Candida flosculorum sp. nov. and *Candida floris* sp. nov., two yeast species associated with tropical flowers

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Two ascomycetous yeast species, *Candida flosculorum* sp. nov. and *Candida floris* sp. nov., were isolated from tropical flowers and their associated insects. *C. flosculorum* was isolated from flower bracts of *Heliconia velloziana* and *Heliconia episcopalis* (Heliconiaceae) collected from two Atlantic rain forest sites in Brazil. *C. floris* was isolated from flowers of *Ipomoea* sp. (Convolvulaceae) growing on the banks of the river Paraguai in the pantanal ecosystem in Brazil and from an adult of the stingless bee *Trigona* sp. and a flower of *Merremia quinquefolia* (Convolvulaceae) in Costa Rica. *C. flosculorum* belongs to the Metschnikowiaceae clade and *C. floris* belongs to the *Starmerella* clade. The type strain of *C. flosculorum* is UFMG-JL13^T (=CBS 10566^T=NRRL Y-48258^T) and the type strain of *C. floris* is UWO(PS) 00-226.2^T (=CBS 10593^T=NRRL Y-48255^T).

Flowers of plant species belonging to the Convolvulaceae and Heliconiaceae families are a rich source of novel yeast species. Most of the novel yeast species isolated from these plants belong to the *Metschnikowia*, *Wickerhamiella* and *Starmerella* clades. In ephemeral flowers of the Convolvulaceae, the yeasts are transported by pollinating and non-pollinating flies, beetles and bees that deposit them in the corolla. In the longer-lasting flowers of the Heliconiaceae, yeasts are probably introduced by a different and more diverse set of animal vectors and they may grow on the sugary compounds present in nectar (Lachance *et al.*, 1998, 1999, 2001; Ruivo *et al.*, 2006; Rosa *et al.*, 2007).

In this paper, we describe two novel yeast species associated with flowers and related insects of these two plant families. The first species was found inhabiting flowers of *Heliconia velloziana* and *Heliconia episcopalis* in two Atlantic rain forest sites in Brazil. The second species was isolated from flowers of *Ipomoea* sp. on the banks of the river Paraguai,

Brazil, and from a stingless bee and a flower of *Merremia quinquefolia* (wood rose) in Costa Rica. Analysis of the sequences of the D1/D2 domains of the large-subunit rDNA showed that these species represent distinct species of the Metschnikowiaceae and *Starmerella* clades, respectively. These novel species are described as *Candida flosculorum* sp. nov. and *Candida floris* sp. nov.

The strains considered in this study are listed in Table 1. Two strains of *C. flosculorum* were isolated from nectar present in flower bracts of *H. velloziana*, collected in the Picinguaba area, an Atlantic rain forest site at the Serra do Mar state park in São Paulo state, in September 2000 (Ruivo *et al.*, 2006). Five strains came from flower bracts of *H. episcopalis* in Parque Estadual do Rio Doce, an Atlantic rain forest in the state of Minas Gerais, collected in July 2002. Isolates of *C. floris* were obtained from flowers of *Ipomoea* sp. collected at the banks of the Paraguai river near the city of Corumbá, in the state of Mato Grosso do Sul, Brazil, in an area of pantanal ecosystem, in February 2002, and from a stingless bee (*Trigona* sp.) and a flower of *M. quinquefolia* collected in 2000 and 2001, respectively, in Santa Rosa National Park, Costa Rica. The yeasts were isolated on yeast extract-malt extract agar (YMA, 1 %

The GenBank/EMBL/DDBJ accession numbers for the gene sequences of the D1/D2 domain of the 26S rDNA of strains UWO(PS) 00-226.2^T and UFMG-JL13^T are AF313353 and EF137918, respectively.

Table 1. Sources of isolation of *Candida flosculorum* and *Candida floris*

UFMG, Universidade Federal de Minas Gerais, Brazil; CBS, Centraalbureau voor Schimmelcultures; UWO(PS), Yeast Culture Collection of the Department of Biology, University of Western Ontario, Canada.

Name	Source	Strain number	Location
<i>C. flosculorum</i>	Flower bract of <i>Heliconia episcopalis</i>	UFMG-JL11, UFMG-JL13 ^T (=CBS 10566 ^T), UFMG-JL36, UFMG-JL45, UFMG-JL52	Parque Estadual do Rio Doce Atlantic rain forest, state of Minas Gerais, Brazil
	Flower bract of <i>H. velloziana</i>	UNESP-00-91, UNESP-00-92	Picinguaba Atlantic rain forest site, Parque Estadual da Serra do Mar, state of São Paulo, Brazil
<i>C. floris</i>	Flowers of <i>Ipomoea</i> sp.	UFMG-C16, UFMG-C37, UFMG-C139, UFMG-C141, UFMG-C144, UFMG-C151	Paraguai River, pantanal ecosystem, state of Mato Grosso do Sul, Brazil
	<i>Trigona</i> sp. from <i>Merremia quinquefolia</i>	UWO(PS) 00-226.2 ^T (=CBS 10593 ^T)	Santa Rosa National Park, Guanacaste, Costa Rica
	Flower of <i>M. quinquefolia</i>	UWO(PS) 01-159.2	Santa Rosa National Park, Guanacaste, Costa Rica

glucose, 0.5 % peptone, 0.3 % yeast extract, 0.3 % malt extract and 2 % agar) containing 100 mg chloramphenicol l⁻¹. Sampling methods were as described previously (Lachance *et al.*, 1998; Rosa *et al.*, 2003; Ruivo *et al.*, 2006). Plates were incubated at room temperature (25 ± 3 °C) for 3–8 days. Each different yeast morphotype was purified and maintained on YMA slants or liquid nitrogen for later identification. The yeasts were characterized by standard methods (Yarrow, 1998). Identifications followed the keys of Kurtzman & Fell (1998).

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 6.0 (Lynnon BioSoft). Existing sequences for other related yeasts were retrieved from GenBank. The basic local alignment search tool (BLASTN) available at <http://www.ncbi.nlm.nih.gov> was used for this task. The CLUSTAL W software (Thompson *et al.*, 1994) incorporated

in DNAMAN was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations.

Species delineation, classification and ecology

Candida flosculorum belongs to the Metschnikowiaceae clade and is phylogenetically related to *Candida* sp. BCC 7717, an undescribed species isolated from plant materials in Thailand (Fig. 1). These species showed more than 10 % sequence divergence in the D1/D2 regions of the 26S rDNA large-subunit. The sequences of the D1/D2 domains of three isolates of *C. flosculorum* (UFMG-JL13^T, UFMG-JL52 and UNESP-00-92) were identical. All isolates of *C. flosculorum* had identical physiological profiles. *C. flosculorum* was isolated from two different *Heliconia* species and may be widely distributed in tropical rain forests. The *Heliconia* species sampled in our work occur in South-eastern Brazil and in Amazonia and *H. episcopalis* is also used as an ornamental plant in Florida, Hawaii and Costa Rica. Several insects, mainly species of Diptera, feed on the plant leaves and bracts and may be the most important vectors of this yeast. Several species of *Merosargus* (Diptera) use bracts and flowers of *H. episcopalis* for

Fig. 1. Neighbour-joining tree showing the placement of *Candida flosculorum* among related species in the Metschnikowiaceae clade. Bootstrap values of 50 % or above are shown. Bar, 0.05 substitutions per nucleotide position.

feeding and breeding (J. C. R. Fontenelle, unpublished data). Other possible vectors of the yeast community associated with the nectar of *Heliconia* bracts are species of hummingbirds. In the Parque Estadual do Rio Doce, four species of hummingbirds are known to visit *H. episcopalis* flowers. These include *Glaucis hirsuta* and *Phaethornis idaliae* (A. C. F. Alves & J. C. R. Fontenelle, unpublished data). Three other novel yeast species (*Candida heliconiae*, *Candida picinguabensis* and *Candida saopaulonensis*) were isolated from *H. velloziana* by Ruivo *et al.* (2006). This result suggests that *Heliconia* species may be an interesting yeast habitat that deserves further study.

Candida floris is related to *Candida etchellsii*, as these species showed 5.8% sequence divergence in the D1/D2 region of the rDNA large-subunit. Both species belong to the *Starmerella* clade (Fig. 2). Strain UWO(PS) 00-226.2^T differed by two substitutions in the D1/D2 region from the Brazilian strain UFMG-C151 (GenBank accession number EF679791). Our strains of *C. floris* were isolated directly from Convolvulaceae flowers and from a meliponine (stingless) bee captured on such a flower in Brazil and in Costa Rica. An association of *C. floris* with stingless bees that visit Convolvulaceae flowers is not unexpected as these insects are known to be an important source of yeast species in the *Starmerella* clade and are frequent visitors to these flowers. A mutually beneficial interaction may exist between them (Rosa *et al.*, 2003; Pimentel *et al.*, 2005).

Isolates of *C. floscolorum* and *C. floris* were examined after growth, individually or mixed in pairs, on several common

sporulation media (cornmeal agar, dilute V8 agar, 5% malt extract agar and yeast carbon base agar supplemented with 0.01% ammonium sulphate, among others), but asci or signs of conjugation were not seen. These species probably occur in nature in the asexual form.

Latin diagnosis of *Candida floscolorum* Rosa, Pagnocca, Lachance, Ruivo & Medeiros sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (2–3 × 2–5 µm). *Post unum mensem sedimentum formatur. Cultura in agar malti post dies 14* (17 °C) *parva, convexa, glabra et candida. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formantur. Glucosum fermentatur. Glucosum, sucrosus, maltosus, cellobiosus, melezitosis (lente), L-sorbusus, D-xylosus (lente), glycerolus, ribitolus, xylitolus, mannitolus, glucitolus, acidus succinicus, acidus citricus, 2-keto-glucuronatus assimilantur, at non galactosus, trehalosus, inulinus, raffinosis, melibiosus, lactosus, methyl α-D-glucosidus, amylosus solubilis, L-rhamnosus, L-arabinosus, D-arabinosus, D-ribosus, ethanolus, methanolus, erythritolus, galactitolus, meso-inositolus, acidus lacticus, acidus gluconicus, glucosaminus, N-acetylglucosaminus, acetonus, ethyl acetatus, nec hexadecanus. Ethylaminus, lycinus et cadaverinus assimilantur at non natrium nitricus nec natrium nitrosus. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C. Habitat floris *Heliconia episcopalis* et *H. velloziana* in Brazil. Typus UFMG-JL13^T (=NRRL Y-48258^T=CBS 10566^T)*

Fig. 2. Neighbour-joining tree showing the placement of *Candida floris* among related species in the *Starmerella* clade. Bootstrap values of 50% or above are shown. Bar, 0.05 substitutions per nucleotide position.

Description of *Candida flosculorum* Rosa, Pagnocca, Lachance, Ruivo & Medeiros sp. nov.

Candida flosculorum (flos.cu.lo'rum. L. gen. masc. pl. n. *flosculorum* of little flowers, refers to florets or flowers from where most of the strains were isolated).

In yeast extract-glucose broth (0.5%:2%, w/v) after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–5 µm). Budding is multilateral (Fig. 3a). A sediment is formed after a month, but no pellicle is observed. On YMA after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. Glucose is fermented. The following carbon compounds are assimilated: glucose, sucrose, maltose, L-sorbose, cellobiose, melezitose (slow), D-xylose, glycerol, salicin and xylitol. No growth occurs on galactose, trehalose, inulin, raffinose, melibiose, lactose, methyl α -D-glucoside, soluble starch, L-rhamnose, D-arabinose, L-arabinose, D-ribose, ribitol, ethanol, methanol, 2-propanol, 2-propanol, erythritol, galactitol, D-mannitol, D-glucitol, succinic acid, citric acid, lactic acid, gluconic acid, *myo*-inositol, glucosamine, *N*-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine and negative for 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 YMA with 5% sodium chloride is positive, but negative at 10% sodium chloride. No growth in glucose-yeast extract broth (50%:0.5%, w/v). Starch-like compounds are not produced. No growth with 1000 µg cycloheximide ml⁻¹. Urease activity is negative. Diazonium blue B reaction is negative. The habitat is flower bracts of *Heliconia* spp. in Brazil.

The type strain of *Candida flosculorum*, UFMG-JL13^T (=NRRL Y-48258^T=CBS 10566^T), was isolated from a flower bract of *H. episcopalis* in the state of Minas Gerais, Brazil.

Fig. 3. Phase-contrast micrograph of budding cells of *Candida flosculorum* UFMG-JL13^T (a) and *C. floris* UWO(PS) 00-226.2^T (b) on yeast carbon base agar with 0.01% ammonium sulphate after 3 days at 22 °C. Bar, 5 µm.

Latin diagnosis of *Candida floris* Rosa, Pagnocca & Lachance sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (2–3 × 2–4 µm). *Post unum mensem sedimentum formatur. Cultura in agar malti post dies 14* (17 °C) *parva, convexa, glabra et candida. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formantur. Glucosi fermentatio variabilis. Glucosum, galactosum, L-sorbosum, glycerolum (aliquando exigue), xylitolum (lente), mannitolum, glucitolum et acidum gluconicum assimilantur, at non sucrosus, trehalosus, maltosus, melezitosus, inulinus, raffinosis, melibiosis, lactosus, methyl α -D-glucosidus, amyllum solubile, cellobiosus, salicinus, D-xylosus, L-arabiosus, L-rhamnosus, D-arabiosus, D-ribosus, ethanolum, methanolum, erythritolum, galactitolum, ribitolum, meso-inositolum, acidum lacticum, acidum succinicum, acidum citricum, 2-keto-gluconatum, glucosaminum, N-acetylglucosaminum, xylitolum, acetone, ethyl acetas, nec hexadecanum. Ethylaminum, lysinum et cadaverinum assimilantur at non natrium nitricum nec natrium nitrosus. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C. Habitat floris in Brazil et Costa Rica. Typus UWO(PS) 00-226.2^T (=CBS 10593^T=NRRL Y-48255^T).*

Description of *Candida floris* Rosa, Pagnocca & Lachance sp. nov.

Candida floris (flo.ris. L. gen. masc. n. *floris* of a flower, from where this yeast was isolated).

In yeast extract-glucose (0.5%:2%, w/v) broth after 3 days at 25 °C, the cells are ovoid to ellipsoidal (2–3 × 2–4 µm). Budding is multilateral (Fig. 3b). A sediment is formed after a month, but no pellicle is observed. On YMA after 2 days at room temperature, colonies are white, convex, smooth and opalescent. In Dalmau plates after 2 weeks on cornmeal agar, pseudomycelia or true mycelia are not formed. Glucose fermentation is variable. The following carbon compounds are assimilated: glucose, galactose (variable), L-sorbose (variable) glycerol (occasionally weak), D-mannitol, D-glucitol and D-gluconate. No growth occurs on inulin, raffinose, melibiose, sucrose, trehalose, maltose, lactose, melezitose, D-xylose, L-arabinose, ethanol, ribitol, xylitol, succinic acid, citric acid, methyl α -D-glucoside, soluble starch, cellobiose, salicin, L-rhamnose, D-arabinose, D-ribose, methanol, 2-propanol, 2-propanol, erythritol, galactitol, *myo*-inositol, lactic acid, glucosamine, *N*-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: positive for lysine, ethylamine-HCl and cadaverine, and negative for 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 YMA with 10% sodium chloride is positive. Growth in yeast extract-glucose (0.5%:50%, w/v) broth is weak to strong. Starch-like compounds are not produced. Growth is positive in 1000 µg cycloheximide ml⁻¹. Urease activity is negative.

Diazonium blue B reaction is negative. The habitat is flowers of *Ipomoea* sp. and *M. quinquefolia* in Brazil and Costa Rica.

The type strain of *Candida floris*, UWO(PS) 00-226.2^T (=CBS 10593^T=NRRL Y-48255^T), was isolated from an adult of the stingless bee *Trigona* sp. from a *M. quinquefolia* flower in Costa Rica.

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