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-T200.1^T (=CBS 10936^T=NRRL Y-48478^T) and the type strain of *Candida jalapaonensis* is UFMG-03-T210^T (=CBS 10935^T=NRRL Y-48477^T).

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 falcaomoraisii*, *Candida azymoides*, *Metschnikowia cerradonensis* and *Moniliella fONSECAE*, 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 °C) 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,

The GenBank/EMBL/DBJ accession numbers for the sequences reported in this paper are EU580139 and EU580140.

Table 1. List of isolates of *Wickerhamomyces queroliae* and *Candida jalapaonensis*

Strain number*	Source	Location
<i>W. queroliae</i> UFMG-05-T200.1 ^T (=CBS 10936 ^T =NRRL Y-48478 ^T) and UFMG-05-T407	Larvae of <i>Anastrepha mucronata</i> (Diptera: Tephritidae) collected from ripe fruit of <i>Peritassa campestris</i> ('Bacupari', Hippocrateaceae)	Ipuca forest fragment, Lago Verde estate, Tocantins, Brazil
<i>Candida jalapaonensis</i> UFMG-03-T210 ^T (=CBS 10935 ^T =NRRL Y-48477 ^T), UFMG-03-T211, UFMG-03-T213 and UFMG-03-T214	Flowers of <i>Centropogon cornutus</i> (Campanulaceae)	Road approximately 100 m from the first fall of the waterfall Cachoeira da Velha, State Park of Jalapão, Tocantins, Brazil

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Gorodkova 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-1 α 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

Fig. 1. Neighbour-joining tree based on large-subunit rRNA genes showing the placement of *Wickerhamomyces queroliae* among related species in the *Wickerhamomyces* clade. Bootstrap values greater than 50 % are shown. Bar, 5 substitutions per 100 nucleotides.

melezitose, which is positive for *W. anomalus* and *W. ciferrii* and negative for the novel species.

Candida jalapaonensis sp. nov. belongs to the *Wickerhamiella* clade and is a sister species to *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 *Centropogon cornutus* in the State Park of Jalapão, state of Tocantins, Brazil. Yeast species from the *Wickerhamiella* clade are frequently associated with flowers and floricolous insects (Lachance *et al.*, 1998, 2000). Flowers of *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 *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 Gorodkova agars, but no asci or signs of conjugation were observed.

Latin diagnosis of *Wickerhamomyces queroliae* Rosa, Morais, Lachance et 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 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 ner pseudomycelium non-formantur. Species homothallica. Asci inconjugati et stabiles, et habentes 2–4 ascospores petasiformes. Glucosum, maltosum et sucrosum fermentantur. Glucosum, maltosum, sucrosum, trehalosum, D-xylosum, L-arabinosum, D-ribosum, L-rhamnosum, ethanolum (lente), glycerolum, erythritolum, ribitolum, mannitolum, glucitolum, salicinum, acidum lacticum (variabile), acidum succinicum, acidum citricum (exiguum et lente), xylitolum et acidum gluconicum assimilantur, at non-galactosum, L-sorbosum, raffinolum, inulinum, melibiosum, lactosum, melezitosum, cellobiosum, amyllum solubile, D-arabinosum, methanolum, 2-propanolum, galactitolum,*

*myo-inositolum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetolum, ethyl acetat ner hexadecanum. Ethylaminum, lysinum, cadaverinum, natrium nitricum et natrium nitrosum assimilantur. Ad crescentiuam vitamini externa necessaria sunt. Augmentum in 37 °C. Habitat congregationem *Anastrepha mucronata* in Brazil. Typus UFMG-05-T200.1^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10936^T typus stirps deposita est.*

Description of *Wickerhamomyces queroliae* Rosa, Morais, Lachance & Pimenta sp. nov.

Wickerhamomyces queroliae (que.ro'li.ae. N.L. gen. fem. sing. n. *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, pseudomycelia or true mycelia 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

Fig. 2. Neighbour-joining tree based on large-subunit rRNA genes showing the placement of *Candida jalapaonensis* among related species in the *Wickerhamiella* clade. Bootstrap values greater than 50 % are shown. Bar, 5 substitutions per 100 nucleotides.

Fig. 3. Phase-contrast micrographs of cells of *Wickerhamomyces queroliae* UFMG-05-T200.1^T 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. Bars, 5 µm.

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 100 µg 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-T200.1^T, 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 10936^T (=NRRL Y-48478^T).

Latin diagnosis of *Candida jalapaonensis* Rosa, Morais, Lachance et 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. Ascospores non-formantur. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, ethanolum (exiguum), glycerolum, mannitolum, glucitolum, acidum succinicum, acetonum et hexadecanum assimilantur, at non-sucrosum, maltosum, trehalosum, raffinolum, inulinum, melibiosum, lactosum, melezitolum, cellobiosum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, methanolum, 2-propanolum, erythritolum, ribitolum, galactitolum, myo-inositolum, 2-ketogluconatum, salicinum, acidum lacticum, acidum citricum, xylitolum, glucosaminum, N-acetylglucosaminum, acidum gluconicum ner ethyl acetat. Ethylaminum, lysinum et cadaverinum assimilantur at non-natrium nitricum et natrium nitrosum. Ad crescentiam vitamina externa necessaria sunt. Augmentum in 37 °C (variabile). Habitat floris in Brazil. Typus UFMG-03-T210^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 10935^T typus stirps deposita est.

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 Dalmat plates after 2 weeks on cornmeal agar, pseudomycelia 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-xylose, 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. 4. Phase-contrast micrograph of *Candida jalapaonensis* UFMG-03-T210^T 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-T210^T, 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 10935^T (=NRRL Y-48477^T).

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