Jaminaea phylloscopi sp. nov. (Microstromatales), a basidiomycetous yeast isolated from migratory birds in the Mediterranean basin

Nicola Francesca,[1,2] Marco Alexandre Guerreiro,[2,3] Cláudia Carvalho,[2] Marco Coelho,[2] Antonio Alfonzo,[1] Walter Randazzo,[1] José Paulo Sampaio[2] and Giancarlo Moschetti[1]

Correspondence
Nicola Francesca
nicola.francesca@unipa.it

[1]Dipartimento Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy

[2]UCIBIO-REQUIMTE, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

[3]Geobotany, Faculty for Biology and Biotechnology, Ruhr-Universität Bochum, Bochum, Germany

During a survey of yeasts vectored by migratory birds in the Mediterranean basin, isolations from the cloacae of members of the order Passeriformes collected in Ustica (Italy) were performed. Based on phylogenetic analysis of the D1/D2 domain of the 26S rRNA gene and the internal transcribed spacer ITS1-5.8S rRNA gene-ITS2 region, five yeast isolates clustered in a new lineage within the Microstromatales clade. The DNA sequences of these isolates differed from those of their closest relatives, *Jaminaea angkorensis* and *Jaminaea lanaiensis*, by 20 and 25 nt substitutions in the D1/D2 domain and 119 and 131 nt substitutions in the complete ITS region, respectively. In addition, the five isolates showed phenotypic characteristics not observed in their closest relatives, such as the ability to grow at 44 °C and at pH 2.5, which suggests a possible adaptation to the bird gastrointestinal tract. On the basis of the isolation source, phenotypic features and molecular strain typing carried out with randomly amplified polymorphic DNA (RAPD)-PCR and mini-satellite-primed (MSP)-PCR analysis, the five isolates were characterized as five distinct strains of a novel species formally described as *Jaminaea phylloscopi* sp. nov., with 551B6^T (=PYCC 6783^T=CBS 14087^T) as the type strain. The Mycobank accession number is MB811984.

The Microstromatales represent an order that contains several teleomorphic and anamorphic genera. Among them, the genera *Quambalaria* and *Sympodiomycopsis* include the largest number of species, with five and three taxa, respectively, whereas the genus *Jaminaea*, the latest to be described (Sipiczki & Kajdacsi, 2009), has two recognized species at the time of writing. The genus *Quambalaria* was erected by Simpson (2000) to accommodate three species, *Q. pitereka*, *Q. eucalypti* and *Q. pusilla*, commonly recognized as eucalypt pathogens. Recently, *Q. cyanescens* (de Beer *et al.*, 2006) and *Q. coyrecup* (Paap *et al.*, 2008) have been also been described as plant pathogens. The genus *Sympodiomycopsis* was proposed by Sugiyama *et al.* (1991) and, at the time of writing, includes three described species: *S. paphiopedili* (Sugiyama *et al.*, 1991), *S. kandeliae* (Wei *et al.*, 2011) and *S. yantaiensis* (Chen *et al.*, 2013). The genus *Jaminaea* comprises *J. angkorensis* (Sipiczki & Kajdacsi, 2009) and *J. lanaiensis* (Wei *et al.*, 2011). This last species was originally described as *Sympodiomycopsis lanaiensis* by Mahdi *et al.* (2008).

Recent studies have been focused on the diversity and ecology of ascomycetous yeasts carried by migratory birds (Cafarchia *et al.*, 2006a; Cafarchia *et al.*, 2006b; Francesca *et al.*, 2010, 2012). More recently, our group described a new ascomycetous species, *Wickerhamomyces silviae* (Francesca *et al.*, 2013), isolated from birds during the 2012

Abbreviations: MSP, mini-satellite-primed; RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain sequences of the 26S rRNA gene of strains 551B6^T, 551B4-B, 551B4, 551B5 and 551B6-B are KF916680, KF916677, KF916678, KF916679 and KF916681, respectively; those for the ITS1-5.8S-ITS2 region of the same strains are KF916685, KF916682, KF916683, KF916684 and KF916686, respectively. The Mycobank accession number is MB811984.

Three supplementary figures are available with the online Supplementary Material.

autumnal migration, in Ustica island (Italy). However, very few studies have reported on basidiomycetous yeasts isolated from migratory birds.

During an investigation of fungal diversity associated with cloacae of migratory birds in Ustica, several species of filamentous fungi were isolated and characterized (Alfonzo *et al.*, 2013). Among them, five phenotypically similar isolates were preliminarily classified as basidiomycetous yeasts and stored for further investigation. In the present study, we show that these isolates represent five distinct strains of a novel species of the order Microstromatales.

Birds were sampled during the migration from sub-Saharan areas to Europe in Ustica (38° 42′ N 13° 10′ 48″ E; sample date: 2–4 May 2009) (Alfonzo *et al.*, 2013), an island north of Sicily (Italy) that represents one of the most important stop-over sites in the Mediterranean basin (Francesca *et al.*, 2012). Animals were captured, ringed and identified following the procedures reported by Mullarney *et al.* (1999) and Kaiser (1993). Cloacae of the birds were analysed for the presence of fungi as reported by Alfonzo *et al.* (2013). Yeast cultures were purified on malt extract agar.

The yeast isolates were identified by genotypic analysis. DNA was extracted by cell lysis using the InstaGene Matrix kit (Bio-Rad Laboratories) according to the manufacturer's instructions and the ITS1-5.8S-ITS2 region and the D1/D2 domain of the 26S rRNA gene were sequenced (Settanni *et al.*, 2012). The identities of the generated sequences were determined by BLASTN search (www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed with CLUSTAL W (BioEdit V7.0.9) (Thompson *et al.*, 1997).

The phylogenetic trees were obtained using three different methods: (i) Bayesian inference employed MrBayes 3.1.2 software (Ronquist & Huelsenbeck, 2003) and was carried out using 1 000 000 generations with four independent chains and the GTR model. Substitution-rate variation among sites was modelled by a discrete approximation of the gamma-distribution with a proportion of invariable sites (I1G). The resultant trees were sampled every 100 generations with trees sampled during the first 2000 generations discarded as burn-in (the burn-in period was estimated by plotting the likelihood of the sampled trees). Relationships among the remaining trees were summarized using a majority-rule consensus method with clade probabilities determined using MrBayes 3.1.2. Phylogenetic trees were saved and edited for publication in Adobe Photoshop CS6 (Adobe Systems); (ii) maximum-likelihood statistical methods (Saitou & Nei, 1987) with 1000 bootstrap iterations and (iii) neighbour-joining with 1000 bootstrap replications (Felsenstein, 1985) were both carried out using MEGA software v5.10 (Tamura *et al.*, 2011). Model parameters were calculated in Modeltest (Tamura *et al.*, 2011).

The discrimination at strain level of the isolates was carried out through two different PCR fingerprinting assays with primers $(GTG)_5$ (Sampaio *et al.*, 2001) and M13 (Stenlid *et al.*, 1994; Valmorri *et al.*, 2010). All patterns were analysed using the Gelcompare II software version 6.5 (Applied-Maths, Sin Marten Latem, Belgium).

All five isolates were phenotypically investigated. Colony and cell morphology, hyphae and pseudohyphae were examined as reported by Yarrow (1998) and Kurtzman *et al.* (2011). The fermentation of carbohydrates and the assimilation of carbon and nitrogen compounds were determined according to standard protocols (Yarrow, 1998). Additional tests such as growth in the presence of 50 % glucose, 0.1 % and 0.01 % cycloheximide, and in vitamin-free and amino acid-free medium, and the Diazonium Blue B reaction, as well as tests for starch formation, gelatin liquefaction and urea hydrolysis were conducted (Kurtzman *et al.*, 2011).

To test the ability of the isolates to tolerate the stress conditions of the bird's digestive tract and cloacae, growth in liquid medium at 37, 40, 42 and 44 °C, at pH 2.5 and pH 3.0, as well as on 1 % acetic acid agar (Yarrow, 1998) was assessed for all the yeasts isolated in the present study. The tests were carried out individually and by combining the temperatures of 25, 37, 40, 42 and 44 °C with low pH and the presence of acetic acid. Microbial growth was estimated in the same way as in the assimilation tests. The results of the tests were evaluated as 'positive' when intense growth with a strong cell sediment was observed; 'weak' for growth with limited cell sediment; and 'negative' when no growth was observed.

Five cultures (551B4-B=PYCC 6785, 551B4=PYCC 6784, 551B5=PYCC 6786, 551B6=PYCC 6783^T and 551B6-B=PYCC 6787) that were isolated from two individuals of *Phylloscopus sibilatrix* (wood warbler), showed identical D1/D2 domain and ITS sequences. Searches in the GenBank and MycoBank databases showed 97 % D1/D2 sequence identity (19 nt differences) to strain 8A1 of *Sympodiomycopsis* sp. (GenBank accession number AM946764) and to three strains of *Q. cyanescens* (GenBank accession numbers DQ823440, DQ82344 and DQ823442). Furthermore, sequences of the five isolates differed from all type strains of species of the genera *Sympodiomycopsis* and *Quambalaria* by more than 19 nt substitutions.

The final phylogenetic tree was built using Bayesian inference and was based on a combined alignment of the ITS region and D1/D2 domain (Fig. 1). The isolates were placed in a separate lineage of the Microstomatales supported by high posterior probability values. The five isolates, together with strain 8A1, that represents an undescribed species of the genus *Jaminaea* (erroneously labelled at NCBI as *Sympodiomycopsis*), clustered together within the Microstomatales, and the most closely related species were *J. angkoriensis* and *J. lanaiensis*. The results obtained by maximum-likelihood and neighbour-joining algorithms using the same dataset were congruent with the Bayesian analysis shown in Fig. 1 and Figs S1 and S2 (available in the online Supplementary Material).

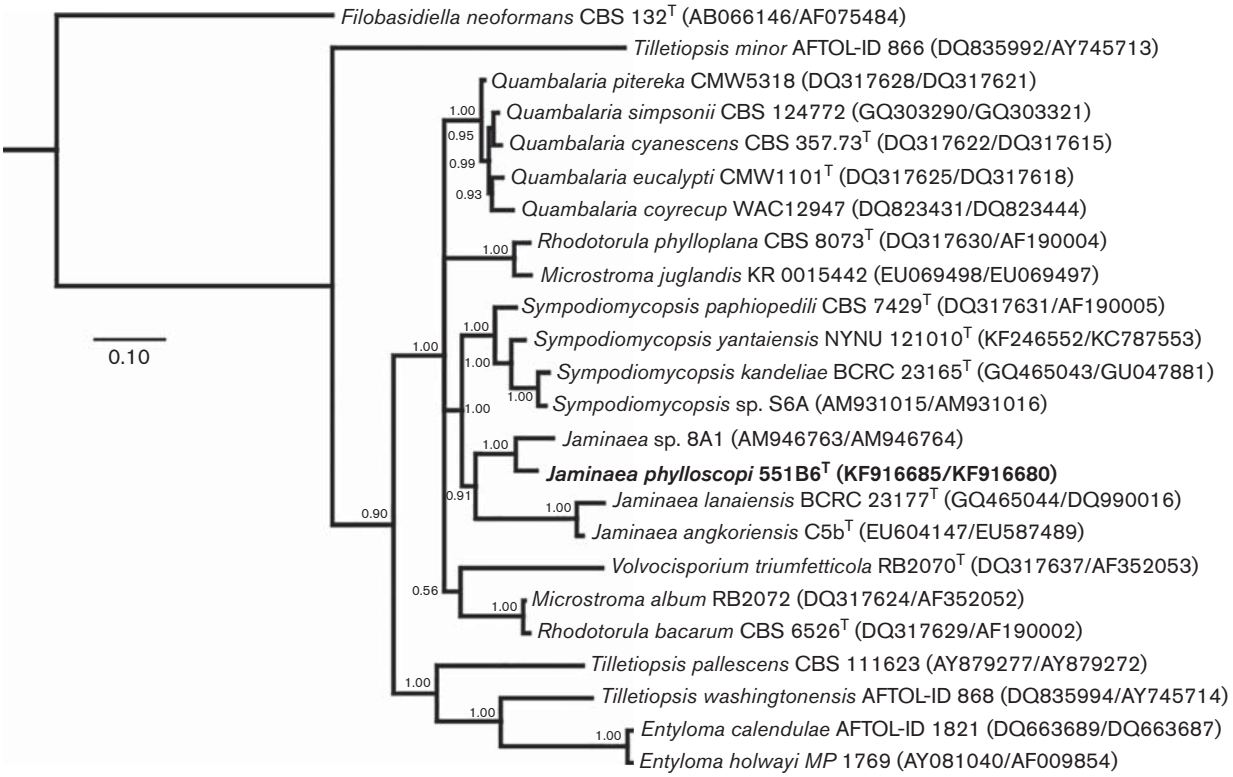

Fig. 1. Bayesian phylogeny of *Jaminaea phylloscopi* sp. nov. and closest relatives based on concatenated sequences of ITS1-5.8S-ITS2 rRNA region and D1/D2 domain of 26S rRNA gene (GenBank accession numbers of both regions are shown in parentheses). Numbers at nodes refer to posterior probabilities determined by sumt command of MrBayes 3.1.2 software; values <50 % are not shown. Strain 81A, incorrectly designated *Sympodiomyces* in the GenBank database, is assigned to the genus *Jaminaea*. Bar, 0.1 substitutions per nucleotide position.

The D1/D2 sequences of the novel isolates differed from *J. angkorensis* and *J. lanaiensis* by 20 and 25 nt substitutions, respectively. In addition, a high number of differences were found between the ITS sequences of the novel isolates and those of *J. angkorensis* (119 substitutions) and *J. lanaiensis* (131 substitutions). Within the Microstromatales, the comparison of ITS sequences among closely related species (i.e. species belonging to the genera *Sympodiomycopsis* and *Quambalaria*) shows a high number of nucleotide differences and a much higher variability than that of the D1/D2 domain. Phenotypic characterization of the novel isolates revealed several features commonly reported for the genus *Jaminaea*. Cells were ovoid to elongate and budding was polar (Fig. 2a). Fermentation was negative and several compounds were assimilated as reported in Table 1. On the other hand, several assimilation discrepancies were found between the isolates and the two known species of the genus *Jaminaea* (Table 1). Moreover, the novel isolates showed notable growth in liquid medium at 37, 40, 42 and 44 °C and at pH 3.0 and 2.5, but no growth was observed in the presence of 1 % acetic acid. In addition, among the isolates, phenotypic variability was detected mainly in terms of growth in the presence of 50 % glucose, growth at 44 °C, and at pH 3.0 and 42 °C, as well as in terms of assimilation of D-galactose, D-arabinose, salicin, starch, ribitol, xylitol, protocatechuic acid, L-malic acid and L-lysine.

J. lanaiensis and *J. angkorensis* were originally found in driftwood and decaying leaves, respectively. The genus *Sympodiomycopsis*, that is sister to *Jaminaea*, appears to be associated with flowers (Sugiyama *et al.*, 1991; Wei *et al.*, 2011) and insects. The recent description of *S. yantaiensis* (Chen *et al.*, 2013) showed that the presence of these yeasts in insect frass is probably due to the feeding activities of these insects on flowers. It has been reported that several insects (e.g. *Drosophila*, *Apis* and *Vespa*) carry yeasts on their bodies (Snowdon & Cliver, 1996) and in their gut (Ricci *et al.*, 2011) contributing to their dissemination although these animal vectors do not travel long distances. Furthermore, some yeast communities seem to be mutualistic with insects (Starmer *et al.*, 2003).

In the present work, we assume that the yeasts detected in the cloacae of wood warbler birds were first ingested and then survived gastric transit, thus withstanding the high body temperature of 41 ± 2 °C and low pH of the digestive tract and cloacae of migratory birds (Gwinner, 1990). At the same time, the novel isolates were unable to grow

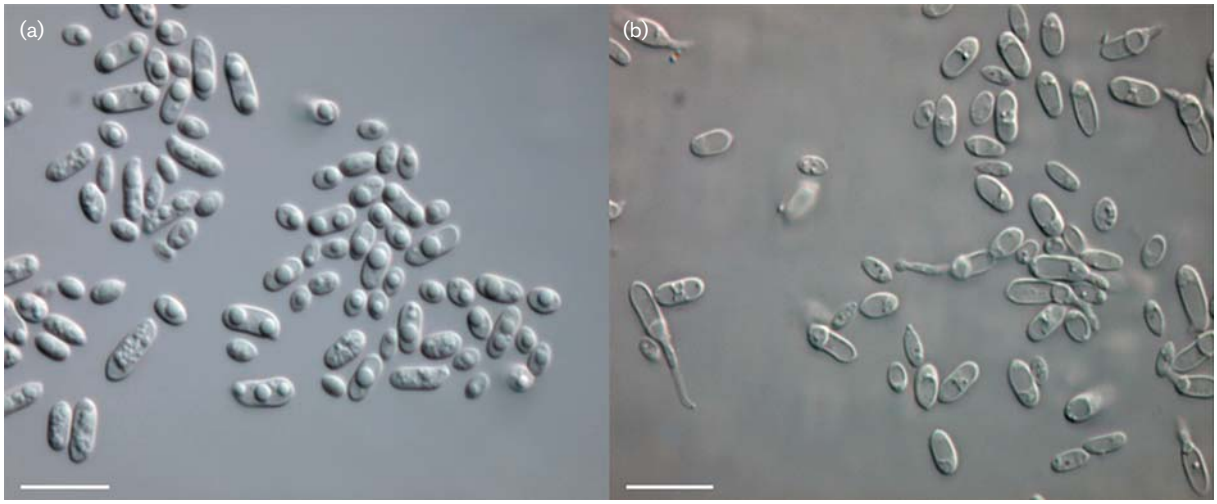

Fig. 2. *Jaminaea phylloscopi* sp. nov. 551B6^T (=PYCC 6783^T=CBS 14087^T) vegetative cell (a) after 3 days at 25 °C on 5 % malt extract agar; blastoconidia and conidiogenous cells (b) after 15 days at 25 °C on corn meal agar. Bars, 10 μm.

in the presence of 1 % acetic acid. These results were comparable to that found by Francesca *et al.* (2014) for many yeast isolates collected by migratory birds.

The wood warbler belongs to the Passerideae family and migration includes a round trip to the resting areas and a return to the territories of nesting, which occur in autumn and spring, respectively (Snow and Perrins, 1998). The wood warbler is widespread in deciduous forests in Europe, and the islands of the Mediterranean Basin are used during migrations to rest and to replace fat lost during flight. The migratory movements follow the seasonality of food resources and, as other Passerideae, are insectivore in spring and granivorous in autumn (Snow & Perrins, 1998). It is possible that *J. phylloscopi* is associated with the food sources of wood warblers but we have not tested this hypothesis.

The hypothesis of a potential adaptation of yeasts to the particular conditions of birds digestive tract was put forward by Francesca *et al.* (2013) based on the finding that eight strains of the novel species *Wickerhamomyces sylviae* were able to grow at 42 °C and at pH 2.5, a trait not exhibited by other species of the genus. These phenotypic features are not commonly reported for basidiomycetous yeasts that normally have maximum growth temperatures between 30 °C and 35 °C (Magan, 2008), except for pathogenic species like *Cryptococcus neoformans* (Kraus *et al.*, 2004). Therefore, the finding that the five novel isolates could grow well at 37 °C and weakly at 42 °C, even at low pH, argues in favour of an adaptation to the bird gastrointestinal tract and expands the range of high temperatures to which basidiomycetous yeasts are adapted.

The novel cultures were also characterized at the strain level by randomly amplified polymorphic DNA (RAPD)-PCR and mini-satellite-primed (MSP)-PCR. Results from the analysis both of RAPD and MSP profiles (Fig. S3) showed that the novel isolates were divided into five clusters at a similarity level higher than 90 %. In addition to the results from molecular strain typing, the different phenotypic traits and the different individual birds used as isolation sources, suggest little clonal relatedness between the novel isolates. On the basis of the results above, *Jaminaea phylloscopi* sp. nov. is proposed (type strain 551B6^T= PYCC 6783^T=CBS 14087^T).

Description of *Jaminaea phylloscopi* Francesca, Guerreiro, Carvalho, Sampaio & Moschetti sp. nov.

Jaminaea phylloscopi [phyl.lo.sco′pi. N.L. gen. n. *phylloscopi* of the bird *Phylloscopus sibilatrix*, Bechstein 1793 (wood warbler), from which the type strain was isolated].

After 3 days at 25 °C on 5 % malt extract agar, colonies are shiny with smooth texture and cream colour that becomes light pink (salmon colour) after incubation for 7 days. The margin is entire with a small elevation of the transversal section. Pseudohyphae and/or hyphae are not formed. Cells are ovoid to elongate (3–7 × 1–3 μm) and divide by polar budding (Fig. 2a). After 15 days at 25 °C on Dalmau plates, hyphae and pseudohyphae are not produced and on corn meal agar blastoconidia and conidiogenous cells are observed (Fig. 2b). Sexual structures are not detected. Fermentation is negative. The following compounds are assimilated: D-glucose, D-ribose, L-arabinose, sucrose, maltose, α,α-trehalose, methyl α-D-glucoside, cellobiose, lactose, raffinose, melezitose, inulin, glycerol, erythritol, D-glucitol, D-mannitol, succinate, ethanol and nitrate (all positive); D-galactose, D-xylose and L-malic acid (positive or delayed); nitrite (delayed); D-arabinose, protocatechuic acid, ribitol and xylitol (delayed or delayed but weak); salicin (delayed or negative); L-sorbose and

Table 1. Relevant physiological differences among the five isolates of *Jaminaea phylloscopi* sp. nov. and the species *Jaminaea angkorensis* and *Jaminaea lanaiensis*

Taxa: 1, 551B4 (=PYCC 6784) (data from this study); 2, 551B4-B (=PYCC 6785) (this study); 3, 551B5 (=PYCC 6786) (this study); 4, 551B6^T (=PYCC 6783^T=CBS 14087^T) (this study); 5, 551B6-B (=PYCC 6787) (this study); 6, *J. angkorensis* (Sipiczki & Kajacsi, 2009); 7, *J. lanaiensis* (Wei *et al.*, 2011). +, Positive; −, negative; D, delayed (rapid development of a positive reaction after a lag period); DW, delayed but weak (development of a weak reaction after a lag period); W, weak; ND, not determined.

Characteristic	1	2	3	4	5	6	7
D-Galactose	D	D	+	DW	DW	+	−
D-Ribose	+	+	+	+	+	+	−
D-Xylose	D	+	+	+	+	+	−
L-Arabinose	+	+	+	+	+	+	−
D-Arabinose	DW	DW	D	DW	DW	+	−
L-Rhamnose	−	−	−	−	−	+	−
α,α-Trehalose	+	+	+	+	+	+	−
Cellobiose	+	+	+	+	+	+	−
Salicin	DW	DW	−	−	−	+	−
Melibiose	−	−	−	−	−	+	+
Lactose	+	+	+	+	+	+	−
Melezitose	+	+	+	+	+	+	−
Inulin	+	+	+	+	+	+	−
Starch	−	W	W	W	W	+	+
Erythritol	+	+	+	+	+	+	−
Ribitol	DW	DW	DW	D	D	+	−
Xylitol	DW	DW	DW	D	D	+	ND
D-Glucuronate	−	−	−	−	−	+	−
Citrate	−	−	−	−	−	+	+
Methanol	−	−	−	−	−	+	−
Ethanol	+	+	+	+	+	+	−
L-Malic acid	D	+	+	+	+	ND	ND
Protocatechuic acid	D	D	D	DW	DW	ND	ND
Nitrite	D	D	D	D	D	−	ND
L-Lysine	DW	W	W	DW	DW	+	ND
DBB reaction	−	−	−	−	−	ND	+
Growth at:							
37 °C	W	+	+	+	+	−	−
40 °C	W	W	W	W	W	−	−
42 °C	W	W	W	W	W	−	−
44 °C	W	−	W	W	W	−	−
pH 3.0 and 25 °C	W	W	W	W	W	−	−
pH 3.0 and 37 °C	W	W	W	W	W	−	−
pH 3.0 and 40 °C	W	W	W	W	W	−	−
pH 3.0 and 42 °C	W	−	W	W	W	−	−
pH 2.5 and 25 °C	W	W	W	W	W	−	−
pH 2.5 and 37 °C	W	W	W	W	W	−	−
pH 2.5 and 40 °C	W	W	W	W	W	−	−
pH 2.5 and 42 °C	W	W	W	W	W	−	−

DL-lactate (slow); and L-lysine (weak or delayed but weak). L-Rhamnose, melibiose, D-glucosamine, galactitol, D-glucono-1,5-lactone, D-gluconate, D-glucuronate, *myo*-inositol, citrate, methanol, L-tartaric acid, ethylamine, cadaverine, creatine, creatinine, cycloheximide (0.1 % and 0.01 %) and ethylamine are not assimilated. Starch assimilation is negative or weak. Growth both on vitamin- and amino acid-free media is variable. Growth is positive or weak in the presence of 50 % glucose, and the Diazonium Blue B reaction, gelatin liquefaction and starch formation are negative. Hydrolysis of urea is positive. Growth at 37 °C and 44 °C is positive or weak and weak or negative, respectively. Growth in liquid medium at pH 3 and pH 2.5 up to 42 °C is weak. Growth on 1 % acetic acid agar is negative.

The type strain, 551B6^T, was isolated from a trans-Saharan migratory bird [*Phylloscopus sibilatrix*, Bechstein 1793 (wood warbler)] in May 2009 in Ustica, Sicily (Italy), by C. Sannino. The type strain has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands as strain CBS 14087^T, and in the Portuguese Yeast Culture Collection, Caparica, Portugal as strain PYCC 6783^T. The Mycobank accession number is MB811984.

Acknowledgements

The authors are grateful to Professor Bruno Massa for his help in bird capture and analysis of data. This work was partially financially supported by FCT (Portugal) through project UID/Multi/04378/2013 (Portugal) and by Provincia Regionale of Palermo (Italy) for ringing of birds in Ustica.

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.

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.

Chen, L., Zhang, L., Li, Z. H. & Hui, F. L. (2013). *Sympodiomycopsis yantaiensis* sp. nov., a basidiomycetous yeast isolated from insect frass. *Int J Syst Evol Microbiol* **63**, 3501–3505.

de Beer, Z. W., Begerow, D., Bauer, R., Pegg, G. S., Crous, P. W. & Wingfield, M. J. (2006). Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. *Stud Mycol* **55**, 289–298.

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.

Francesca, N., Carvalho, C., Almeida, P. M., Sannino, C., Settanni, 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.

Francesca, N., Carvalho, C., Sannino, C., Guerreiro, M.A., Almeida, P.M., Settanni, L., Massa, B, Sampaio, J.P. & Moschetti, G. (2014). Yeasts vectored by migratory birds collected in the Mediterranean island of Ustica and description of *Phaffomyces usticensis* f.a. sp. nov., a new species related to the cactus ecoclade. *FEMS Yeast Res* **14**, 910–921.

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

Kaiser, A. (1993). A new multi-category classification of subcutaneous fat deposits of songbirds. *J Field Ornithol* **64**, 246–255.

Kraus, P. R., Boily, M. J., Giles, S. S., Stajich, J. E., Allen, A., Cox, G. M., Dietrich, F. S., Perfect, J. R. & Heitman, J. (2004). Identification of *Cryptococcus neoformans* temperature-regulated genes with a genomic-DNA microarray. *Eukaryot Cell* **3**, 1249–1260.

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.

Magan, N. (2008). Ecophysiology: impact of environment on growth, synthesis of compatible solutes and enzyme production. In *Ecology of Saprotrophic Basidiomycetes*, pp. 63–78. Edited by L. Boddy, J. Frankland & P. van West. London: Academic Press Elsevier.

Mahdi, L. E., Statzell-Tallman, A., Fell, J. W., Brown, M. V. & Donachie, S. P. (2008). *Sympodiomycopsis lanaiensis* sp. nov., a basidiomycetous yeast (Ustilaginomycotina: Microstromatales) from marine driftwood in Hawai'i. *FEMS Yeast Res* **8**, 1357–1363.

Mullarney, K., Svensson, L., Zetterstrom, D. & Grant, P. (1999). *Bird Guide*. London: Harper Collins Publisher.

Paap, T., Burgess, T. I., McComb, J. A., Shearer, B. L., St, J. & Hardy, G. E. (2008). *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia. *Mycol Res* **112**, 57–69.

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.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

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.

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.

Simpson, J. A. (2000). *Quambalaria*, a new genus of eucalypt pathogens. *Australasian Mycologist* **19**, 57–62.

Sipiczki, M. & Kajdacsi, E. (2009). *Jaminaea angkorensis* gen. nov., sp. nov., a novel anamorphic fungus containing an S943 nuclear small-subunit rRNA group IB intron represents a basal branch of Microstromatales. *Int J Syst Evol Microbiol* **59**, 914–920.

Snow, D. W. & Perrins, C. M. (1998). *The Birds of the Western Palearctic: Concise Edition*. Oxford: Oxford University Press.

Snowdon, J. A. & Cliver, D. O. (1996). Microorganisms in honey. *Int J Food Microbiol* **31**, 1–26.

Starmer, W. T., Schmedicke, R. A. & Lachance, M. A. (2003). The origin of the cactus-yeast community. *FEMS Yeast Res* **3**, 441–448.

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

Sugiyama, J., Tokuoka, K., Suh, S. O., Hirata, A. & Komagata, K. (1991). *Sympodiomycopsis*: a new yeast-like anamorph genus with basidiomycetous nature from orchid nectar. *Antonie van Leeuwenhoek* **59**, 95–108.

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.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

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.

Wei, Y. H., Liou, G. Y., Liu, H. Y. & Lee, F. L. (2011). *Sympodiomycopsis kandeliae* sp. nov., a basidiomycetous anamorphic fungus from mangroves, and reclassification of *Sympodiomycopsis lanaiensis* as *Jaminaea lanaiensis* comb. nov. *Int J Syst Evol Microbiol* **61**, 469–473.

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.