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ORIGINAL PAPER

Description of *Groenewaldozyma* gen. nov. for placement of *Candida auringiensis*, *Candida salmanticensis* and *Candida tartarivorans*

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Abstract DNA sequence analyses have demonstrated that species of the polyphyletic anamorphic ascomycete genus *Candida* may be members of described teleomorphic genera, members of the *Candida tropicalis* clade upon which the genus *Candida* is circumscribed, or members of isolated clades that represent undescribed genera. From phylogenetic analysis of gene sequences from nuclear large subunit rRNA, mitochondrial small subunit rRNA and cytochrome oxidase II, *Candida auringiensis* (NRRL Y-17674^T, CBS 6913^T), *Candida salmanticensis* (NRRL Y-17090^T, CBS 5121^T), and *Candida tartarivorans* (NRRL Y-27291^T, CBS 7955^T) were shown to be members of an isolated clade and are proposed for reclassification in the genus *Groenewaldozyma* gen. nov. (MycoBank MB 815817). Neighbouring taxa include species of the *Wickerhamiella* clade and *Candida blankii.*

Keywords Candida · *Groenewaldozyma* gen. nov. · Phylogenetics · Yeasts

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Introduction

Characterization of yeasts from DNA sequence analysis has resulted in accurate identification of species and an estimation of their phylogenetic placement (Kurtzman and Robnett 1998, 2013). DNA comparisons also showed that species of the genus *Candida* are distributed throughout the Saccharomycotina with some species seen as members of ascosporic genera, whereas others form isolated lineages that represent undescribed genera (Kurtzman and Robnett 1998). Because the D1/D2 LSU rRNA gene sequence used in many comparisons is relatively short (ca. 600 nucleotides), support for resulting clades is often weak, which has demonstrated the need for more extensive datasets to verify the placements suggested from D1/D2. In addition to the need for more robust datasets, until recently, reclassification of *Candida* species was hindered by provisions of the Botanical Code.

Classification of yeasts and other fungi has been governed by the rules of the *International Code of Botanical Nomenclature* (Vienna Code) (McNeill et al. 2006), which did not permit inclusion of teleomorphic and anamorphic species in the same genus, even if demonstrated to be congeneric. The latest edition of the Code, now entitled *International Code of Nomenclature for algae, fungi, and plants* (Melbourne Code) (McNeill et al. 2012), permits inclusion of anamorphic and teleomorphic species in the same genus, resulting in many new species combinations as anamorphs are transferred to teleomorphic genera. The genus

Candida, with over 400 described species (Daniel et al. 2014) presents a unique problem. The genus was circumscribed on *Candida vulgaris* (= *C. tropicalis*), so the majority of *Candida* species are not members of this clade, but under the old Code, anamorphic ascomycete species were assigned to this genus because there were few other options. Now with changes in the Code and the application of phylogenetic analysis of gene sequences, it is possible to group related species, some of which are members of teleomorphic genera, some are members of *Candida* and some represent new genera. Where sufficient data are available, various *Candida* species have been transferred to new genera, such as *Danielozyma*, *Deakozyma* and *Middelhovenomyces* (Kurtzman and Robnett 2014). Multilocus DNA sequence analysis has demonstrated that *Candida auringiensis*, *Candida salmanticensis* and *Candida tartarivorans* are members of an isolated clade unrelated to other described genera, and it is proposed to assign these species to a new genus.

Materials and methods

Gene sequences for the nearly entire nuclear LSU rRNA, mitochondrial SSU rRNA and cytochrome oxidase II that were compared in this study had been determined earlier by Kurtzman and Robnett (2007) and that paper gives sequencing conditions, primers, and GenBank accession numbers for the sequences determined. For phylogenetic analysis, sequences were aligned using Muscle, which is in the MEGA version 5.2 software package (Tamura et al. 2011), and the alignments were visually adjusted. Phylogenetic relatedness among species was determined from the maximum likelihood program included in MEGA using the Hasegawa–Kishino–Yano model, and bootstrap support was determined from 1000 replicates. Species tested as the outgroup in the analyses included *Trigonopsis variabilis*, *Tortispora caseinolytica* and *Schizosaccharomyces pombe*.

Results and discussion

C. auringiensis and *C. salmanticensis* were recognized as an isolated pair of neighbouring species from analysis of gene sequences for D1/D2 domain LSU rRNA (Kurtzman and Robnett 1998) and for 18S rRNA (Suzuki et al. 1999). Soon after, Fonseca et al. (2000) added *C. tartarivorans* to the clade. A multilocus study by (Kurtzman and Robnett 2007) strongly supported the clade (100 % bootstrap) and provided information on neighbouring species. Now, due to adoption of the Melbourne Code, it is possible to transfer these three *Candida* species to a new genus (Fig. 1). which is described as follows.

Groenewaldozyma Kurtzman gen. nov

Description of the genus

Growth is by multilateral budding on a narrow base and cells may be globose, ovoid or elongate. Pseudohyphae and true hyphae may be formed. An ascosporic state is unknown.

Species ferment glucose, galactose and trehalose, but fermentation of sucrose, maltose, lactose and raffinose is species and strain variable. Dien et al. (1996) reported some strains of *C. auringiensis* to ferment l-arabinose, a property useful for biomass utilization. Many of the hexoses, pentoses, disaccharides, sugar alcohols and organic acids commonly used in yeast taxonomy are assimilated, but there is no growth on methanol. Nitrate is not utilized as a sole source of nitrogen. Data for fermentation and growth reactions are from Lachance et al. (2011). The genus can be separated from other members of the Saccharomycotina by gene sequence analysis.

Phylogenetic placement: Saccharomycetales, Saccharomycotina, Ascomycota. The genus *Groenewaldozyma*, among currently recognized taxa, appears most closely related to species of the *Wickerhamiella* clade and to *Candida blankii* (Fig. 1, (Kurtzman and Robnett 2007).

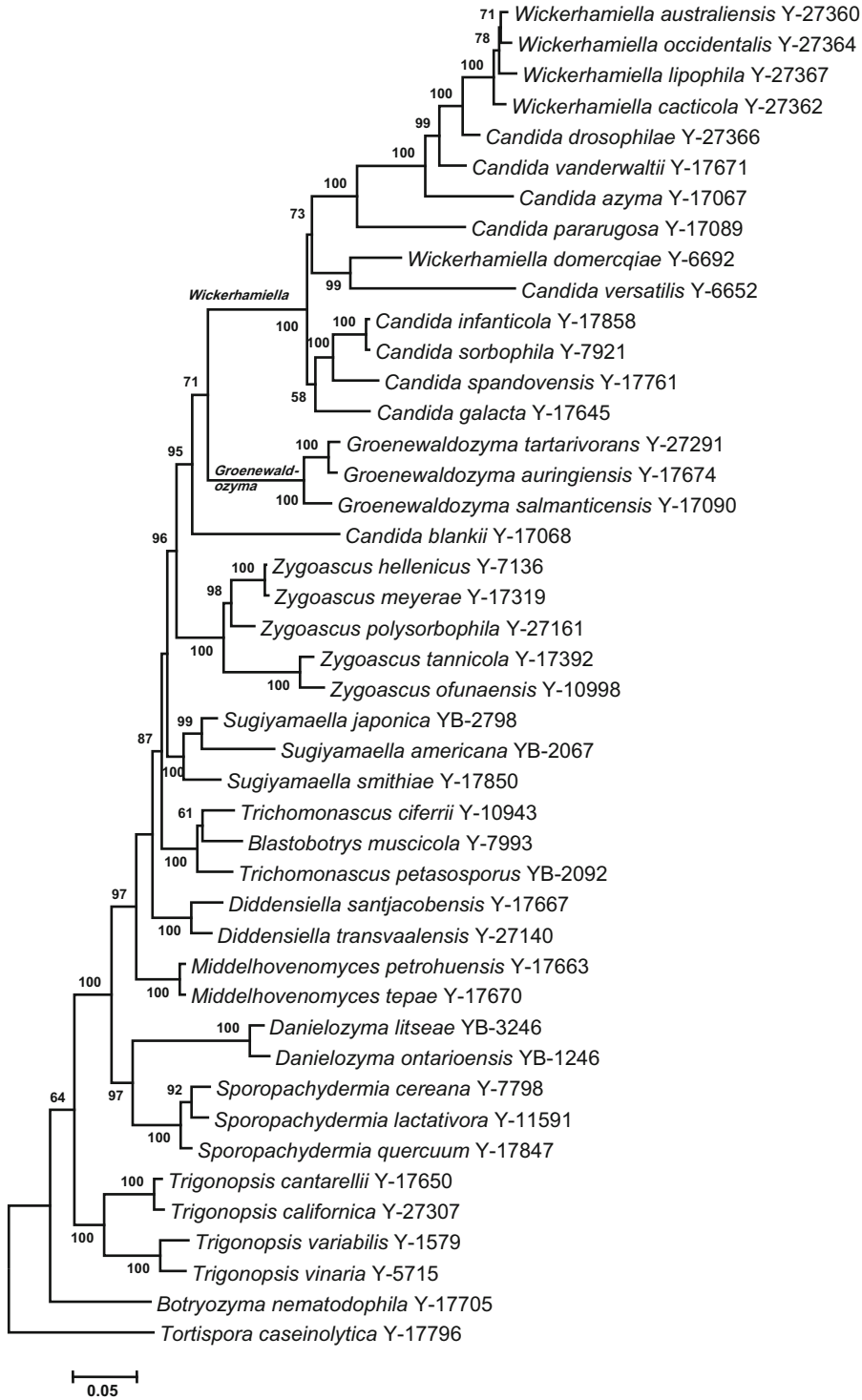
Type species: *G. auringiensis* (Santa María) Kurtzman comb. nov.

MycoBank number: MB 815817.

Etymology: The genus is named in honor of Dr. Marizeth Groenewald, Centraalbureau voor Schimmelcultures Biodiversity Center, Utrecht, The Netherlands, for her many contributions to yeast systematics and yeast biodiversity.

Species of *Candida* proposed for transfer to the genus *Groenewaldozyma* as new combinations:

G. auringiensis (Santa María) Kurtzman comb. nov.



Basionym: *C. auringiensis* Santa María (1978). Commun Inst Nac Invest Agron, Serie General 3:55.

Type strain: NRRL Y-17674, CBS 6913.

Mycobank No.: MB 815818.

G. salmanticensis (Santa María) Kurtzman comb. nov.

◀**Fig. 1** Phylogenetic tree showing placement of the genus *Groenewaldozyma* among neighboring taxa. The tree was derived from maximum likelihood analysis of concatenated gene sequences from nearly entire nuclear LSU rRNA, mitochondrial small rRNA and cytochrome oxidase II. Bootstrap values >50 % are given at nodes and based on 1000 replicates. *T. caseinolytica* served as outgroup species in the analysis but replacement of *T. caseinolytica* with either *T. variabilis* or *S. pombe* had no effect on tree topology or branch support. All species are represented by type strains and are maintained in the ARS Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL USA

Basionym: *Torulopsis salmanticensis* Santa María (1963). Antonie van Leeuwenhoek 29:330.

Type strain: NRRL Y-17090, CBS 5121.

Mycobank No.: MB 815819.

G. tartarivorans (Fonseca, Fell, Kurtzman & Spencer-Martins) Kurtzman comb. nov.

Basionym: *C. tartarivorans* (Fonseca et al. 2000) Int J Syst Evol Microbiol 50:390.

Type strain: NRRL Y-27291, CBS 7955.

Mycobank No.: MB 815820.

At present, *Groenewaldozyma* is a small genus with relatively little known about its ecology and geographical distribution. The description of *G. auringiensis* was based on three strains (CBS 6913^{T}, CBS 6919, CBS 6920) that had been isolated from alpechin in Spain (Santa María 1978), but Lachance (Lachance et al. 2011) isolated additional apparently new species of *Groenewaldozyma* that are near *G. auringiensis*. These included UWO(PS)85–228.1.3 (GenBank AF530606) from sap fluxes of mesquite (*Prosopis juliflora*) growing in Arizona, USA and Baja California, Mexico, UWO(PS)93–324.2 from the rot of *Agaves* sp. in the Bahamas, and UWO(PS)99–304.7 (GenBank AF530605) from sap fluxes of the guapinol tree (*Hymenacea courbaril*) in Costa Rica. *G. salmanticensis* was also described by Santa María (1963) and had been isolated from alpechin in Spain. Since then, Lachance (Lachance et al. 2011) reported isolation of two additional strains, UWO(PS)85–301.1 from *Drosophila carbonaria* collected in sap flux of a mesquite (*Prosopis juliflora*), Arizona, USA, and UWO(PS)03–325y3 from sap flux of red oak (*Quercus rubra*), Ontario, Canada. The third species assigned to *Groenewaldozyma*, *G. tartarivorans*, was isolated from dried wine lees in Portugal (Fonseca et al. 2000). Three additional isolates are reported in GenBank: Y0 285 (GenBank LC015275) from fermentation silage of sugar cane, Brazil; IMUFRJ 51977 (GenBank FN428939) from a sugar cane field in Brazil; and NU28S61 (GenBank HM461710) from soil in Taiwan. From strain histories, it appears that distribution of *Groenewaldozyma* is worldwide. A majority of substrates from which strains have been isolated are characterized by low water activity suggesting additional species might be found in moderate to high osmotic habitats. Species of the neighbouring *Wickerhamiella* clade are often isolated from similar habitats (Lachance and Kurtzman 2011; Lachance et al. 2011) On the basis of D1/D2 analysis, there are presently 30 species assigned to the *Wickerhamiella* clade (Dayo-Owoyemi et al. 2014; Khunnamwong et al. 2014; Ren et al. 2014).

Phylogenetic placement of the genus *Groenewaldozyma* among other members of the Saccharomycotina is somewhat uncertain, but the multigene analysis of Kurtzman and Robnett (2007) placed the three species now assigned to *Groenewaldozyma* near *Wickerhamiella* and *C. blankii*. The analysis presented in Fig. 1 mirrors the earlier analysis, but with fewer included species. Consequently, *Groenewaldozyma* appears well isolated from *Wickerhamiella* and *C. blankii*, and it is likely that this separation will be maintained as more extensive datasets become available. The present work, as well as other recent studies (e.g., Kurtzman 2015; Kurtzman and Robnett 2014; Lachance and Kurtzman 2013), mark the beginning of the dissolution of the polyphyletic genus *Candida* into phylogenetically circumscribed genera, although placement of many *Candida* species must await more robust datasets.

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