Wickerhamiella pagnoccae sp. nov. and Candida tocantinsensis sp. nov., two ascomycetous yeasts from flower bracts of Heliconia psittacorum (Heliconiaceae)

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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).

Flower bracts of plants of the genus Heliconia (Heliconiaceae) form a complex micro-environment containing several species of insects and micro-organisms (Ruivo et al., 2006). Heliconia, the single genus within the family Heliconiaceae, has a primarily neotropical distribution with approximately 215 species native to Central and South America and six species native to the South Pacific. Heliconia species are herbaceous monocots characterized by banana-like leaves, a pseudostem and inflorescences consisting of thick, coriaceous bracts that subtend partial florescences of cincinni (Schumann, 1900; Weberling, 1982). The actual flower emerges in an inflorescence from the large showy bracts, almost always at the end of long, leafy shoots. Flowers of New World taxa are morphologically specialized for hummingbird pollination (Stiles, 1975; Gill, 1987; Taylor & White, 2007).

In their natural habitat, Heliconia species typically occupy clearings on the forest floor in humid tropical rainforests, especially in places where sunlight can penetrate through the leaf canopy, and also along river banks. The bracts are often filled with water and house a distinctive aquatic micro-ecosystem colonized by insects and a diverse microbiota. The nectar in the bracts is the source of food for these organisms (Schnittler & Stephenson, 2002). The flower bracts are also visited by hummingbirds (Stiles, 1975). Insects and hummingbirds may act as vectors of the micro-organisms among different plants. Yeasts are components of the microbial communities associated with these flowers. Four Candida species, Candida flosculorum, Candida heliconiae, Candida picinguabensis and Candida saopaulonensis, have been isolated from flower bracts of Heliconia species in Brazil (Ruivo et al., 2006; Rosa et al., 2007).

During a study on yeasts associated with flowers, 13 isolates of an ascosporogenous yeast were obtained from 10 flower bracts of Heliconia psittacorum collected in the riparian forest of Formiga Falls, a Cerrado ecosystem in the State of Tocantins, Brazil. These strains are shown to represent a novel species; its closest relative was Candida jalapaonensis of the Wickerhamiella clade. Another two isolates of a novel asexual ascomycetous yeast species were also obtained from two flowers of H. psittacorum. This novel species belongs to the Metschnikowiaceae clade. The names proposed for the novel species are Wickerhamiella pagnoccae sp. nov. and Candida tocantinsensis sp. nov.

Samples of flower bracts of Heliconia psittacorum were collected in the riparian forest of Formiga Falls, a Cerrado ecosystem in the Jalapão Region, Brazil. The Cerrado...
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 micro-organisms 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

![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.](https://www.microbiologyresearch.org/doi/abs/10.1099/00169565-62-4-460)
the sequence. The mating types of the novel species were crossed with the type strain of Candida jalapaonensis, but ascii 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 $h^+$. The designated allotype is strain UFMG-F8F2; five other strains (UFMG-F1C1, F7A3, A13A1, F20C3 and F22A1) have the same mating type. The two isolates were examined (Image 51x52 to 363x214). 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 lipophila 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 could not be used to identify a sister species with any degree of confidence. The two isolates were examined either individually or mixed on cornmeal, V8, dilute V8, 5 % malt extract, yeast carbon base supplemented with 0.01 % ammonium sulfate, and Gorodkowa agars incubated at 20 °C or 28 °C for 30 days, but ascii or signs of conjugation were not seen. The name Candida tocatinsensis sp. nov. is proposed for these two isolates.

Both novel species were obtained from flower bracts of H. psittacorum, a plant species that possesses ephemeral flowers and long-standing bracts with extrafloral 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 saopaulonensis, 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 tocatinsensis 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**

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

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

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**Fig. 2.** Phylogenetic placement of *Candida tocatinsensis* sp. nov. based on maximum-parsimony analysis of sequences of the D1/D2 domains of the large-subunit rRNA gene. A bootstrap consensus tree from 100 replicates is shown; bootstrap values are shown at nodes. The tree was obtained using the Close-Neighbour-Interchange algorithm (Nei & Kumar, 2000) with search level 2 with initial trees obtained by random addition. A total of 374 positions were used in the analysis conducted with the program MEGA4 (Tamura et al., 2007). Bar, number of nucleotide changes.

Description of Wickerhamiella pagnoccaae
Barbosa, Morais, Morais, Rosa, Pimenta, Lachance & Rosa sp. nov.

Wickerhamiella pagnoccaae (pag.noc’ca.e. N.L. gen. nom. m. sing. n. pagnoccaae of Pagnocca, referring to Professor Fernando Carlos 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 opaque. In Dalmau 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, zygoites and asc containing one ascospore are also present (Fig. 3). Ascospores are liberated and agglutinate. Glucose is not fermented. Assimilates the following carbon compounds: glucose, L-sorosum, D-mannitol, D-glucitolum and succinic acid. No growth occurs on galactose, maltose, sucrose, trehalose, raffinose, D-xylose, L-arabinosum, D-arabinosum, D-ribose, L-rhamnosum, ethanol, glycerol, erythritolum, ribitol, salicin, lactic acid, citric acid, xylitol, gluconic acid, inulin, melibiosum, lactose, melezitose, cellobiosum, soluble starch, methanol, 2-propanolum, galactitolum, myo-inositolum, 2-ketogluconate, D-gluconate, N-acetylglucosamine, acetonom, ethyl acetate or hexadeceanum. Assimilates lysinum, but not ethylamine hydrochloride, cadaverinum, nitrato or nitritum. 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-F18Cl1T (=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, Lachance & Rosa sp. nov.

In medio liquido glucosum et extractum levidinis post dies tres cellulae singulares 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 pseudomyelium non formatur. Ascosporae non formatur. Glucosum fermentatur. Glucosum, galactosum, L-sorosum, maltosum, sucrorum, trehalosum (lente), melezitosum, D-xylosum (exigue), D-arabinosum (exigue), ethanolum, ribitolum, mannitolum, glucitolum, acidum succinicum et xylitolum assimilantur, at non raffinosum, L-arabinosum, D-ribosum, L-rhamnosum, glycerolom, erythritolum, salicinum, acidum lacticum, acidum citricum, acidum gluconicum, inulinum, melibiosum, lactosum, cellobiosum, amylose solubile, methanolum, 2-propanolum, galactitolum, myo-inositolum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetonom, ethyl acetate nec hexadeceanum. Lysinum, ethylaminum et

Fig. 3. Phase-contrast micrographs of cells and asc of the complementary mating types of Wickerhamiella pagnoccaae 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, asci, deliquesced asci and released ascospores. Bars, 5 μm.
Candida tocantinsensis (to.can.tins.en’sis. N.L. nom. fem. sing. adj. tocan.tins.en’sis 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 x 3.0–4.0 μm), isolated or in pairs. Budding is multilateral (Fig. 4). Sediment is formed after 3 days. Bar, 5 μm.

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.

cadaverinum assimilantur, non natrium nitricum nec natrium nitrosum. Augmentum in 37 °C. Habitat congregation in Heliconia psittacorum in Brazil. Typus: UFMG-F16D1T. In collectione zymotica Centraalbureau voor Schimmecultures, 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. tocantin.sen’sis 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 x 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. On YM agar after 2 weeks on cornmeal agar, pseudomycelium or true mycelia are not formed. Sexual spores are not observed. Glucose is fermented. Assimilates the following carbon compounds: glucose, galactose, L-sorbitose, maltose, sucrose, trehalose (slow), melezitose, D-xylene (weak), D-arabinose (weak), ethanol, ribitol, mannitol, glucitol, sucinic acid and xyitol. No growth occurs on raffinose, L-arabinose, D-ribose, L-rihamnose, glycerol, erythritol, salicin, lactic acid, citric acid, gluconate, inulin, melibiose, lactose, cellobiose, 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. 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.

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References


