***Candida orba* sp. nov., a new cactus-specific yeast species from Queensland, Australia**

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A new species of yeast from decaying cladodes of *Opuntia* cactus, *Candida orba*, is described. This species is a member of a four-species clade of cactophilic yeasts. The new species has only been found in one region of Queensland, Australia, where it was presumably introduced during attempts to eradicate prickly pear cactus. DNA–DNA relatedness, phylogenetic analysis, physiological differences, killer-sensitivity profiles and mating reactions establish the distinctness of the taxon as a new species. *C. orba* is most closely related to *Phaffomyces thermotolerans*, a species found associated with columnar cacti in the North American Sonoran Desert. The type strain of *C. orba*, isolated from rotting cladodes of *Opuntia stricta* in the State of Queensland, Australia, is strain UCD-FST 84-833.1^T (= CBS 8782^T = NRRL Y-27336^T = ATCC MYA-341). Only the h⁺ mating type of the species has been recovered. The lack of the opposite mating type could be the result of a bottleneck during its introduction to Australia. The original geographic/host distribution of this species in the Americas is unknown.

Keywords: *Candida orba* sp. nov., large subunit rDNA analysis, yeasts, DNA–DNA hybridization, phylogeny of *Phaffomyces* spp.

INTRODUCTION

Following an extensive survey of yeasts associated with cactophilic *Drosophila* and their cereoid host plants in the American Sonoran Desert by Heed *et al.* (1976), an extended survey was made of decaying *Opuntia* cladode rots in Australia, an as yet unexplored habitat for cactophilic yeasts (Barker *et al.*, 1983, 1984). Starmer *et al.* (1979) described a new heterothallic yeast, *Pichia opuntiae*, that was repeatedly isolated from *Opuntia* necroses in widely separated areas of eastern Australia. These authors also described a closely related yeast found in cereoid cactus rots from various locations in the North American Sonoran Desert, which they named *Pichia opuntiae* var. *thermotolerans*, resulting in two varieties of *P. opuntiae*, differing in physiology, habitat and geographic distribution. Subsequently, Holzschu *et al.* (1985) raised *P. opuntiae* var. *thermotolerans* to the rank of species based on low DNA relatedness (approx. 28% between the two varieties), different biogeography and host plants, and differences in

electrophoretic mobilities of 12 metabolic enzymes. Starmer *et al.* (1984) described another heterothallic species, *Pichia antillensis*, that was most closely related to *P. opuntiae* (DNA relatedness about 50%) and more distantly to *Pichia thermotolerans* (about 26%). Its occurrence in columnar cacti on several islands in the West Indies of the Caribbean Sea separated the three species geographically, by host plant and by a few physiological characteristics, including the production of killer toxins by *Pichia antillensis* but not by the other two species.

Yamada *et al.* (1997) reported that *P. opuntiae* and *P. thermotolerans* were only distantly related to *Pichia membranifaciens* (the type species of the genus *Pichia*) based on a comparison of partial base sequences of 18S rRNA and proposed a new genus, *Phaffomyces*, for *P. opuntiae* and *P. thermotolerans*. In a subsequent paper, Yamada *et al.* (1999) also transferred *P. antillensis* to the genus *Phaffomyces* based on the complete sequences of 18S rRNA fragments and showed that the genus *Phaffomyces* is monophyletic and distinct from other ascomycetous genera.

Their conclusions are in agreement with the placement of the above three species in a small clade very distant

The GenBank accession number for the 26 rDNA D1/D2 sequence of *Candida orba* UCD-FST 84-833.1^T is AF229034.

from *P. membranifaciens* by Kurtzman & Robnett (1998), based on analysis of 26S rRNA partial sequences.

In the present paper, yet another species belonging to the above group is described that has about 50% DNA relatedness to *Phaffomyces thermotolerans* and approximately 25% to both *Phaffomyces opuntiae* and *Phaffomyces antillensis*. Currently, 14 strains of this species have been isolated from *Opuntia* necroses in the State of Queensland, Australia. All of the isolates were found to be of the single mating type h^- based on their interaction with the two mating types of *P. antillensis* (see below). The name *Candida orba* is proposed for the new species, the specific epithet reflecting that currently the species is deprived of its complementary mating type h^+ .

METHODS

Strain isolation. Samples of necrotic cactus tissue yielding the new yeast were collected in 1978, 1984 and 1996 from *Opuntia* species in several locations in Queensland, Australia. The techniques used for yeast isolation have been described by Starmer & Phaff (1983). Morphological and physiological characteristics of the isolates were determined by methods currently used in yeast taxonomy (Yarrow, 1998). Among the many strains isolated, 14 strains were representative of *Candida orba* (Table 1).

DNA base composition. Nuclear DNA base composition determination of strains was carried out by the buoyant density method in CsCl as described by Price *et al.* (1978).

DNA purification and procedure for reassociation. DNA extraction and purification were carried out by using the methods described by Price *et al.* (1978) except that the

Table 1 Strain numbers, host plants and locations of *C. orba* isolates

Strain*	Host plant	Location†
UCD 78-529.3	<i>Opuntia stricta</i>	Grandchester
UCD 84-833.1*	<i>Opuntia stricta</i>	Hemmant
UCD 84-837.1	<i>Drosophila buzzatii</i>	Hemmant
UCD 84-860.2	<i>Opuntia stricta</i>	Hemmant
UCD 84-853.3	<i>Opuntia stricta</i>	Hemmant
UCD 84-866.1	<i>Opuntia stricta</i>	Hemmant
UCD 99-1	<i>Opuntia stricta</i>	Hemmant
UCD 99-2	<i>Opuntia tomentosa</i>	Marburg-Minden
UCD 99-3	<i>Opuntia tomentosa</i>	Brigalow
UCD 99-4	<i>Opuntia tomentosa</i>	Brigalow
UCD 99-5	<i>Opuntia tomentosa</i>	Brigalow
UCD 99-6	<i>Opuntia tomentosa</i>	Brigalow
UCD 99-7	<i>Opuntia tomentosa</i>	Brigalow
UCD 99-8	<i>Opuntia tomentosa</i>	Brigalow

* UCD (= UCD-FST), Culture Collection of the Department of Food Science & Technology, University of California, Davis, CA, USA.

† All locations are in the State of Queensland, Australia.

Table 2 Differences in the physiological abilities of the three *Phaffomyces* species and *C. orba*

Species: 1, *P. antillensis*; 2, *P. opuntiae*; 3, *P. thermotolerans*; 4, *C. orba*. +, Growth is good under those conditions; l (latent), growth is good, but occurs after 7 d; w, growth is weak, but not latent; –, no growth occurs; combined symbols, e.g. l/w, some strains are latent and some strains are weak.

Characteristic	1	2	3	4
Cellobiose	–	l/–	+	+
Salicin	–	+	+	+
L-Rhamnose	l	l/w	l/w	–
D-Mannitol	l/w/–	l/–	l	l/–
Citrate	+	+	–	–
Cycloheximide (1 µg ml ⁻¹)	+	–	–	–
Triterpene glycosides (2%) at 20 °C	–	–	–	+
Triterpene glycosides (2%) at 30 °C	–	–	+	+
33 °C	+	+/-	+	+
37 °C	+/-	–	+	–
39 °C	+/-	–	–	–

purified DNA was concentrated by electrophoresis in a concentrator (ISCO) and the reference DNA was further purified by banding in a CsCl gradient using a model L ultracentrifuge prior to labelling with ¹²⁵I as described by Holzschu *et al.* (1979). Denaturation of DNA, incubation for reassociation and analysis of the renaturation kinetics, reannealing reactions, and quantification of single- and double-stranded DNAs were done following the protocol of Price *et al.* (1978). Samples were counted with a gamma ray counter (90% efficiency).

DNA sequence analysis. The D1 and D2 variable domains of the large subunit rDNA were amplified by PCR from whole cells as described by Lachance *et al.* (1999). The amplified DNA was concentrated and cleaned on Qiaquick PCR columns (Qiagen) and sequenced in an ABI sequencer at the John P. Robarts Research Institute, London, Ontario, Canada. The sequences were edited with the program DNAMAN version 4.0 (Lynnon BioSoft). Existing sequences for other yeasts were retrieved from GenBank, where they had been deposited by Kurtzman & Robnett (1998). The CLUSTAL W (Thompson *et al.*, 1994) algorithm provided in the DNAMAN package was used to align the sequences and construct a neighbour-joining tree with 1000 bootstrap iterations.

Detection of killer strains. Testing was done according to Starmer *et al.* (1987). YM (Difco) agar buffered to pH 4.2 with 0.05 M citrate buffer and containing 0.003% (w/v) methylene blue was the assay medium. Yeast strains to be tested for sensitivity to potential killer strains were spread to a density of approximately 10⁴ cells per plate. Potential killer strains were inoculated onto the lawn at high density. Both lawn and test strains were taken from exponentially growing cultures (1–3 d after inoculation). Incubation was done at 22 °C for 2–3 d.

Fig. 1. Dendrograms of the four species relationships based on Euclidean distances: DNA distance [100–relative binding (%)], physiological distance (data from Table 2), mean taxon responses for killer abilities (rows of Fig. 2) and sensitivity (columns of Fig. 2). Bars, Euclidean distances for each character.

Resistance to triterpene glycosides. Ability of yeasts to grow at 25 °C in the presence of toxic triterpene glycosides, which are present in cactus species belonging to the subtribe Stenocereinae, was tested in YM agar supplemented with 1 or 2% dried pulverized tissue of *Stenocereus gummosus* (agria) (Starmer *et al.*, 1980). The cactus powder may be replaced by 8 mg digitonin l⁻¹ (Sigma).

Distance determination. Comparisons among the four species for DNA relatedness, physiological abilities, killer ability and killer sensitivity were made by calculating distance matrices for each trait and using a least-squares fitting program (FITCH of PHYLIP 3.5c; Felsenstein, 1989) to construct dendrograms. All distances were determined as Euclidean distances. DNA distances between taxa were calculated as 100–relative binding (%) from data in the literature (Starmer *et al.*, 1984; Holzschu *et al.*, 1985) and Table 4. Physiological distance was determined by using Table 2 to assign relative scores (+ = 4; 1 = 2; w = 1; – = 0) for each trait. In a similar manner, the distance between taxa for killer ability was calculated by assigning relative scores (strong = 3; moderate killing = 2; weak killing = 1; no killing = 0) for the mean within taxon responses given in Fig. 1. Sensitivity to killer toxin was computed by comparing pairs of columns, whereas killer activity was computed by comparing pairs of rows.

RESULTS

Strains of the new species described here were originally recognized as atypical isolates of *Pichia opuntiae* that differed phenotypically from that species by their good growth on cellobiose and lack of growth on citrate as sole carbon sources. Table 2 lists the salient physiological differences among the three species of *Phaffomyces* and *C. orba*. A dendrogram (unrooted),

produced using Euclidean distances based on those physiological differences (Fig. 1) among the four species, shows *C. orba* to be most similar to *P. thermotolerans*, while *P. opuntiae* and *P. antillensis* form another similar pair. All strains of *C. orba* were found to be killer strains of *Candida glabrata* (Y-55 = NCYC 388; obtained from T. W. Young) in contrast to *P. opuntiae*, which does not kill *C. glabrata*. However, all species of the *Phaffomyces* clade make mycocins that kill other yeasts and exhibit cross-killing activity. Table 3 lists four strains of each species that were used in reciprocal killer tests. These tests, depicted in Fig. 2, show *C. orba* is a killer of *P. opuntiae* and *P. antillensis*, but does not kill *P. thermotolerans*.

The proposed transfer of *Pichia opuntiae*, *Pichia thermotolerans* and *Pichia antillensis* to the new genus *Phaffomyces* by Yamada *et al.* (1997, 1999) has been accepted in this report. The present paper provides a description of an additional species belonging to this clade, for which the name *Candida orba* is proposed. The new species has been isolated repeatedly from *Opuntia* necroses and associated *Drosophila* in several locations in Queensland, Australia (Table 1). Attempts to obtain sporulation of *C. orba* strains on various sporulation media were unsuccessful. Since all species of *Phaffomyces* are heterothallic, the *C. orba* strains were mixed in all possible combinations on sporulation media, but no evidence of conjugation or sporulation was found. Mating the strains with mating types of the three *Phaffomyces* species was attempted and it was found that all strains of *C. orba* produced zygotes on dilute (25%) V-8 juice agar with the h⁺ mating strain of *P. antillensis*, but not with the h⁻ mating type of that species. Spores, however, were not observed in the zygotes or in the mixed cultures after extended incubation. Strains of the new species are therefore assigned the mating type h⁻.

Confirmation of the novelty of *C. orba* was obtained from a DNA hybridization experiment involving ¹²⁵I-labelled DNA from strain 84-833.1^T of *C. orba* and unlabelled DNAs from the species of *Phaffomyces*. The results, presented in Table 4, clearly show that *C. orba* is distinct from the members of the genus *Phaffomyces* and represents a new species.

The phylogenetic relationships among members of the genus *Phaffomyces* and *C. orba* based on the D1/D2 divergent domains of the large ribosomal subunit are shown in Fig. 3. *C. orba* exhibited 9.8% sequence divergence (53 base substitutions) from its sister species *P. thermotolerans*, confirming that the two taxa represent related, but distinct evolutionary lineages. *C. orba* differs from *P. opuntiae* and *P. antillensis* by 67 and 70 base substitutions, respectively, in the D1/D2 domain.

Latin description of *Candida orba* sp. nov.

In medio liquido cum glucoso, peptono et extracto levedinis (post dies 3 ad 30 °C), cellulae ovoideae, elongatae vel clavatae, 2–6 × 3–10 µm, singulae, binae,

Table 3 Representative strains of the four species used in the cross-killing study, their host source and locality of isolation

Species and strain	Designation in Fig. 2	Host plant	Locality
<i>P. opuntiae</i>			
84-677.2	opt-1	<i>Opuntia stricta</i>	O'Hara, NSW, Australia
84-684.3	opt-2	<i>Opuntia stricta</i>	O'Hara, NSW, Australia
84-869.3	opt-3	<i>Opuntia tomentosa</i>	Warwick, Qld, Australia
84-876.1	opt-4	<i>Opuntia tomentosa</i>	Warwick, Qld, Australia
<i>C. orba</i>			
85-1	orb-1	<i>Opuntia stricta</i>	Borallon, Qld, Australia
84-833.1 ^T	orb-2	<i>Opuntia stricta</i>	Hemmant, Qld, Australia
84-860.2	orb-3	<i>Opuntia stricta</i>	Hemmant, Qld, Australia
84-866.1	orb-4	<i>Opuntia stricta</i>	Hemmant, Qld, Australia
<i>P. antillensis</i>			
82-550A	ant-1	<i>Cephalocereus royenii</i>	Montserrat, Lesser Antilles
82-592B	ant-2	<i>Cephalocereus royenii</i>	Beef Island, Tortola, BVI
82-589B	ant-3	<i>Cephalocereus royenii</i>	Beef Island, Tortola, BVI
82-568B	ant-4	<i>Cephalocereus royenii</i>	Montserrat, Lesser Antilles
<i>P. thermotolerans</i>			
83-501.1	thr-1	<i>Carnegiea gigantea</i>	Ironwood, Tucson, AZ, USA
83-505.1	thr-2	<i>Carnegiea gigantea</i>	Ironwood, Tucson, AZ, USA
83-510.1	thr-3	<i>Carnegiea gigantea</i>	Ironwood, Tucson, AZ, USA
83-124	thr-4	<i>Stenocereus thurberi</i>	Organpipe Natl Monument, AZ, USA

Fig. 2. Cross-killing responses of strains (Table 3) of the three *Phaffomyces* species and *C. orba*.

gregatim cellularum 6–10, *pellicula nulla*. *Post dies 7 pellicula nitida madita crescit*. *Cultura in agar malti (post dies 21 ad 25 °C) eburnea, butyrosa vel mollis*,

*convexa, non-patula, glabra cum lineis radiatis, seminidata, margine glabro vel undulato. Cellulae haploideae sexualis solae h⁻. Pseudomycelium in agar farinae Zea nullum vel rudimentarius. Fermentatio glucosi nullum. Glucosum, cellobiosum, ethanolum, glycerolum, D-glucitolium exigue aut nullum, salicinum, methyl β-D-glucosidum, glucono-δ-lactonum, acidum lacticum, acidum succinicum, 2-propanolum (exiguum), at non galactosum, L-sorbosum, maltosum, saccharosum, trehalosum, lactosum, melibiosum, raffinose, melezitose, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, i-erythritolum, ribitolium, galactitolium, D-mannitolium, methyl α-D-glucosidum, 2-keto-gluconatum, 5-keto-gluconatum, acidum citricum, i-inositolium, methanolum, acidum gluconicum, glucosaminum, N-acetyl-glucosaminum, hexadecanum, nec acetoneum. Kalium nitricum, natrium nitrosum non assimilantur; ethyl aminum, lysinum, cadaverinum assimilantur. Crescere potest in 30 °C at non in 37 °C. G+C acidi deoxyribonucleati 33.4–34.0 mol% (5 stirpes, vide tabulum 4). Ad crescentiam additae vitaminarum necessariae sunt. Habitatio in cactis Australiensis. Typus: UCD-FST 84-833.1^T h⁻ ex tabidosus sacculis cacti *Opuntia stricta* isolata est. *Cultura auxiliaris sexus alterius h⁺ carens*. In *Collezione Zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum* (CBS 8782^T) deposita est.*

Description of the type strain of *Candida orba* sp. nov.

Candida orba (or'ba. L. fem. adj. *orba* deprived of, indicating loss of its complementary mating type).

Table 4 Reassociation of ^{125}I -labelled nuclear DNA isolated from *C. orba* 84-833.1^T with DNAs of other cactophilic yeast strains

^{125}I -labelled DNA (0.2 µg) and unlabelled DNA (200 µg) were incubated at 61.5 °C for 25 h in 0.5 ml 280 mM phosphate buffer, pH 6.8.

Organism or tissue	Strain	G + C (mol %)	Relative binding (%) to 84-833.1 ^T *
<i>C. orba</i>	84-833.1 ^T	33.6 ± 0.4	100
<i>C. orba</i>	84-866.1	33.5 ± 0.4	103
<i>C. orba</i>	84-853.3	33.4 ± 0.3	102
<i>C. orba</i>	84-860.2	34.0 ± 0.5	100
<i>C. orba</i>	78-529.3	33.7 ± 0.5	100
<i>P. thermotolerans</i>	76-211 ^T	33.2 ± 0.1	52
<i>P. opuntiae</i>	77-40 ^T	33.9 ± 0.3	27
<i>P. antillensis</i>	82-651.1 ^T	33.6 ± 0.6	24
<i>Candida lusitanae</i>	80-84	45.2 ± 0.1	0
Calf thymus DNA			3

* Corrected for zero-time binding (1.06 %) and for self-reassociation of labelled DNA (4 %) (Price *et al.*, 1978).

Fig. 3. Neighbour-joining tree showing the phylogenetic placement of *C. orba* based on the sequence of the D1/D2 divergent domains of the large rDNA subunit. The percentage bootstrap values were obtained from 1000 iterations. Bar, 5% sequence divergence. The sequences for other species were determined by Kurtzman & Robnett (1998).

In YM (Difco) liquid medium after 3 d at 30 °C, the cells are ovoid, elongate or club-shaped, 2–6 × 3–10 µm, single, in pairs and clusters of 6–10 cells, reproducing by multilateral budding; a sediment is formed, a pellicle is lacking. After 7 d, a glistening, moist pellicle develops. The streak culture on malt agar after 3 weeks at 25 °C is cream coloured, butyrous to soft, convex, non-spreading, smooth with slight radial striations, semi-glossy, border smooth to slightly lobulate. On cornmeal agar after 2 weeks, pseudomycelium is absent with occasional rudimentary tufts of growth. Cells are heterothallic but only one mating type has been found (h^-) based on zygote formation with the h^+ mating type of the related species *P. antillensis*. All of our isolates of *C. orba* reacted in a similar way. Glucose is not fermented. The following carbon compounds are assimilated: glucose, cellobiose, ethanol, glycerol, salicin, methyl β -D-glucoside, D-mannitol (latent or negative), D-glucitol (weak or negative), glucono- δ -lactone, lactate and succinate. The following are not assimilated: D-galactose, L-sorbose, maltose, sucrose, trehalose, lactose, meli-

biose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, i-erythritol, ribitol, galactitol, methyl α -D-glucoside, 2-keto-D-gluconate, 5-keto-D-gluconate, gluconate, citrate, i-inositol, methanol, D-glucosamine, N-acetyl-D-glucosamine, hexadecane and acetone. Potassium nitrate and sodium nitrite are not utilized as sole sources of nitrogen; ethylamine, cadaverine and L-lysine are utilized. Does not grow in vitamin-free medium. Grows in amino acid-free medium. Does not grow in the presence of 100, 10 or 1 µg cycloheximide ml⁻¹. Weak growth on YM agar containing 5% NaCl; no growth on YM agar with 7.5% NaCl. Does not grow in the presence of 50% (w/w) glucose. Does not hydrolyse casein or gelatin. Does not produce urease or lipolytic activity. Grows at 30 °C; no growth at 37 °C. Growth is not inhibited at 25 °C on a complete medium supplemented with 2% agria cactus powder. Kills standard test strain of *Candida glabrata* Y-55. Diazonium blue B reaction is negative. Nuclear DNA base composition 33.6 mol% (buoyant density method). Habitat: necrotic cladodes of *Opuntia stricta* in the State of Queensland, Australia. The holotype strain of mating type h^- is UCD-FST 84-833.1^T, which has been deposited in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, as strain CBS 8782^T, in the American Type Culture Collection as strain ATCC MYA-341^T and in the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA, as NRRL Y-27336^T. The isotype of mating type h^+ has not been discovered among the isolates so far recovered.

DISCUSSION

Explorations in 1978, 1984 and 1996 of the yeast biota associated with Australian *Opuntia* cactus necroses resulted in the recognition of an additional new species of the recently proposed genus *Phaffomyces* by

Yamada *et al.* (1997, 1999). These authors transferred three cactophilic yeast species previously placed in the genus *Pichia* into the new genus *Phaffomyces* as *P. opuntiae* comb. nov., *P. thermotolerans* comb. nov. and *P. antillensis* comb. nov. Fourteen strains (Table 1) of the new species were collected from *Opuntia* necroses or associated *Drosophila buzzatii* in the State of Queensland and are described here as *Candida orba*.

The four species are considered to be distinct species regardless of the species concept one employs: biological species (Mayr, 1963), recognition species (Paterson, 1985), phylogenetic species (Cracraft, 1987) or cohesion species (Templeton, 1989). This is because they: (1) show either pre or post-mating reproductive isolation; (2) live in distinct host species and/or live in distinct geographic regions; and (3) have distinct phenotypes (physiological and DNA–DNA relatedness) that are more similar within a taxon than between taxa.

The cladogram in Fig. 3 confirms the interspecific relationships inferred on the basis of mating compatibility, DNA reassociation and other biological characteristics of *Phaffomyces* species. It is also in perfect agreement with the phylogeny proposed by Shen & Lachance (1993) on the basis of restriction maps of the whole rDNA gene cluster (in that paper, *C. orba* was referred to as 'Hemmant strains'). The observation of a 9.8% D1/D2 sequence difference between *C. orba* and *P. thermotolerans* is of special interest when contrasted to the DNA reassociation value of 52%. The latter characteristic could be interpreted, in some cases, as a basis to consider such taxa as very close relatives, perhaps even as varieties of the same species. Taken in isolation, a sequence divergence near 10% would not suggest such a close relationship. These observations suggest that numerical scales of relatedness obtained by various methods should not be regarded as absolutes and that their application in evolutionary taxonomy should always take into account the whole biology of organisms.

Because no sexual interaction was observed by mixing cells of the isolates in all possible combinations, the mating type of the isolates was elucidated from mating attempts with mating types of the three species of *Phaffomyces*. Although DNA relatedness, physiological similarity and killer-sensitivity profiles (Fig. 2) show that *P. thermotolerans* is most closely related to *C. orba*, mating reactions with that species were unsuccessful because of its very weak intraspecific mating ability (Starmer *et al.*, 1979). However, strong mating, although limited to zygote formation, was observed with the h^+ mating type of *P. antillensis*, indicating that all isolates of *C. orba* were of mating type h^- . In the absence of sporulation, the isolates from Australian *Opuntia* necroses described here are considered anamorphs and therefore are classified in an anamorphic ascomycetous genus, in this case *Candida* Berkhout. However, overwhelming evidence, presented in this paper, clearly shows that *C. orba* is a

close relative of the other three species of the genus *Phaffomyces*. In our opinion, placing the new species in the anamorphic genus *Candida* may create taxonomic confusion, rather than clarifying its taxonomic position as a close relative of its sister species *P. thermotolerans*. Unfortunately, the complementary mating type h^+ of *C. orba* has not been discovered and therefore, by the International Code of Botanical Nomenclature, the species must be placed in the genus *Candida*. Unequal distribution of mating types in some habitats has been observed before, such as in the case of *Clavispora opuntiae* (Phaff *et al.*, 1986). This species also occurs in Australian *Opuntia* necroses where it was originally discovered. The ratio of mating types of Australian isolates was 150 h^+ strains to 5 h^- strains, the latter occurring in a small area near Borallon in Queensland, whereas the h^+ strains came from a vast area in eastern Australia. In contrast, the two mating types of *C. opuntiae* coexist in other parts of the world, occasionally in the same host plant (Lachance *et al.*, 1994). During the prickly pear eradication programme (ca. 1920–1930), infested plants containing the destructive moth, *Cactoblastis cactorum*, and other insects parasitic on cactus, such as cochineal (*Dactilopius opuntiae*), were shipped to Australia from both Argentina and various areas in the Southern United States (Dodd, 1940; Murray, 1982). It is speculated that this eradication programme could also be responsible for the observed one-sided mating type distribution of *C. orba*. The origin of *C. orba* in the Americas is unknown. However, since *C. orba* is most closely related to *P. thermotolerans* (Table 4 and Fig. 2) and is resistant to triterpene glycosides at 20–30 °C, it is possible that *C. orba* originated in cereoid columnar cacti of the subtribe Stenocereinae rather than in *Opuntia* of the New World. This might explain its spotty occurrence in Australia.

C. orba can be differentiated from *P. opuntiae* (27% DNA relatedness) by lack of growth on citrate and good growth on cellobiose; it also is a killer of *Candida glabrata* strain Y-55. It differs from *P. thermotolerans* (52% DNA relatedness) by host plant, geography, lack of growth at 37 °C and resistance to triterpene glycosides at 20 °C. It differs from *P. antillensis* (24% DNA relatedness) by host plant, geography, good growth on cellobiose and salicin, and lack of growth on citrate. Comparisons of the three taxa of *Phaffomyces* and *C. orba* by DNA–DNA relatedness, physiology and killer-sensitivity profiles show concordant dendrograms (Fig. 1), indicating the utility of these methods for differentiating species. However, the dendrogram based on killer ability was not concordant and cast doubt on killer profiles as a useful tool to discriminate taxa at this level.

The previously described species of *Phaffomyces* form a cactus-specific genus most closely related to *Pichia* (*Komagataella*) *pastoris* (Kurtzman & Robnett, 1998). *P. pastoris* occurs in tree-flux habitats bordering the cactus habitat (Ganter *et al.*, 1986), suggesting a likely origin of this independent cactophilic-yeast genus in a

tree-flux habitat and presupposing the greater age of the tree-flux habitat.

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