerhamomyces* described by Kurtzman *et al.* (2008). Pairwise sequence alignment of D1/D2 sequences in the GenBank (http://www.ncbi.nlm.nih.gov) and MycoBank (http://www.mycobank.org/) databases revealed that the most closely related species were *Wickerhamomyces lynferdii*, *Wickerhamomyces subpelliculosus* and *Wickerhamomyces anomalus*, from which our sequences differed in 16 (2.95 %), 17 (3.13 %) and 18 (3.32 %) positions. According to Kurtzman & Robnett (1998) and Sugita *et al.* (1999), these results suggested that our isolates were different from other species of ascomycetous yeasts. In order to determine the phylogenetic position of our isolates, a neighbour-joining phylogenetic tree was reconstructed (Fig. 1) using the D1/D2 sequences of the eight isolates and their closest relatives from the genus *Wickerhamomyces*. Interestingly, the phylogenetic analysis placed all eight isolates into a single cluster separated from the other species of the genus *Wickerhamomyces*.

The eight isolates were then subjected to phenotypic tests commonly applied for yeast characterization. They formed

Table 1. Strains of *W. sylviae* sp. nov. and characteristics of birds from which they were isolated

Strain	Bird species	Bird sample
U13B1	*Sylvia atricapilla*, L. 1758 (blackcap)	4G3301
U78B1	*Sylvia atricapilla*, L. 1758 (blackcap)	LS02312
U78B2	*Sylvia atricapilla*, L. 1758 (blackcap)	LS02312
U80B1	*Erithacus rubecula*, L. 1758 (robin)	8A04063
U80B2	*Erithacus rubecula*, L. 1758 (robin)	8A04063
U88A1	*Sylvia communis*, Latham 1787 (whitethroat)	4G3305
U88A2^T	*Sylvia communis*, Latham 1787 (whitethroat)	4G3305
U92A1	*Erithacus rubecula*, L. 1758 (robin)	8A04070

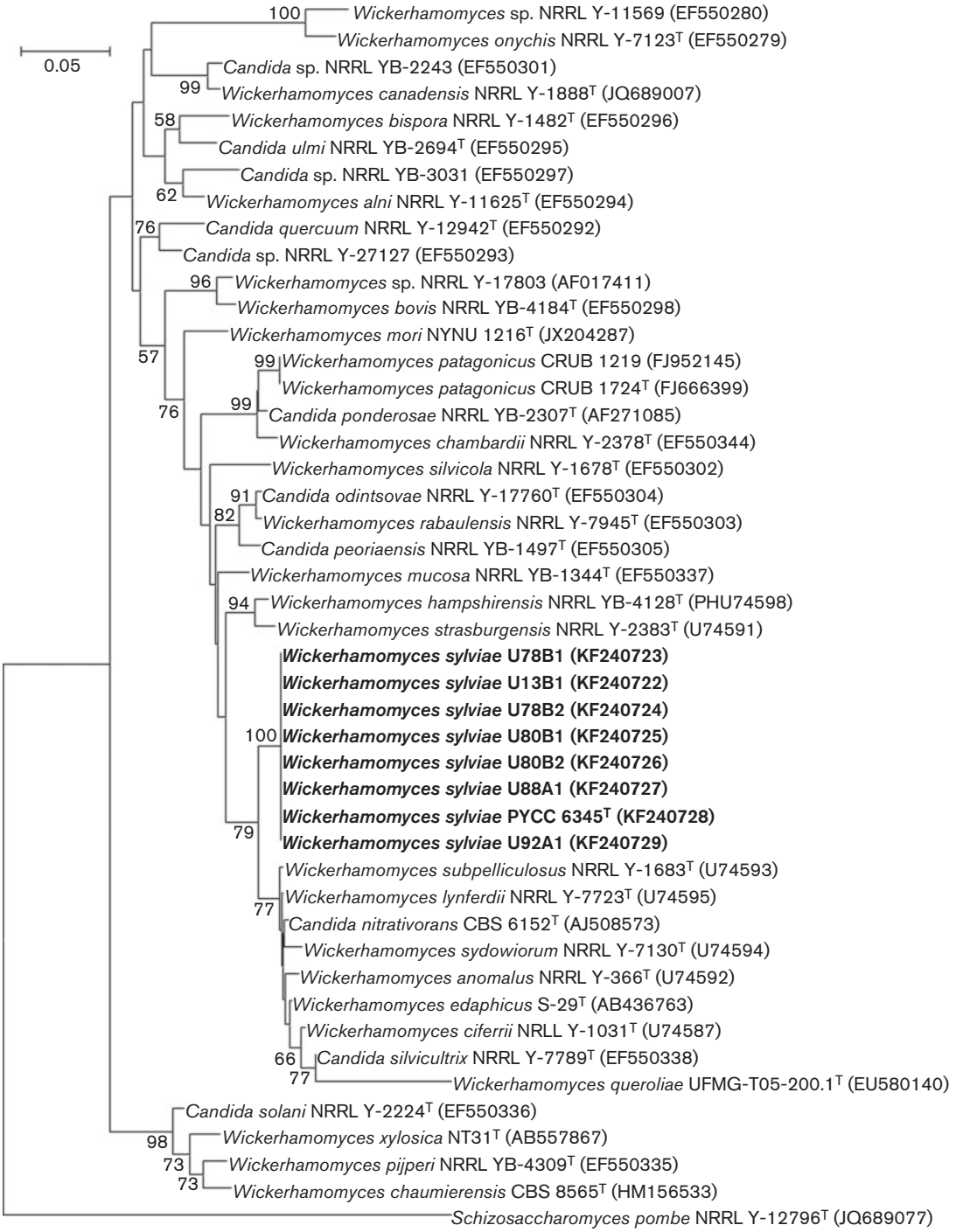

Fig. 1. Neighbour-joining phylogenetic tree based on sequences of the D1/D2 domains of the 26S rRNA genes of the eight strains of *W. sylviae* sp. nov. and their closest relatives. Bootstrap values (1000 replicates) >50 % are shown on branches. Bar, 0.05 changes per nucleotide position.

spherical-elongate cells with multilateral budding, fermented glucose, were unable to utilize methanol and hexadecane and had a negative Diazonium blue B reaction (Kurtzman, 2011a). Although asci or signs of conjugation were not observed after testing all strains singly, in pairs and in mass matings, the species under study was assigned to the genus *Wickerhamomyces* following the Melbourne Code (McNeill *et al.*, 2011) and similar cases concerning yeast taxa (Badotti *et al.*, 2013). Consistent with this, the term 'f.a.' (*forma asexualis*) has been added to name of the

Table 2. Salient physiological differences among the eight strains of *W. sylviae* sp. nov. and closely related species

Species/strains: 1, U13B1; 2, U78B1; 3, U78B2; 4, U80B1; 5, U80B2; 6, U88A1; 7, U88A2^T; 8, U92A1; 9, *W. anomalus*; 10, *W. linferdii*; 11, *W. subpelliculosus*. +, Positive; −, negative; d, delayed; w, weak; v, variable; ND, no data available. Data for reference species were taken from Kurtzman *et al.* (2011).

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Assimilation of:											
D-Galactose	−	−	w	−	+	w	+	+	v	+	+
D-Ribose	d	d	d	−	−	−	−	−	v	v	v
L-Rhamnose	−	−	−	+	−	−	−	−	−	−	−
Sucrose	−	−	−	w	−	−	−	−	+	+	+
α,α-Trehalose	d	d	d	+	w	d	d	d	+	+	+
Methyl α-D-glucoside	−	−	−	−	−	−	d	−	+	+	+
Cellobiose	−	−	d	−	−	−	−	−	+	+	v
Salicin	w	+	−	+	−	w	+	w	+	+	v
Lactose	−	−	−	−	−	−	−	−	−	+	+
Raffinose	−	−	−	−	−	−	−	−	+	v	v
Melezitose	−	d	d	+	d	w	w	d	+	+	v
Inulin	−	−	−	−	−	−	−	−	−	+	+
Soluble starch	w	+	w	+	w	+	+	w	+	−	v
Glycerol	−	−	−	d	−	−	−	d	+	−	v
Erythritol	+	+	d	+	d	−	w	d	+	+	+
Ribitol	d	−	d	−	−	−	−	−	v	+	+
Xylitol	d	d	d	−	d	−	−	−	v	v	v
D-Glucitol	−	−	−	−	−	−	−	−	+	+	+
D-Mannitol	−	−	−	−	−	−	−	−	+	+	+
D-Gluconate	d	+	−	+	−	d	+	d	v	+	+
Citrate	−	−	−	−	−	−	−	−	+	+	+
Ethanol	w	w	−	w	−	−	−	−	+	+	+
Salicylic acid	−	−	−	−	−	−	−	−	+	+	+
Nitrate	w	w	−	+	+	+	w	−	+	+	+
Growth on/at:											
Vitamin-free medium	+	+	+	+	+	+	+	+	+	+	−
37 °C	+	+	+	+	+	+	+	+	v	−	v
40 °C	+	+	+	+	+	+	w	w	ND	ND	ND
42 °C	+	−	w	w	−	−	w	−	ND	ND	ND

present novel species in order to indicate the description of its asexual form.

Except for isolate U88A1, all isolates showed formation of pseudohyphae, but not true hyphae, on Dalmau plates after 15 days at 25 °C. The physiological characteristics of the eight isolates are reported in Table 2. Notable differences were registered in terms of assimilation of D-galactose, salicin, melezitose, soluble starch, erythritol, D-gluconate, ethanol and nitrate. The eight isolates differed from their closest relatives mainly in terms of fermentation of sucrose, methyl α-D-glucoside, cellobiose, xylitol, D-glucitol, D-mannitol, citrate and salicylic acid, which gave negative results for the majority of our isolates. In contrast to the common behaviour of their closest relatives, all eight isolates of the novel species showed notable growth in glucose-nitrogen base broth after 3 days at 37 °C. As reported by Gwinner (1990), birds are characterized by high body temperature (42 ± 1 °C) and, for this reason, we decided to assess the ability of our isolates to grow at 40 and 42 °C. Isolates U13B1, U78B2, U80B1 and U88A2 were able to grow at 40 °C and could even grow at 42 °C. Thus, the capacity of our isolates to grow at temperatures higher than 37 °C may indicate that the novel species is adapted to bird body temperature. Furthermore, the eight yeasts were collected from five animals belonging to three different bird species, which could support the hypothesis of a specific association between the novel species and migratory birds.

Additionally, several authors have reported the isolation of other species of the genus *Wickerhamomyces* from animals such as the gut of insects (Rosa *et al.*, 2009; Hui *et al.*, 2013) or reported strains of *W. anomalus* as potential endosymbionts of mosquitos (Ricci *et al.*, 2011). Although the body temperature of these insects is lower than that of birds, the stressing conditions that characterize the insect gut as well as the bird cloaca could result in the selection of yeast species and, consequently, in the adaptation of yeasts to their hosts.

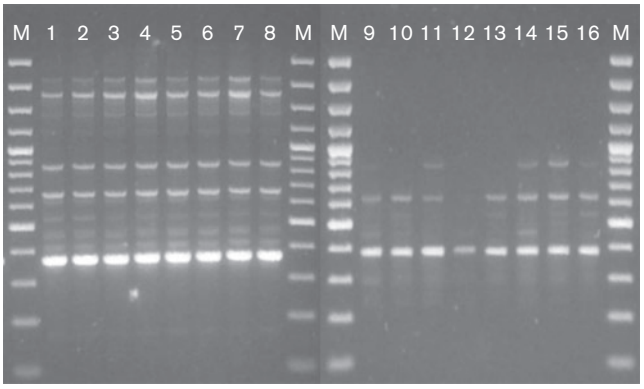

Fig. 2. PCR fingerprints of the eight strains of *W. sylviae* sp. nov. Lanes: 1–8, RAPD-PCR profiles generated using primer M13 from strains U13B1 (1), U78B1 (2), U78B2 (3), U80B1 (4), U80B2 (5), U88A1 (6), U88A2^T (7) and U92A1 (8); 9–16, MSP-PCR profiles generated with primer $(GTG)_5$ from strains U13B1 (9), U78B1 (10), U78B2 (11), U80B1 (12), U80B2 (13), U88A1 (14), U88A2^T (15) and U92A1 (16); M, molecular marker GeneRuler 100 bp DNA ladder (Fermentas).

The eight isolates were characterized genetically by fingerprinting PCR analysis. MSP-PCR determined their separation into three clusters, while RAPD-PCR showed no differences among the eight isolates (Fig. 2). However, in addition to the MSP-PCR profiles, the different biochemical features and the different birds (individuals and species) used as sources of isolation excluded clonal relatedness among isolates U13B1, U78B1, U78B2, U80B1, U80B2, U88A1, U88A2^T and U92A2, which represent eight different strains of the same species.

On the basis of molecular and phenotypic characteristics and according to the criteria suggested by Kurtzman (2011b), we conclude that all eight strains characterized in this study represent a novel ascomycete yeast species, for which we propose the name *Wickerhamomyces sylviae* sp. nov., with the type strain U88A2^T (=PYCC 6345^T=CBS 12888^T).

Description of *Wickerhamomyces sylviae* Francesca, Moschetti & Sampaio f.a., sp. nov.

Wickerhamomyces sylviae [syl′vi.ae. N.L. gen. n. *sylviae* of the bird *Sylvia communis* Latham 1787 (whitethroat), from which the type strain was isolated].

After 3 days at 25 °C on 5 % malt extract agar, colonies are white, smooth, faintly glistening and butyrous; the margin of colonies is entire and, after 10 days, pseudohyphae are formed on the margin. Cells are ovoid to elongate (2–3 × 2–4 μm) and multiply by multilateral budding (Fig. 3a). On Dalmau plates after 15 days at 25 °C, growth under the coverslip shows pseudohyphae but not true hyphae (Fig. 3b). No asci or signs of conjugation are detected after testing strains singly, in pairs as well as in mass crossings.

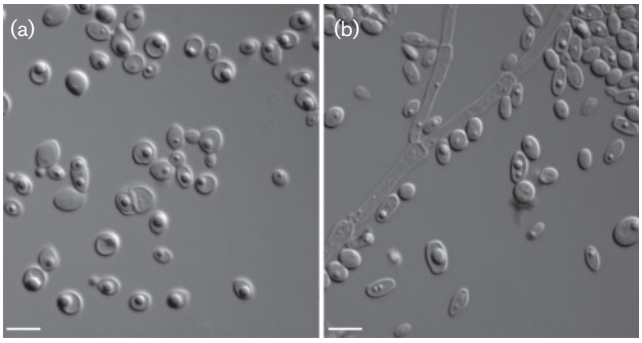

Fig. 3. Micrographs of growth of strain U88A2^T. (a) Vegetative cells after 3 days at 25 °C on 5 % malt extract agar; (b) pseudohyphae after 15 days at 25 °C on Dalmau plates. Bars, 10 μm.

D-Glucose, D-galactose, maltose and α,α-trehalose are fermented but not sucrose, lactose or raffinose. D-Glucose, α,α-trehalose (positive), soluble starch (positive and occasionally weak), L-arabinose, D-arabinose, DL-lactate and succinate (occasionally positive or weak) are assimilated as carbon sources. Assimilation of D-galactose, salicin, maltose, melezitose, erythritol and D-gluconate is variable. Assimilation of ethanol and sucrose is negative and occasionally weak. Assimilation of L-rhamnose is negative and occasionally positive. Assimilation of D-ribose, methyl α-D-glucoside, cellobiose, glycerol, ribitol, D-glucono-1,5-lactone and 5-keto-D-gluconate is negative and occasionally delayed; assimilation of D-xylose and xylitol is delayed and occasionally negative. L-Sorbose, melibiose, lactose, raffinose, inulin, D-glucitol, D-mannitol, galactitol, *myo*-inositol, 2-keto-D-gluconate, citrate, methanol, salicylic acid, hexadecane and saccharate are not assimilated as carbon sources. Nitrate (variable), D-glucosamine (negative and occasionally delayed) and *N*-acetyl-D-glucosamine (negative and occasionally weak) are assimilated as nitrogen sources. Grows in the presence of 10 % NaCl/5 % glucose and on vitamin-free medium as well as at 37 and 40 °C. Growth at 42 °C is variable. Starch formation, gelatin liquefaction and the Diazonium blue B reaction are negative.

The type strain U88A2^T (=PYCC 6345^T=CBS 12888^T) was isolated in 2012 by C. Sannino from a trans-Saharan migratory bird [*Sylvia communis*, Latham 1787 (whitethroat)] on the island of Ustica (38° 42′ 31.66″ N 13° 10′ 46.62″ E) (Sicily, Italy). The Mycobank accession number is MB 804762.

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References

Alfonzo, A., Francesca, N., Sannino, C., Settanni, L. & Moschetti, G. (2013). Filamentous fungi transported by birds during migration across the Mediterranean sea. *Curr Microbiol* **66**, 236–242.

Badotti, F., Silva, P. A., Mendonça, M. C., Gomes, F. C. O., Morais, P. B., Lachance, M.-A. & Rosa, C. A. (2013). *Wickerhamiella dulcicola* sp. nov. and *Wickerhamiella cachassae* sp. nov., yeasts isolated from cachaça fermentation in Brazil. *Int J Syst Evol Microbiol* **63**, 1169–1173.

Cafarchia, C., Camarda, A., Romito, D., Campolo, M., Quaglia, N. C., Tullio, D. & Otranto, D. (2006a). Occurrence of yeasts in cloacae of migratory birds. *Mycopathologia* **161**, 229–234.

Cafarchia, C., Romito, D., Iatta, R., Camarda, A., Montagna, M. T. & Otranto, D. (2006b). Role of birds of prey as carriers and spreaders of *Cryptococcus neoformans* and other zoonotic yeasts. *Med Mycol* **44**, 485–492.

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.

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

Francesca, N., Chiurazzi, M., Romano, R., Aponte, M., Settanni, L. & Moschetti, G. (2010). Indigenous yeast communities in the environment of ''Rovello Bianco'' grape variety and their use in commercial white wine fermentation. *World J Microbiol Biotechnol* **26**, 337–351.

Francesca, N., Canale, D. E., Settanni, L. & Moschetti, G. (2012). Dissemination of wine-related yeasts by migratory birds. *Environ Microbiol Rep* **4**, 105–112.

Goymann, W., Spina, F., Ferri, A. & Fusani, L. (2010). Body fat influences departure from stopover sites in migratory birds: evidence from whole-island telemetry. *Biol Lett* **6**, 478–481.

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

Gwinner, E. (editor) (1990). *Bird Migration: Physiology and Ecophysiology*. Berlin: Springer.

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.

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. (2011a). Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. *Antonie van Leeuwenhoek* **99**, 13–23.

Kurtzman, C. P. (2011b). *Wickerhamomyces* Kurtzman, Robnett & Basehoar-Powers (2008). In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 899–917. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

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 sequence analysis, and the proposal of *Barnettozyma* gen. nov., *Lindnera* gen. nov. and *Wickerhamomyces* gen. nov. *FEMS Yeast Res* **8**, 939–954.

Kurtzman, C. P., Fell, J. W., Boekhout, T. & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 87–110. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: 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.

McNeill, J., Barrie, F. R., Buck, W. R., Demoulin, V., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Marhold, K. & other authors (2011). *International Code of Nomenclature for Algae, Fungi, and Plants (Melbourne Code)*. Königstein: Koeltz Scientific.

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.

Ninomiya, S., Mikata, K., Kajimura, H. & Kawasaki, H. (2013). Two novel ascomycetous yeast species, *Wickerhamomyces scolytoplatypi* sp. nov. and *Cyberlindnera xylebori* sp. nov., isolated from ambrosia beetle galleries. *Int J Syst Evol Microbiol* **63**, 2706–2711.

Ricci, I., Mosca, M., Valzano, M., Damiani, C., Scuppa, P., Rossi, P., Crotti, E., Cappelli, A., Ulissi, U. & other authors (2011). Different mosquito species host *Wickerhamomyces anomalus* (*Pichia anomala*): perspectives on vector-borne diseases symbiotic control. *Antonie van Leeuwenhoek* **99**, 43–50.

Robert, V. (2003). Data processing. In *Yeasts in Food: Beneficial and Detrimental Aspects*, pp. 139–169. Edited by T. Boekhout & V. Robert. Hamburg: Behrs Verlag.

Robert, V., Evrard, P. & Hennebert, G. L. (1997). BCCM/Allev 2.00, an automated system for the identification of yeasts. *Mycotaxon* **64**, 455–463.

Rosa, C. A., Morais, P. B., Lachance, M. A., Santos, R. O., Melo, W. G. P., Viana, R. H. O., Bragança, M. A. & 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., 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.

Settanni, L., Sannino, C., Francesca, N., Guarcello, R. & Moschetti, G. (2012). Yeast ecology of vineyards within Marsala wine area (western Sicily) in two consecutive vintages and selection of autochthonous *Saccharomyces cerevisiae* strains. *J Biosci Bioeng* **114**, 606–614.

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.

Stenlid, J., Karlsson, J. O. & Hogberg, N. (1994). Intraspecific genetic variation in *Heterobasidium annosum* revealed by amplification of minisatellite DNA. *Mycol Res* **98**, 57–63.

Sugita, T., Nishikawa, A., Ikeda, R. & Shinoda, T. (1999). Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. *J Clin Microbiol* **37**, 1985–1993.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Valmorri, S., Tofalo, R., Settanni, L., Corsetti, A. & Suzzi, G. (2010). Yeast microbiota associated with spontaneous sourdough fermentations in the production of traditional wheat sourdough breads of the Abruzzo region (Italy). *Antonie van Leeuwenhoek* **97**, 119–129.

Yarrow, D. (1998). Methods for the isolation and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.