Two novel yeast species were isolated from nectar of flower bracts of Heliconia psittacorum (Heliconiaceae) collected in a Cerrado ecosystem in the state of Tocantins, northern Brazil. *Wickerhamiella pagnoccae* sp. nov., which is closely related to *Candida jalapaonensis*, is heterothallic and produces one spheroid ascospore per ascus. *Candida tocantinsensis* sp. nov. belongs to the Metschnikowiaceae clade and its nearest relative is *Candida ubatubensis*, but the sequence identity (%) in the D1/D2 domains of the rRNA gene is low. The type strain of *W. pagnoccae* is UFMG-F18C1T (=CBS 12178T =NRRL Y-48735T) and the type strain of *C. tocantinsensis* is UFMG-F16D1T (=CBS 12177T =NRRL Y-48734T).
ecosystem is one of the richest tropical savannas and is considered to be a biodiversity ‘hotspot’. The Jalapão region is a protected area of almost 53 340.90 km² located in the eastern part of Tocantins. Although the Jalapão is within the Cerrado biome, this area is strongly influenced by neighbouring ecosystems such as the Amazon forest to the north, the Cerrado (Brazilian savanna) to the south and west and also the caatinga (semi-desert) to the east (Colli et al., 2009). The Formiga Falls area is close to the Formiga River within the sandy terrain of Jalapão State Ecological Park.

Fifteen flower bracts of H. psittacorum were collected in April 2009. The extrafloral nectaries were gently scraped with a sterile inoculation loop and streaked on plates of YM agar (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1.0 % glucose, 2 % agar and 0.02 % chloramphenicol). In Heliconia, within each bract there is a coil of inconspicuous flowers. The flowers are arranged in two whorls of three and form a lower lip subtended by ridged bracteoles; the septal nectaries are present in the syncarpous inferior ovary. Hence, although the actual sampled substrate was the nectary of each inflorescence, there may have been cross-contamination with microorganisms present in the tiny flowers. 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 for later identification. The yeasts were characterized using standard methods (Yarrow, 1998). Preliminary identities were determined using the keys of Kurtzman & Fell (1998). Sporulation was investigated using malt extract, glucose-yeast extract, yeast carbon base plus 0.01 % ammonium sulfate (YCBAS), dilute (1 : 9) V8, Fowell’s acetate and YM agars at 22 °C for up to 4 weeks.

The ITS, 5.8S rRNA gene and D1/D2 variable domains of the large subunit of the rRNA gene 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). Sequences were assembled, edited and aligned with the program MEGA4 (Tamura et al., 2007). Phylogenetic placements of the novel species were based on maximum-parsimony analysis of D1/D2 large-subunit rRNA gene sequences. The tree is a bootstrap consensus tree obtained using the Close-Neighbour-Interchange algorithm (Nei & Kumar, 2000) with search level 2 with initial trees obtained by random addition.

**Species delineation and ecology**

Several different yeast species were isolated from the H. psittacorum bracts. These species included *Aureobasidium pullulans*, *Candida flosculorum*, *Candida intermedia*, *Candida species*, *Cryptococcus species*, *Pichia fermentans*, *Pseudozyma antarctica*, *Pseudozyma hubeiensis* and *Wickerhamiella species*. The species identified as a member of the genus *Wickerhamiella* was isolated from 10 flowers and produced conjugated asci with one ascospore per ascus. Analysis of D1/D2 large-subunit rRNA gene sequences (Fig. 1) confirmed that this yeast belonged to the *Wickerhamiella* clade and that it was phylogenetically related to *Candida jalapaonensis*. This species differed from *Candida jalapaonensis* by seven nucleotide substitutions spread among six locations along

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**Fig. 1.** Phylogenetic placement of *Wickerhamiella pagnoccae* sp. nov. based on maximum-parsimony analysis of sequences of the D1/D2 domains of the large-subunit rRNA gene. The bootstrap consensus tree was inferred from 1000 replicates; bootstrap values are shown at nodes. The tree was obtained using the Close-Neighbour-Interchange algorithm (Nei & Kumar, 2000). Positions with less than 95 % site coverage were eliminated. The final alignment contained 486 positions. The program MEGA4 (Tamura et al., 2007) was used. Bar, number of nucleotide changes.
the sequence. The mating types of the novel species were crossed with the type strain of *Candida jalapaonensis*, but asci or signals of conjugation were not seen. The name *Wickerhamiella pagnoccae* sp. nov. is proposed to accommodate these isolates.

*W. pagnoccae*, a heterothallic, haplontic species, formed conjugation tubes and one spheroid ascospore per ascus after 20 h of incubation on dilute (1 : 9) V8 agar at 24 °C. The type strain, UFMG-F18C1T, was assigned arbitrarily to mating type *h* + of the species; six other isolates (UMFG-F1B1, F7A2, F8F1, F13A2, F20C3 and F22A1) have the same mating type. The designated allotype is strain UFMG-F8F2; five other strains (UMFG-F1C1, F7A3, A13A1, F20C3 and F22A2) have the same mating type (*h* -). As the mating types of the novel species appear to be distributed more or less equally, we conclude that the species is sexually active. *W. pagnoccae* differed from its nearest relative by its inability to assimilate galactose, glycerol, acetone and hexadecane, which are positive for *Candida jalapaonensis*, and its ability to grow on 10 % NaCl plus 5 % glucose medium. This novel species is the second nitrate-negative species in the genus (*Wickerhamiella lipothila* is the other nitrate-negative species); nitrate is assimilated by most species of the genus *Wickerhamiella* (Lachance & Kurtzman, 2011).

Two isolates of a species putatively assigned to the genus *Candida* were also obtained from two different flowers of *H. psittacorum*. The sequences of the D1/D2 domains showed that the species occupied a basal position in the Metschnikowiaceae clade (Fig. 2). The novel species differed by 43 or more substitutions in the D1/D2 sequence from other species and the phenetic sequence distance differed by 43 or more substitutions in the D1/D2 sequence of the *H. psittacorum*. The sequences of the D1/D2 domains of the large-subunit rRNA gene (Lachance & Kurtzman, 2011). In contrast, only two isolates of *Candida tocantinsensis* were obtained from 15 different samples of flower bracts of *H. psittacorum*, making it more difficult to speculate about the habitat of this novel species. However, other species in the same subclade have been isolated from *Heliconia* flower bracts, suggesting that this group of species may occur in *Heliconia* flower bracts and similar substrates in the Brazilian Cerrado ecosystem.

Both novel species were obtained from flower bracts of *H. psittacorum*, a plant species that possesses ephemeral flowers and long-standing bracts with extraloral nectaries. Flowers of *Heliconia* are a rich source of yeast species, as shown by Ruivo et al. (2006), who described *Candida heliconiae*, *Candida picinguabensis* and *Candida saopaulensis*, and Rosa et al. (2007), who reported the first occurrence of *Candida flosculorum* in *Heliconia velloziana* and *Heliconia episcopalis* in rainforests of south-eastern Brazil. It is likely that yeasts and bacteria are vectored by hummingbirds and insects to the nectar found in the bracts and grow in this substrate. As 13 strains of *W. pagnoccae* were isolated from 10 flower bracts of *H. psittacorum*, we infer that the yeast is autochthonous to this plant species in the Cerrado region of Jalapão, northern Brazil. Other known heterothallic *Wickerhamiella* species also occur in association with flowers (Lachance et al., 1998). *Wickerhamiella* species are highly specialized nutritionally and ecologically. The restricted physiology and the strong association with floricolous insects seem to be characteristic of most other members of the clade (Lachance & Kurtzman, 2011). In contrast, only two isolates of *Candida tocantinsensis* were obtained from 15 different samples of flower bracts of *H. psittacorum*, making it more difficult to speculate about the habitat of this novel species. However, other species in the same subclade have been isolated from *Heliconia* flower bracts, suggesting that this group of species may occur in *Heliconia* flower bracts and similar substrates in the Brazilian Cerrado ecosystem.

**Latin diagnosis of Wickerhamiella pagnoccae**

**Barbosa, Morais, Morais, Rosa, Pimenta, Lachance & Rosa sp. nov.**

*In medio liquido glucosum et extractum levidinis post dies tres cellulae singulae aut binae, cellulae ovoidae (1.5–3.0 x 2.0–4.0 μm).* Post unum mensem sedimentum formatur. *Cultura in agar mali post dies 2 (25 °C) parva, convexa, glabra et candida.* In agar farinaceus Zea mays post dies 14 (17 °C) mycelium nec pseudomycelium non formantur. *Species*

Description of Wickerhamiella pagnoccae Barbosa, Morais, Morais, Rosa, Pimenta, Lanchance & Rosa sp. nov.

Wickerhamiella pagnoccae (pag.noc’ca.e. N.L. gen. nom. m. sing. n. pagnoccae of Pagnocca, in recognition of his contributions to yeast systematics and ecology in Brazil). In 2 % glucose–0.5 % yeast extract broth after 3 days at 25 °C, cells are ovoid (1.5–3.0 × 2.0–4.0 μm), isolated or in pairs. 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 Dalmat plates after 2 weeks at 17 °C on cornmeal agar, pseudohyphae or true hyphae are not formed. Species is heterothallic. After 1 day on dilute (1:9) V8 agar, mixed strains of complementary mating types give rise to short conjugation tubes. Conjugated pairs, zygothe and asc containing one ascospore are also present (Fig. 3). Ascospores are liberated and agglutinated. Glucose is not fermented. Assimilates the following carbon compounds: glucose, L-sorbose, D-mannitol, D-glucitol and succinic acid. No growth occurs on galactose, maltose, sucrose, trehalose, raffinose, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol, salicin, lactic acid, citric acid, xylitol, gluconic acid, inulin, melibiose, lactose, melezitose, cellobiose, soluble starch, methanol, 2-propanol, galactitol, myo-inositol, 2-ketogluconate, D-glucoamine, N-acetylg glucosamine, acetone, ethyl acetate or hexadecane. Assimilates lyeine, but not ethylamine hydrochloride, cadaverine, nitrate or 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 % NaCl is positive. Growth in 50 % glucose–0.5 % yeast extract 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 flower bracts of Heliconia psittacorum collected in a riparian area of Cerrado ecosystem, in the state of Tocantins, Brazil.

The type strain (h+) is UFMG-F18ClT (=CBS 12178T =NRRL Y-48735T), isolated from flowers of Heliconia psittacorum collected in the riparian area of Formiga Falls, Brazil. The designated allotype (h−), UFMG-F8F2 (=CBS 12179=NRRL Y-48736), was recovered from flower bracts of H. psittacorum at the same collection site.

Latin diagnosis of Candida tocantinsensis Barbosa, Morais, Morais, Rosa, Pimenta, Lanchance & Rosa sp. nov.

In medio liquido glucosum et extractum levidinis post dies tres cellulae singulaires aut binae, cellulae ovoidae (2.0–3.0 × 3.0–4.0 μm). Post unum mensem sedimentum formatum. 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 formatur. Ascoporae non formatur. Glucosum fermentatur. Glucosum, galactosum, L-sorbosum, maltosum, sucrosum, trehalosum (lente), melezitosum, D-xylose (exigve), D-arabinosum (exigve), ethanolum, ribitolum, mannitolum, glucitolum, acidum succinicum et xylitolum assimilantur, at non raffinosum, L-arabinosum, D-ribose, L-rhamnosum, glycerolum, erythritolum, salicinum, acidum lacticum, acidum citricum, acidum gluconicum, inulinum, melibiosum, lactosum, cellobiosum, amyrum solubile, methanolum, 2-propanolum, galactitolum, myo-inositolum, 2-ketogluconatum, glucosaminum, N-acetylg glucosaminum, acetomon, ethyl acetum nec hexadecanum. Lysinum, ethylaminum et

Fig. 3. Phase-contrast micrographs of cells and asci of the complementary mating types of Wickerhamiella pagnoccae sp. nov. grown on dilute (1:9) V8 agar after 24 h. (a) Vegetative and conjugative cells; (b) vegetative cells and an ascus with an emerging ascospore; and (c) vegetative cells, asc, deliquesced asc and released ascospores. Bars, 5 μm.
In 2% glucose–0.5% yeast extract broth after 3 days at 25 °C. Habitat congregation in Heliconia psittacorum in Brazil. Typus: UFMG-F16D1T. In collectione zymotica Centraalbureau voor Schimmelcultures, Trajectum ad Rhenum, sub no. CBS 12177T typus stirps deposita est.

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

*Candida tocantinsensis* (to.can.tins.en’sis. N.L. nom. fem. sing. adj. tocantinsensis of or belonging to the state of Tocantins, Brazil, where this yeast was found).

In 2% glucose–0.5% yeast extract broth after 3 days at 25 °C, cells are ovoid (2.0–3.0 × 3.0–4.0 μm), isolated or in pairs. Budding is multilateral (Fig. 4). Sediment is formed after 1 month, but no pellicle is observed. In 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. Sexual spores are not observed. Glucose is fermented. Assimilates the following carbon compounds: glucose, galactose, L-sorbose, maltose, sucrose, trehalose (slow), melezitose, D-xylose (weak), D-arabinose (weak), ethanol, ribitol, mannitol, glucitol, succinic acid and xylitol. No growth occurs on raffinose, L-arabinose, D-ethanol, ribitol, mannitol, glucitol, succinic acid and myo-inositol, myo-inositol, 2-ketogluconate, glucosamine, N-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilates lysine, ethylamine hydrochloride and cadaverine; negative for nitrate and nitrite assimilation. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth on YM agar and on raffinose with 0.01% cycloheximide ml⁻¹ growth is positive. Urease activity is negative. Diazonium Blue B reaction is negative. Habitat is flower bracts of *Heliconia psittacorum* collected in a riparian area of Cerrado ecosystem, in the state of Tocantins, Brazil.

The type strain is UFMG-F16D1T (=CBS 12177T=NRRL Y-48734T), isolated from flowers of *H. psittacorum* collected in the riparian area of Formiga Falls, Brazil.

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**References**


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**Fig. 4.** Phase-contrast micrograph of cells of *Candida tocantinsensis* sp. nov. grown on yeast nitrogen base with 0.01% ammonium sulfate after 3 days. Bar, 5 μm.

