Wickerhamomyces queroliae sp. nov. and Candida jalapaonensis sp. nov., two yeast species isolated from Cerrado ecosystem in North Brazil

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Two novel yeast species, Wickerhamomyces queroliae sp. nov. and Candida jalapaonensis sp. nov., were isolated, respectively, from larvae of Anastrepha mucronata (Diptera: Tephritidae) collected from ripe fruit of Peritassa campestris (‘Bacupari’, Hippocrateaceae) and from flowers of Centropogon cornutus (Campanulaceae) in the Cerrado ecosystem of the state of Tocantins, Brazil. Analysis of the D1/D2 large-subunit rRNA gene sequences placed W. queroliae in the Wickerhamomyces clade near Wickerhamomyces ciferri and Candida silvicultrix. Candida jalapaonensis belongs to the Wickerhamiella clade and is related to Candida drosophilae. The type strain of Wickerhamomyces queroliae is UFMG-05-T2001T (=CBS 10936T=NRRL Y-48478) and the type strain of Candida jalapaonensis is UFMG-03-T2101T (=CBS 10935T=NRRL Y-48477).

The Brazilian Cerrado is considered to be a biodiversity hotspot (Myers et al., 2000). This ecosystem is characterized by the occurrence of a large number of endemic species of plants and animals. However, extensive studies of the microbial diversity from this ecosystem are scarce. Only four novel yeast species, Ogataea falcaoamoraisii, Candida azymoides, Metschnikowia cerradonensis and Moniliella fonsecaei, have been described from natural sources in the Cerrado ecosystem (Morais et al. 2004; Rosa et al., 2006, 2007, 2008). During a survey of yeasts associated with various substrates from the Cerrado ecosystem in the state of Tocantins, northern Brazil, several strains were isolated. Two isolates were obtained from larvae of Anastrepha mucronata (Diptera: Tephritidae) collected from ripe fruit of Peritassa campestris (‘Bacupari’, Hippocrateaceae). Other isolates were from flowers of Centropogon cornutus (Campanulaceae). Analysis of the sequences of the D1/D2 domains of the large-subunit rDNA showed that these strains represent phylogenetically distinct species, the first isolate being related to the Wickerhamomyces clade and the second to the Wickerhamiella clade. In this paper, we describe two novel species, for which the names Wickerhamomyces queroliae sp. nov. and Candida jalapaonensis sp. nov. are proposed.

Details of the strains considered in this study are given in Table 1. Larvae of A. mucronata (Tephritidae) were collected directly from decaying fruit of P. campestris in an Ipuca forest fragment from the Cerrado ecosystem, in October 2005 (Rosa et al. 2006). The larvae were collected with sterile forceps, surface-sterilized by immersion in 70 % ethanol for 1 min, and placed on plates of YM agar (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1.0 % glucose, 2 % agar, and 100 mg chloramphenicol L−1). Flowers of Centropogon cornutus (Campanulaceae) were collected near a road at a distance of approximately 100 m of a waterfall (Cachoeira da Velha) in the State Park of Jalapão, state of Tocantins, in September 2003. The nectary region of the flowers was scraped gently with a sterile loop and streak-inoculated onto YM agar. The plates were incubated at room temperature (25 ± 3 ºC) for 3–8 days. Each different yeast morphotype was purified and maintained on YM slants or in liquid nitrogen storage for later identification. The yeasts were characterized using standard methods (Yarrow, 1998). Identities were verified using the keys of Kurtzman & Fell (1998). Sporulation was investigated using malt extract, glucose-yeast extract, yeast carbon base plus 0.01 % ammonium sulfate, V8, cornmeal,
Gorodkowa and YM agars at 22 °C for 3 weeks. The heat treatment technique described by Wickerham & Burton (1954) was used to determine whether W. queroliae sp. nov. was homothallic or heterothallic.

The D1/D2 variable domains of the large-subunit rDNA were amplified by PCR directly from whole cells, as described previously (Lachance et al., 1999). The amplified DNA was concentrated and cleaned on QIAquick PCR columns (Qiagen) and sequenced using an ABI sequencer at the John P. Robarts Research Institute (London, Ontario, Canada). The sequence was edited with the program DNAMAN, version 6 (Lynnon BioSoft). Sequences for other yeasts were retrieved from GenBank.

CLUSTAL W software (Thompson et al., 1994) was used to align the sequences and a neighbour-joining tree was constructed based on 1000 bootstrap iterations.

**Classification, ecology and species delineation**

The genus *Wickerhamomyces* was proposed by Kurtzman et al. (2008) to accommodate 17 phylogenetically related species formerly assigned to *Pichia*, *Williopsis* and *Hansenula*. The genus was phylogenetically circumscribed from an analysis of large-subunit and small-subunit rRNA and EF-1α gene sequences and is a sister genus to *Lindera*, another collection of species formerly assigned to the three aforementioned taxa. The type species of *Wickerhamomyces* is *Wickerhamomyces canadensis*. Based on the sequences of the D1/D2 region of the large-subunit rRNA gene, *W. queroliae* sp. nov. occupied a basal position with respect to several species that included *W. ciferrii* and *Candida silvicultrix* (Fig. 1). In terms of sequence divergence, *W. queroliae* sp. nov. differed by 21 substitutions and 31 gaps from *Candida silvicultrix*, and by 23 substitutions and 30 gaps from *W. ciferrii* in the D1/D2 region of the large-subunit rRNA gene. The isolates of this novel yeast species were obtained from larvae of *A. mucronata*, suggesting that the yeast is associated with this insect. *A. mucronata* is a frugivorous fly, and other species of this genus are considered pests for many different kinds of fruits (Cruz-López et al. 2006). However, there is no record of significant economic damage being caused by this fly species. *A. mucronata* was collected from fruit of *P. campestris* and also probably attacks other fruits of the Cerrado ecosystem. The habitat of *W. queroliae* sp. nov. could probably be this fly and fruit of the Cerrado ecosystem. Two to four hat-shaped ascospores were produced from single colonies of *W. queroliae* sp. nov. when grown on dilute (1 : 19) V8 agar. After heat treatment, according to Wickerham & Burton (1954), 15 colonies of *W. queroliae* were transferred individually to plates of dilute V8 agar. The plates were incubated for 14 days at 25 °C, and ascospores were produced, showing that *W. queroliae* sp. nov. is homothallic. *W. queroliae* sp. nov. can be distinguished from *W. anomalus* and *W. ciferrii* based on growth on cellobiose, raffinose and...
melezitose, which is positive for \textit{W. anomalus} and \textit{W. ciferrii} and negative for the novel species.

\textit{Candida jalapaonensis} sp. nov. belongs to the \textit{Wickerhamiella} clade and is a sister species to \textit{Candida drosophilae}. The two species differed in 14 bases and two gaps in the D1/D2 region of the large-subunit rDNA (Fig. 2). Four strains of the novel species were isolated from flowers of \textit{Centropogon cornutus} in the State Park of Jalapão, state of Tocantins, Brazil. Yeast species from the \textit{Wickerhamiella} clade are frequently associated with flowers and floricolous insects (Lachance \textit{et al.}, 1998, 2000). Flowers of \textit{Centropogon cornutus} are frequently visited by insects and also by hummingbirds, and these probably serve as vectors of this yeast in the Cerrado ecosystem. Isolates of \textit{Candida jalapaonensis} sp. nov. were examined individually or as a mixture of two strains on cornmeal, V8, dilute V8, 5 % malt extract, yeast carbon base supplemented with 0.01 % ammonium sulphate and Gorodkowa agars, but no asci or signs of conjugation were observed.

\textbf{Latin diagnosis of \textit{Wickerhamomyces queroliae} Rosa, Morais, Lachance & Pimenta sp. nov.}


\textbf{Description of \textit{Wickerhamomyces queroliae} Rosa, Morais, Lachance & Pimenta sp. nov.}

\textit{Wickerhamomyces queroliae} (que.ro.li.ae. N.L. gen. fem. sing. n. \textit{queroliae} of Querol, referring to Amparo Querol, in recognition of her contributions to yeast systematics and fermentation).

In glucose (2 %), yeast extract (0.5 %) broth after 3 days at 25 °C, cells are ovoid to ellipsoidal (2–3 × 2–4 μm). Budding is multilateral. Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 2 days at 25 °C, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomyelia or true myelia are not formed. Homothallic. After 5 days on diluted (1 : 19) V8 agar, cells give rise to asci containing two to four hat-shaped ascospores (Fig. 3). Asci are unconjugated and ascospores are not liberated. Glucose, maltose and sucrose are fermented. Assimilation of carbon compounds: glucose, maltose, sucrose, trehalose, D-xylose, L-arabinose, D-ribose, L-rhamnose, ethanol (slow), glycerol, erythritol, ribitol, D-mannitol, D-glucitol, salicin, lactic acid (variable), succinic acid, citric acid (weak and slow), xylitol and gluconic acid. No growth occurs on galactose, l-sorbose, raffinose, inulin, melibiose, lactose, melezitose, cellobiose, soluble starch, D-arabinose, methanol, 2-propanol, galactitol, myo-inositol, 2-ketogluconate, glucosamine, N-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl, cadaverine, nitrate and nitrite. Growth in vitamin-free medium is
negative. Growth in amino acid-free medium is positive. Growth at 37 °C is positive. Growth on YM agar with 10 % sodium chloride is negative. Growth in 50 % glucose/yeast extract (0.5 %) is negative. Starch-like compounds are not produced. In 100μg cycloheximide ml⁻¹ growth is variable. Urease activity is negative. Diazonium Blue B reaction is negative.

Habitat is larvae of Anastrepha mucronata (Diptera: Tephritidae) collected from fruit of Peritassa campestris ('Bacupari', Hippocrateaceae) in Ipuca ecosystem, in the state of Tocantins, Brazil. The type strain is UFMG-05-T200.1T, isolated from larvae of A. mucronata collected from ripe fruit of P. campestris, Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10936T (=NRRL Y-48478T).

**Latin diagnosis of Candida jalapaonensis Rosa, Morais, Lachance & Pimenta sp. nov.**

In medio liquido glucosum et extractum levidinis post dies tres cellulae singulae aut binae; cellulae ovoidae aut ellipsoideae (2–3 × 2–4 μm). Post unum mensem pellicula et sedimentum formantur. Cultura in agaro malti post dies 2 (25 °C) parva, convexa, glabra et candida. In agaro farinae Zea mays post dies 14 mycelium nec pseudomycelium non-formantur. Ascosporae non-formantur. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, ethanolum (exigue), glycerol, mannitolum, glicitolum, acidum succinicum, acetonum et hexadecanum assimilantur, at non-nitratum et nitritum. Growth in vitamin-free medium is

**Description of Candida jalapaonensis Rosa, Morais, Lachance & Pimenta sp. nov.**

*Candida jalapaonensis* (ja.la.pa.o.nen’sis. N.L. nom. masc. sing. adj. jalapaonensis referring to the place where this yeast was found).

In glucose (2 %), yeast extract (0.5 %) broth after 3 days at 25 °C, cells are ovoid to ellipsoidal (2–3 × 2–4 μm). Budding is multilateral (Fig. 4). Sediment and pellicle are formed after 1 month. On YM agar after 2 days at 25 °C, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomyelia or true mycelia are not formed. Ascospores are not formed. Fermentation of glucose is negative. Assimilation of carbon compounds: glucose, galactose, L-sorbose, ethanol (weak), glycerol, D-mannitol, D-glucitol, succinic acid, acetone and hexadecane are assimilated. No growth occurs on sucrose, maltose, trehalose, raffinose, inulin, melibiose, lactose, melezitose, cellobiose, soluble starch, D-xyllose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, 2-propanol, erythritol, ribitol, galactitol, myo-inositol, 2-ketogluconate, salicin, lactic acid, citric acid, xylitol, glucosamine, N-acetylglucosamine, gluconic acid or ethyl acetate. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl, cadaverine, and negative for nitrate and nitrite. Growth in vitamin-free medium is

**Fig. 3.** Phase-contrast micrographs of cells of *Wickerhamomyces queroliae* UFMG-05-T200.1T on dilute (1 : 19) V8 agar after 5 days at 22 °C. (a) Ascus with hat-shaped ascospores; (b) budding cells and an ascus with hat-shaped ascospores. Bar, 5 μm.

**Fig. 4.** Phase-contrast micrograph of *Candida jalapaonensis* UFMG-03-T210T on yeast extract-malt extract agar after 5 days at 22 °C. Bar, 5 μm.
negative. Growth in amino acid-free medium is positive. Growth at 37 °C is variable. Growth on YM agar with 10% sodium chloride is negative. Growth in 50% glucose/yeast extract (0.5%) is negative. Starch-like compounds are not produced. In 100 μg cycloheximide ml⁻¹ growth is negative. Urease activity is negative. Diazonium Blue B reaction is negative.

Habitat is flowers of Centropogon cornutus (Campanulaceae) in the Cerrado ecosystem, in the state of Tocantins, Brazil. The type strain is UFMG-03-T210ᵀ, isolated from flowers of Centropogon cornutus in Brazil. It has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as strain CBS 10935ᵀ (=NRRL Y-48477ᵀ).

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