***Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae* and *Candida lipophila*, five new related yeast species from flowers and associated insects**

Marc-André Lachance,¹ Carlos A. Rosa,² William T. Starmer,³
Birgit Schlag-Edler,¹ J. Stuart F. Barker⁴ and Jane M. Bowles¹

Author for correspondence: Marc-André Lachance. Tel: +1 519 661 3752. Fax: +1 519 661 3935.
e-mail: lachance@julian.uwo.ca

¹ Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada N6A 5B7

² Departamento de Microbiología-ICB, CP 486, Universidade Federal de Minas Gerais, Belo Horizonte-MG, CEP 31270-901, Brazil

³ Department of Biology, Syracuse University, Syracuse, NY 13244, USA

⁴ Department of Animal Science, University of New England, Armidale, NSW 2351, Australia

Five new yeast species, *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae* and *Candida lipophila*, are described to accommodate isolates recovered from flowers and floricolous insects of Australian *Hibiscus* trees, cosmopolitan morning glories (*Ipomoea* spp.) and Brazilian cereoid cacti. The new *Wickerhamiella* species are heterothallic, occur in the haploid condition and are clearly separated reproductively from one another. Although they exhibit little physiological variation, they are easily delineated from *Wickerhamiella domercqiae*, the only species known previously, by their resistance to cycloheximide and the production of strong extracellular lipases. *C. drosophilae* and *C. lipophila* share the latter property, but unlike the *Wickerhamiella* species, they fail to utilize nitrate as sole nitrogen source. PFGE indicates that these yeasts have an unusually low number of chromosomes. The large-subunit rDNA (D1/D2) sequences demonstrate a close relationship between the five species and *Candida vanderwaltii* and *Candida azyma*. Their relationship with *W. domercqiae* is more distant, but all share, with some other *Candida* species, a single monophyletic clade. The type and isotype strains are as follows: *W. australiensis* strains UWO(PS)95-604.3⁺ (h⁺; CBS 8456^T) and UWO(PS)95-631.3⁺ (h⁻; CBS 8457^T); *W. cacticola* strains UFMG96-267⁺ (h⁺; CBS 8454^T) and UFMG96-381⁺ (h⁻; CBS 8455^T); *W. occidentalis* strains UWO(PS)91-698.4⁺ (h⁺; CBS 8452^T) and UFMG96-212⁺ (h⁻; CBS 8453^T); *C. drosophilae* UWO(PS)91-716.3⁺ (CBS 8459^T); and *C. lipophila* UWO(PS)91-681.3⁺ (CBS 8458^T).

Keywords: yeasts, *Wickerhamiella*, *Candida*, flowers, insects

INTRODUCTION

The genus *Wickerhamiella* was until recently known through a single homothallic species, *Wickerhamiella domercqiae* van der Walt & Liebenberg, described on the basis of two strains isolated from heterogeneous sources (van der Walt & Liebenberg, 1973). rDNA sequencing has recently shown that *W. domercqiae* is

moderately related to a small number of *Candida* species, in a clade that lies at the periphery of most ascomycetous yeasts (Kurtzman & Robnett, 1997). In the course of studies of flowers and associated insects in Hawaii, Brazil and Australia, we have isolated a large number of strains with very small cells and which exhibit a very specialized physiological spectrum, notably the production of a strong extracellular lipase. Mating experiments showed that these represented three nitrate-assimilating, ascosporeogenous species typical of the genus *Wickerhamiella*, and two related, nitrate-negative, asporogenous species assignable to

The GenBank accession numbers for the sequences in this paper are AF046033–AF046040.

Table 1. Origin of isolates of *W. australiensis*, *W. cacticola*, *W. occidentalis*, *C. drosophilae* and *C. lipophila*

Site*	Plant	Substrate	Mating type	Strain no.†
<i>W. australiensis</i>				
Balgownie, NSW	<i>Hibiscus heterophyllus</i>	Beetle	h ⁺	95-969.5, 95-970.4
		Flower	h ⁻	95-967.4
		<i>Drosophila</i>	h ⁺	95-984.1
Narrabeen Lagoon, NSW	<i>Ipomoea</i> sp.	Beetle	h ⁻	95-954.3
Port Macquarie, NSW	<i>Ipomoea</i> sp.	Flower	h ⁻	95-1056.2, 95-1058.4
	<i>Hibiscus heterophyllus</i>	Beetle	h ⁺	95-1035.3
		Flower	h ⁺	95-1040.3
Tarrawanna School, NSW	<i>Hibiscus splendens</i>	Beetle	h ⁺	95-964.5
Thora, NSW	<i>Hibiscus heterophyllus</i>	Beetle	h ⁻	95-1065.3, 95-1066.3
		<i>Drosophila</i>	h ⁻	95-1063.3
		Flower	h ⁻	95-1062.3
Beatrice Creek, Qld	<i>Hibiscus heterophyllus</i>	Beetle	h ⁻	95-865.3
		Flower	h ⁺	95-862.2
			h ⁻	95-862.3, 95-863.3
Byfield National Park, Qld	<i>Hibiscus heterophyllus</i>	Flower	h ⁺	95-806.3
Byfield State Forest, Qld	<i>Hibiscus heterophyllus</i>	<i>Drosophila</i>	h ⁺	95-796.3
			h ⁻	95-793.1, 95-794.3, 95-795.1, 95-796.2, 95-815.1
		Flower	h ⁺	95-814.1
Boreen Point, Qld	<i>Hibiscus diversifolius</i>	Beetle	h ⁻	95-706.3
		Flower	h ⁻	95-705.2
Burnett Creek, Qld	<i>Hibiscus heterophyllus</i>	Flower	h ⁺	95-747.5, 95-754.5
			h ⁻	95-740.5
Calen, Qld	<i>Hibiscus heterophyllus</i>	<i>Drosophila</i>	h ⁺	95-852.2
Charlie Moreland State Park, Qld	<i>Hibiscus heterophyllus</i>	Flower	h ⁺	95-609.3, 95-625.3, 95-626.2, 95-627.1, 95-631.4, 95-632.3, 95-633.1
			h ⁻	95-631.3 ¹ (CBS 8457 ¹), 95-651.2
Conondale, Qld	<i>Ipomoea</i> sp.	Beetle	h ⁻	95-606.3
		Flower	h ⁺	95-604.3 ² (CBS 8456 ²)
Childers, Qld	<i>Hibiscus divaricatus</i>	Flower	h ⁺	95-713.4
Eungella National Park, Qld	<i>Hibiscus heterophyllus</i>	<i>Drosophila</i>	h ⁺	95-841.1, 95-842.1
		Flower	h ⁺	95-835.4
Goodna, Qld	<i>Ipomoea</i> sp.	Beetle	h ⁺	95-915.3
Goodnight Scrub State Forest, Qld	<i>Hibiscus splendens</i>	<i>Drosophila</i>	h ⁻	95-737.2
Manoo, Qld	<i>Hibiscus splendens</i>	Flower	h ⁻	95-768.2
Moore Park Beach, Qld	<i>Ipomoea</i> sp.	Flower	h ⁻	95-764.3
Mount Crosby, Qld	<i>Hibiscus heterophyllus</i>	<i>Drosophila</i>	h ⁻	95-906.4
Mount Larcom, Qld	<i>Hibiscus heterophyllus</i>	<i>Drosophila</i>	h ⁻	95-786.1
		Flower	h ⁺	95-775.3
Noosa, Qld	<i>Ipomoea</i> sp.	Flower	h ⁺	95-660.3
Pile Gully State Forest, Qld	<i>Hibiscus heterophyllus</i>	Beetle	h ⁻	95-686.3
		Flower	h ⁺	95-673.3, 95-676.3
<i>W. cacticola</i>				
Marica, RJ	<i>Pilosocereus arrabidaie</i>	Flower	h ⁻	95-343.2
	<i>Cereus pernambucensis</i>	Flower	h ⁺	96-265, 96-267 ² (CBS 8454 ²)
Praia Seca, RJ	<i>Cereus pernambucensis</i>	Flower	h ⁻	96-381 ¹ (CBS 8455 ¹)
Lagoa Santa, MG	<i>Cereus calcirupicola</i>	Flower	h ⁺	96-LS32
			h ⁻	96-LS34

Table 1. (cont.)

Site*	Plant	Substrate	Mating type	Strain no.†
<i>W. occidentalis</i>				
Bird Park, Hawaii	<i>Ipomoea acuminata</i>	<i>Drosophila</i>	h ⁻	87-2197.2
	<i>Nasturtium</i> sp.	Flower	h ⁻	91-610.3
Near Kanuka Natural Area Reserve, Hawaii	<i>Ipomoea acuminata</i>	Flower	h ⁺	91-698.4 ^T (CBS 8452 ^T)
Orchard. Mi 51, Hawaii	<i>Ipomoea acuminata</i>	Flower	h ⁺	91-715.3
Whittington Beach, Hawaii	<i>Ipomoea acuminata</i>	Flower	h ⁻	91-721.2, 91-730.3
Near Kona Garden Estates, Hawaii	<i>Ipomoea acuminata</i>	<i>Drosophila</i>	h ⁺	91-761.3
UFMG, Belo Horizonte, MG	<i>Ipomoea cairica</i>	Flower	h ⁻	95-401.2
Salinas, MG	<i>Ipomoea carnea</i> var. <i>fistulosa</i>	Beetle	h ⁻	96-197
		Flower	h ⁺	96-212 ^I (CBS 8453 ^I)
<i>C. drosophilae</i>				
Bird Park, Hawaii	<i>Ipomoea acuminata</i>	Flower		91-650.2
Orchard. Mi 51, Hawaii	<i>Ipomoea acuminata</i>	Flower		91-716.3 ^T (CBS 8459 ^T)
Near Kanuka Natural Area Reserve, Hawaii	<i>Ipomoea acuminata</i>	<i>Drosophila floricola</i>		91-751.3
Kona Garden Estates, Hawaii	<i>Ipomoea acuminata</i>	<i>Drosophila floricola</i>		91-756.2
Orchard. Mi 51, Hawaii	<i>Ipomoea acuminata</i>	<i>Drosophila floricola</i>		91-764.4
<i>C. lipophila</i>				
Bird Park, Hawaii	<i>Ipomoea acuminata</i>	Flower		87-2173.3, 91-652.2, 91-655.2, 91-672.2, 91-681.3 ^T (CBS 8458 ^T)
		<i>Drosophila floricola</i>		87-2197.1, 87-2229.1, 87-2258.2
		<i>Exalloscaptomyza calliginosa</i>		87-2199.1, 87-2200.1, 87-2203.1, 87-2204.1, 87-2205.1, 87-2209.2, 87-2210.1, 87-2235.2, 87-2245.3, 87-2247.1, 87-2249.2, 87-2250.2
Kona Garden Estates, Hawaii	<i>Ipomoea acuminata</i>	Flower		91-706.3

* MG, Minas Gerais, Brazil; NSW, New South Wales, Australia; Qld, Queensland, Australia; RJ, Rio de Janeiro, Brazil.

† Strain numbers from Brazilian sites (MG, RJ) are UFMG numbers; all others are UWO(PS) numbers. T, Type strain; I, isotype.

the genus *Candida*. We now describe *Wickerhamiella australiensis*, *Wickerhamiella cacticola* [reported previously as '*Candida domercqiae*-like' (Rosa *et al.*, 1994, 1995)], *Wickerhamiella occidentalis*, *Candida drosophilae* and *Candida lipophila* [reported previously as '*Candida species L*' (Lachance *et al.*, 1989)].

METHODS

Isolation and characterization of yeasts. The strains considered in this study are listed in Table 1. They were all collected from flowers and floricolous insects. Samples from the Island of Hawaii were collected from *Ipomoea acuminata* and *Nasturtium* sp., in 1987 and 1991, in the volcanically active Southern region of the island. The drosophilids *Drosophila floricola* and *Exalloscaptomyza calliginosa* that

inhabit the morning glories were also sampled. Brazilian samples were collected in 1995 and 1996 in the coastal area of the state of Rio de Janeiro (Rosa *et al.*, 1994, 1995) and in three localities in the state of Minas Gerais, namely the ecological station of the Universidade Federal de Minas Gerais, Belo Horizonte, the town of Lagoa Santa, near Belo Horizonte, and the town of Salinas. The *Ipomoea* species were herbaceous vines or shrubs, and the cacti were of the genera *Cereus* and *Pilosocereus*. Australian *Hibiscus* and morning glory samples were collected in late spring 1995 over a 6-week period, across a 14° latitude gradient, mostly in the vicinity of the Australian East Coast. Yeasts were cultured the same day, usually within minutes of sampling. The nectary region of morning glory or *Hibiscus* flowers was scraped gently with a sterile loop and streak-inoculated onto acidified YM agar (1.0% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract, 2.0% agar, acidified with HCl to

pH 3.7). *Drosophilids* or beetles were placed in plates of the same medium and kept for 15–60 min before being removed. The material deposited by the insects was spread evenly with a sterile loop. Decaying cactus flower tissue was diluted in sterile water and streak-inoculated as described by Rosa *et al.* (1994). Cultures were maintained in YM slants until preserved in liquid nitrogen using the Microbank system of Pro-Lab diagnostics.

The yeasts were characterized by the standard methods of van der Walt & Yarrow (1984) with a few additional tests (Lachance *et al.*, 1988). Identities were also verified in the keys of Barnett *et al.* (1990). Sexual cross-reactivity was evaluated by mixing pairs of actively growing cultures on GY agar (1.0% glucose, 0.01% yeast extract, 2.0% agar), and observing microscopically for ascus formation after suitable incubation periods at room temperature.

Microscopy. For scanning electron microscopy, material was suspended in sterile water and deposited over a Nuclepore membrane (0.45 µm) on agar. The membrane and its content were fixed in 2.5% glutaraldehyde in 0.3 M cacodylate buffer, pH 7.0, for at least 15 min, rinsed twice in cacodylate buffer, and dehydrated for 15 min in 2,2-dimethoxypropane lightly acidified with HCl. The material was then critical point dried, sputter-coated with gold for 5 min, and observed with a Hitachi S4500 Field Emission scanning electron microscope (Surface Science Western). Images were recorded electronically. For light microscopy, cells were applied to a thin slab of agar (1.8%) and examined with a Leitz Ortholux phase-contrast microscope. The images were recorded on Polaroid type 55 film.

Electrophoretic karyotyping. Karyotypes were obtained using contour-clamped homogeneous electric field electrophoresis (Bio-Rad CHEF DRII). Preparations of intact chromosomes were obtained according to the method of Gardner *et al.* (1993), which does not require cell wall lytic enzymes. The *Saccharomyces cerevisiae* chromosomal DNA standard was obtained from Bio-Rad. Electrophoresis was carried out in a 0.8% (w/v) agarose gel in 1 × TBE buffer under the following conditions: 120 V for 24 h with linear ramping of 100–300 s and 48 h with linear ramping of 400–700 s.

DNA sequence analysis. DNA was extracted as described previously (Lachance, 1990). The D1 and D2 domains of the large-subunit rDNA were amplified using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG) and NL4 (5'-GGTCCGTGTTTCAAGACGG) (O'Donnell, 1993), purchased from Procyon Biopharma. PCR was conducted following the instructions provided by the supplier of *Taq* polymerase (Boehringer Mannheim), in the presence of 1.5 mM MgCl₂, in a Perkin-Elmer System 2400 cyclor. After 2 min denaturation at 95 °C, the mixture was subjected to 35 cycles at 95 °C for 15 s, 53.7 °C for 25 s and 72 °C for 20 s, with a final extension for 5 min at 72 °C. The amplified DNA was concentrated and cleaned by ultrafiltration in MicroCon 100 concentrators (Amicon) and sequenced in an ABI sequencer at the John P. Robarts Research Institute, London, Ontario. The sequences were edited with the program DNAMAN, version 3.2 (Lynnon BioSoft). Known sequences for other yeasts were retrieved from GenBank, where they had been deposited by Kurtzman & Robnett (1997). The CLUSTAL W (Thompson *et al.*, 1994) algorithm

Fig. 1. Phylogeny of *Wickerhamiella* and related *Candida* species based on the D1/D2 variable domains of the large rDNA subunit. The tree was constructed by neighbour-joining analysis of aligned sequences. The numbers at the nodes indicate the percentage bootstrap values for 1000 iterations, and the scale bar shows the proportional sequence divergence.

provided in the DNAMAN package was used to align the sequences.

RESULTS AND DISCUSSION

Species delineation and generic assignment

The new species of *Wickerhamiella* fell into three reproductively isolated units, and therefore represent three distinct biological species. Interspecific mixtures of mating types did not exhibit any signs of conjugation. In each case, mating is apparently controlled by two alleles of a single locus. All strains of the new *Candida* species failed to form conjugation tubes when mixed in every possible pair, within or between species. rDNA sequences (Fig. 1) confirmed that a small amount of divergence (approx. 2–9% sequence difference) has taken place between these species, and showed in addition that the asexual species are phylogenetically distinct and cannot be considered to be the anamorphs of any known *Wickerhamiella* species.

The relatively large genetic distance separating the new species from *W. domercqiae* could be construed, by some, as a basis for creating a new genus. We have considered this possibility, given that direct comparison of *W. domercqiae* and the new species revealed sequence differences of 24–26% in the D1/D2 region, which is comparable in magnitude to the distance separating *S. cerevisiae* from most species included in the analysis. Three important points are relevant to this. First, a biologically valid principle on the basis of which all yeast genera should be separated by a set genetic distance, as measured by the proportion of

Table 2. Differential properties of selected *Wickerhamiella* and *Candida* species

+, Positive; —, negative; w, weak; s, slow; v, variable; Het./hap., heterothallic haploid.

Growth test	<i>W. domercquiae</i>	<i>W. australiensis</i>	<i>W. cacticola</i>	<i>W. occidentalis</i>	<i>C. drosophilae</i>	<i>C. lipophila</i>
Galactose	—	+	w	s	—	—
Cellobiose/salicin	—	—	—	v*	—	—
L-Sorbose	+	+	+	+	+	w
Xylose	—	v	—	/w	w	—
D-Arabinose	—	/w	/w	/w	—	—
Ribose	s	—	—	—	—	—
1/2-Propanol	—	—	—	w/s	—	—
Ribitol	—	s/w	w	w/s	—	—
Xylitol	w/s	s	w/	/s	+	—
Mannitol/glucitol	+	+	+	+	—	—
Succinate	s	+	+	+	w	—
Citrate	+	—	—	—	—	—
2-Ketogluconate	s	w	—	—	—	—
Hexadecane	—	w	—	w/s	w	w
37 °C	—	v	+	v	—	—
Lipase	s	+	+	+	+	+
Nitrate/nitrite	+	+	+	+	—	—
Ethylamine	+	—	—	w	w	—
Cadaverine	+	+	+	+	w	—
NaCl	5% s	10% v	10% s	15% v	15% s	10% +
Glucose, 50%	—	s	—	s	s	w
Cycloheximide:						
100 p.p.m.	—	+	+	+	+	s
1000 p.p.m.	—	+	—	+	s	w
Sexuality	Automatic	Het./hap.	Het./hap.	Het./hap.	Asexual	Asexual
Host plant	?	<i>Hibiscus</i> <i>Ipomoea</i>	Cactus	<i>Ipomoea</i> <i>Nasturtium</i>	<i>Ipomoea</i>	<i>Ipomoea</i>

* Strain 95-401.2 assimilates cellobiose slowly and salicin rapidly.

different bases in aligned sequences, has yet to be formulated. On the contrary, the exploration of sequence divergence among yeasts started with the assumption that well-defined yeast genera would serve as a reference point upon which to understand the significance of genetic divergence in different genera, and not the other way around. Second, as long as a clade remains monophyletic, there is no compelling justification for creating new genera within that clade. The *Wickerhamiella* clade (all species included in Fig. 1 except for *S. cerevisiae*, *Schizosaccharomyces pombe* and new taxa) appears to be monophyletic (Kurtzman & Robnett, 1997). Third, a high degree of homogeneity exists in the physiological (Table 2) and morphological make-up of *Wickerhamiella* species. In particular, the morphology of the asci is both unusual (Fig. 3) and consistent with that given in the original description (van der Walt & Liebenberg, 1973).

As ascospore morphology was in the past given considerable weight in delineating yeast genera, it is worth noting that the ascospores of the new *Wickerhamiella* species are not identical to those of *W.*

domercquiae. In particular, the ascospores of *W. australiensis* (Fig. 4) bear much resemblance to those of *Starmerella bombicola*, the recently discovered teleomorph of *Candida bombicola* (Rosa & Lachance, 1998). However, *Wickerhamiella* and *Starmerella* belong to distinct clades (Kurtzman & Robnett, 1997), leaving little doubt that these yeasts are not close relatives.

The species delineation and the relatedness among the new yeast species are further corroborated by the distribution of chromosome sizes (Fig. 2). All strains examined shared one very large chromosome whose size may be in excess of 5 Mb. In *W. cacticola*, under some conditions, two bands were resolved in that size category. In the other species, additional bands were present in the size range of approximately 1.3–3 Mb. The strains in Fig. 2 (types and isotypes) were selected to show the extent of variation observed within the species. In the case of *W. occidentalis*, the variation in the small chromosomes was correlated with the geographical origin of the strains (i.e. Hawaii vs Brazil).

Fig. 2. Electrokaryotypes of the type (and isotype) strains of *Wickerhamiella* and related *Candida* species. *W. domercqiae* is represented by strain NRRL Y-6692. The results are from two separate experiments.

With respect to intraspecific sequence variation, we have observed three substitutions in *W. cacticola* (one in D1 and two in D2; two strains), one in the D2 region of *W. occidentalis* (three strains) and one in *W. australiensis* (two strains). This adds further support to the generalization made by Kurtzman & Robnett (1997) that strains in a single yeast species have less than 1% sequence difference. Only the type strains of the two *Candida* species were examined by sequencing, as their physiological and morphological characteristics were nearly invariant.

Mating type distributions

As shown in Table 1, the mating types of the *Wickerhamiella* species are distributed more or less equally, suggesting that these species are sexually active. A few strains identified as *W. occidentalis* (not included in Table 1) failed to mate when mixed with others. Clearly, the two *Candida* species may well represent haploid members of other *Wickerhamiella* species with highly disproportionate mating type distributions.

Habitat specificity

The five new taxa described here occur in flowers that are visited by insects, including nitidulid (sap) beetles and a few species of drosophilids. In Australia, where flowers of two unrelated plant genera (*Hibiscus* and *Ipomoea* species) were studied, the distribution seemed independent of host plant. Nitidulid beetles were observed in all cases, including the flowers of Brazilian cacti (Rosa *et al.*, 1994, 1995), where they were believed to serve as vectors. *C. drosophilae* was recovered only

Fig. 3. Comparison of ascus morphology in new *Wickerhamiella* species by phase-contrast microscopy. *W. australiensis*: (a) deliquesced ascus and liberated ascospore with a conspicuous ledge (arrow); (b) mature ascus with two ascospores; (c) deliquesced ascus. *W. cacticola*: (d) mature ascus; (e, f) deliquesced asci. *W. occidentalis*: (g, h) immature asci; (i) mature ascus with emerging ascospore (arrow); (j, k) deliquesced asci. Bar, 10 μ m.

during the 1991 sampling of Hawaiian flowers, and appeared to have displaced *C. lipophila* from *D. floricola*, one of the two drosophilids frequently isolated from Hawaiian morning glories during both samplings. Because of the very slow growth of these

Candida species, it is possible that they were missed in 1987. A more detailed analysis of the ecology of the yeast communities in flowers of morning glory and *Hibiscus* species is in progress.

Latin diagnosis of *Wickerhamiella australiensis* sp. nov.

*In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–3 µm). Post unum mensem sedimentum formatur. Cultura in agar malti post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Post dies unus, cellulae stirpum interfertiliū mixtarum in agar glucosi et extracti levidinis tubi junctionis et asci juncti formantur. Asci ascospores una, aliquando ascospores binae, liberant. Glucosum non fermentatur. Galactosum, L-sorboseum, D-xylosum (variabile), D-arabiosum (variabile et exigue), ethanolum (exigues), glycerolum (lente), ribitolum (lente aut exigues), xylitolum (lente), mannitolum, glucitolum, acidum succinicum, acidum malicum (exigues), glucono-δ-lactonum (variabile et exigues) et hexadecanum (exigues) assimilantur at non inulinum, sucrosū, raffinōsum, melibiosum, lactosum, trehalosum, maltosum, melezitōsum, methyl α-D-glucosidum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, L-arabiosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, galactitolum, meso-inositolum, acidum lacticum, acidum citricum, acidum gluconicum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetone, nec ethyl acetat. Natrium nitricum, natrium nitrosū, lysinum et cadaverinum assimilantur at non ethylaminum. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C (variabile). Habitatio in floribus *Hibiscus* spp. et *Ipomoea* spp. et in scarabeis et *Drosophila hibisci* juncti in Australia. Typus stirps UWO(PS)95-604.3^T (h⁺). Isotypus stirps UWO(PS)95-631.3^I (h⁻). In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8456^T [= UWO(PS)95-604.3^T] et CBS 8457^I [= UWO(PS)95-631.3^I] depositae sunt.*

Description of *Wickerhamiella australiensis* sp. nov.

Wickerhamiella australiensis (aus.tra.li.en'sis. L. nom. fem. adj. *australiensis* of Australia, referring to the geographic distribution of the species).

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are spheroidal to ovoid, occur singly or in parent–bud pairs and measure 1–2 × 2–3 µm. The buds are formed on a short neck. A sediment is formed after a month. On malt agar after 2 weeks at 17 °C, colonies are small, convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae or pseudohyphae are not formed. After 1 d on glucose (1.0%)/yeast extract (0.01%)

Fig. 4. Scanning electron micrographs of *W. australiensis*. The ascospores tend to agglutinate after release (a), and have a deeply convoluted surface and a basal ledge (b). (c) Cell division is by multilateral budding. Bar, 1 µm.

agar, mixed strains of complementary mating types give rise to very short conjugation tubes. Conjugated pairs, zygotes and asci containing one, rarely two ascospores are also present. After 2 d, most asci have released their ascospore(s) through a flared terminal opening. The lobe of the ascus which contains the ascospores is generally twice as long as the other lobe, each lobe representing one of the original conjugating cells (Fig. 3a–c). The ascospores are spheroidal with a deeply convoluted surface and a prominent basal ledge (Figs 3a and 4b). Fermentation is absent. Assimilation of carbon compounds: galactose, L-sorbose (occasionally slow), D-xylose (variable), D-arabinose (variable and weak), ethanol (weak), glycerol (slow), ribitol, xylitol (slow), mannitol, glucitol, succinic acid, malic acid (weak), glucono-δ-lactone (variable and weak) and hexadecane (weak) are assimilated; no growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, methyl α-D-glucoside, cellobiose, salicin, starch, L-rhamnose, L-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, meso-inositol, lactic acid, citric acid, gluconic acid, 2-ketogluconic acid, D-glucosamine, N-acetylglucosamine, acetone or ethyl acetate. Assimilation of nitrogen compounds: sodium nitrate,

Fig. 5. Scanning electron micrographs of *W. cacticola*. (a) Conjugating cells. (b) Empty ascus. (c, d) Released ascospores have a smooth surface and a thin basal ledge. Bar, 1 µm.

sodium nitrite, lysine and cadaverine are utilized; ethylamine is not utilized. Diazonium Blue B reaction is negative. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is variable. Acid formation on chalk agar is negative. Urease activity is negative. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is strong. Production of amyloid compounds is negative. Growth on 50% glucose/yeast extract agar is slow. Growth on YM agar with 10% NaCl is variable. Growth in the presence of 1000 µg cycloheximide ml⁻¹ is positive. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. The habitat is in flowers of *Hibiscus* and *Ipomoea* species, associated beetles and *Drosophila hibisci*, in Australia. The type strain of *W. australiensis* is strain UWO(PS)95-604.3^T. Its mating type was defined arbitrarily as h⁺. It was isolated from an *Ipomoea* sp. flower. The designated isotype, strain UWO(PS)95-631.3¹, has mating type h⁻ and came from a flower of *Hibiscus heterophyllus*. Both are from south Queensland, Australia. These strains have been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strains CBS 8456^T and CBS 8457¹, respectively.

Latin diagnosis of *Wickerhamiella cacticola* sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–3 µm). Post unum mensem sedimentum formatur. Cultura in agar malti

*post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Post dies unus, cellulae stirpium interfertilium mixtarum in agar glucosi et extracti levidinis tubi junctionis et asci juncti formantur. Asci ascospores una liberant. Glucosum non fermentatur. Galactosum (exiguum), L-sorbosum, D-arabinosum (exiguum et variabile), ethanolum (exiguum), glycerolum (lente), ribitolum (exiguum et variabile), xylitolum (variabile et exiguum), mannitolum, glucitolum, acidum succinicum et acidum malicum (exiguum) assimilantur at non inulinum, sucrosum, raffinose, melibiosum, lactosum, trehalosum, maltosum, melezitosum, methyl α-D-glucosidum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, D-xylosum, L-arabinosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, galactitolum, meso-inositolum, acidum lacticum, acidum citricum, acidum gluconicum, glucono-δ-lactonum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetonum, ethylacetat nec hexadecanum. Natrium nitricum, natrium nitrosum, lysinum et cadaverinum assimilantur at non ethylaminum (aliquando exiguum). Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C. Habitatio in floribus cactorum *Pilosocereus arrabidae* et *Cereus* spp. in Brasil. Typus stirps UFMG96-267^T (h⁺). Isotypus stirps UFMG96-381¹ (h⁻). In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8454^T (= UFMG96-267^T) et CBS 8455¹ (= UFMG96-381¹) depositae sunt.*

Description of *Wickerhamiella cacticola* sp. nov.

Wickerhamiella cacticola (cac.ti.co'la. L. nom. fem. adj. *cacticola* cactus-inhabiting, referring to the host plants from which the species was isolated).

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are spheroidal to ovoid, occur singly or in parent-bud pairs and measure 1–2 × 2–3 µm. The buds are formed on a short neck, leaving raised bud scars (Fig. 5a). A sediment is formed after a month. On malt agar after 2 weeks at 17 °C, colonies are small, convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae or pseudohyphae are not formed. After 1 d on glucose (1.0%)/yeast extract (0.01%) agar, mixed strains of complementary mating types give rise to very short conjugation tubes. Conjugated pairs, zygotes and asci containing one ascospore are also present. After 2 d, most asci have released their ascospore(s) through a flared terminal opening. The lobe of the ascus which contains the ascospores is generally 1–1.5 times as long as the other lobe, each lobe representing one of the original conjugating cells (Figs 3d–f and 5b). The ascospores are spheroidal and smooth with an indistinct basal ledge (Fig. 5c, d). Fermentation is absent. Assimilation of carbon compounds: galactose (weak), L-sorbose, D-arabinose (variable and weak), ethanol (weak), glycerol (slow), ribitol (variable and

Fig. 6. Scanning electron micrographs of *W. occidentalis*. (a, b) Released ascospores have a lightly convoluted surface and a double basal ledge. (c) Vegetative cells have elevated bud scars. Bar, 1 µm.

weak). xylitol (variable and weak), mannitol, glucitol, succinic acid and malic acid (weak) are assimilated; no growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, methyl α -D-glucoside, cellobiose, salicin, starch, L-rhamnose, D-xylose, L-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, meso-inositol, lactic acid, citric acid, gluconic acid, glucono- δ -lactone, 2-ketogluconic acid, D-glucosamine, N-acetylglucosamine, acetone, ethyl acetate or hexadecane. Assimilation of nitrogen compounds: sodium nitrate, sodium nitrite, lysine and cadaverine are utilized; ethylamine is not utilized (rarely weak growth). Diazonium Blue B reaction is negative. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is positive. Acid formation on chalk agar is negative. Urease activity is negative. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is strong. Production of amyloid compounds is negative. Growth on 50 % glucose/yeast extract agar is negative. Growth on YM agar with 10 % NaCl is positive or slow; with 15 % NaCl it is negative. Growth in the presence of 100 µg cyclo-

heximide ml⁻¹ is positive; at 1000 µg ml⁻¹ it is negative. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. The habitat is in flowers of columnar cacti *Pilosocereus arrabidaei* and various species of *Cereus*, in Brazil. The type strain of *W. cacticola* is strain UFMG96-267^T. Its mating type was defined arbitrarily as h⁺. The designated isotype, strain UFMG96-381^I, has mating type h⁻. Both are from the cactus *Cereus perambucensis* in the state of Rio de Janeiro, Brazil. These strains have been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strains CBS 8454^T and CBS 8455^I, respectively.

Latin diagnosis of *Wickerhamiella occidentalis* sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–3 µm). Post unum mensem sedimentum formatur. Cultura in agar malti post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Post dies unus, cellulae stirpium interfertilium mixtarum in agar glucosi et extracti levidinis tubi junctionis et asci juncti formantur. Asci ascospora una liberant. Glucosum non fermentatur. Galactosum (lente), L-sorboseum, D-xylosum (variabile et exigue), D-arabinosum (exigue), ethanolum (lente), glycerolum (exigue), ribitolum (lente), xylitolum (lente aut exigue), mannitolum, glucitolum, acidum succinicum, glucono- δ -lactonum (exigue) et hexadecanum (exigue) assimilantur ad non inulinum, sucrosum, raffinoseum, melibiosum, lactosum, trehalosum, maltosum, melezitosum, methyl α -D-glucosidum, cellobiosum (aliquando lente), salicinum (aliquando assimilatum), amyllum solubile, L-rhamnosum, L-arabinosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, galactitolum, meso-inositolum, acidum lacticum, acidum citricum, acidum malicum, acidum gluconicum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetolum, nec ethyl acetat. Natrium nitricum, natrium nitrosum, ethylaminum (exigue), lysinum et cada-verinum assimilantur. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C (variabile). Habitatio in florae et insecta Ipomoea spp. et Nasturtium sp. in Brasil et Hawaii. Typus stirps UWO(PS)91-698.4^T (h⁺). Isotypus stirps UFMG96-212^I (h⁻). In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8452^T [= UWO(PS)91-698.4^T] et CBS 8453^I [= UFMG96-212^I] depositae sunt.

Description of *Wickerhamiella occidentalis* sp. nov.

Wickerhamiella occidentalis (oc.ci.den.ta'lis. L. nom. fem. adj. *occidentalis* Western, referring to the geo-

graphical distribution of the species, in contrast to its Australian sister species).

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are spheroidal to ovoid, occur singly or in parent-bud pairs and measure 1–2 × 2–3 µm. The buds are formed on a short neck, leaving raised bud scars (Fig. 6c). A sediment is formed after a month. On malt agar after 2 weeks at 17 °C, colonies are small, convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae or pseudohyphae are not formed. After 1 d on glucose (1.0%)/yeast extract (0.01%) agar, mixed strains of complementary mating types give rise to very short conjugation tubes. Conjugated pairs, zygotes and asci containing one ascospore are also present. After 2 d, most asci have released their ascospore(s) through a flared terminal opening. The lobe of the ascus which contains the ascospores is at least twice as long as the other lobe, each lobe representing one of the original conjugating cells (Fig. 3g–k). The ascospores are in the shape of a flattened sphere with a slightly convoluted surface and two or more fine basal ledges (Fig. 6a, b). Fermentation is absent. Assimilation of carbon compounds: galactose (slow), L-sorbose, D-xylose (variable and weak), D-arabinose (weak), ethanol (slow), glycerol (weak), ribitol (slow), xylitol (slow or weak), mannitol, glucitol, succinic acid, glucono-δ-lactone (weak) and hexadecane (weak) are assimilated; no growth occurs on inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melzitose, methyl α-D-glucoside, cellobiose (rarely slow), salicin (rarely positive), starch, L-rhamnose, L-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, galactitol, meso-inositol, lactic acid, citric acid, malic acid, gluconic acid, 2-ketogluconic acid, D-glucosamine, N-acetylglucosamine, acetone or ethyl acetate. Assimilation of nitrogen compounds: sodium nitrate, sodium nitrite, ethylamine (weak), lysine and cadaverine are utilized. Diazonium Blue B reaction is negative. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 37 °C is variable. Acid formation on chalk agar is negative. Urease activity is negative. Gelatin liquefaction and casein hydrolysis are negative. Lipolytic activity on Tween 80 agar is strong. Production of amyloid compounds is negative. Growth on 50% glucose/yeast extract agar is slow. Growth on YM agar with 15% NaCl is variable. Growth in the presence of 1000 µg cycloheximide ml⁻¹ is positive. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. The habitat is in flowers and associated insects of *Ipomoea* spp. and *Nasturtium* sp. in Hawaii and Brazil. The type strain of *W. occidentalis* is strain UWO(PS)91-698.4^T. Its mating type was defined arbitrarily as h⁺. It was isolated from an *I. acuminata* flower in Hawaii. The designated isotype, strain UFMG96-212¹, has mating type h⁻ and was isolated from a flower of *Ipomoea fistulosa* in the state of Minas Gerais, Brazil. These strains have been deposited in the collection of the

Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strains CBS 8452^T and CBS 8453¹, respectively.

Latin diagnosis of *Candida drosophilae* sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–3 µm). Post unum mensem sedimentum formatur. Cultura in agar malti post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agar farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Asci non formantur. Glucosum non fermentatur. L-Sorboseum, D-xylosum (exiguum), ethanolum (lente), glycerolum (exiguum), xylitolum, acidum succinicum (exiguum) et hexadecanum (exiguum) assimilantur at non inulinum, sucrosum, raffinoseum, melibioseum, galactosum, lactosum, trehalosum, maltosum, melezitoseum, methyl α-D-glucosidum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, L-arabiosum, D-arabiosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, erythritolum, ribitolum, mannitolum, glucitolum, galactitolum, meso-inositolum, acidum lacticum, acidum citricum, acidum malicum, acidum gluconicum, glucono-δ-lactonum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetolum, nec ethyl acetat. Lysinum, ethylaminum (exiguum) et cadaverinum (exiguum) assimilantur at non natrium nitricum, nec natrium nitrosum. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 30 °C at non in 37 °C. Habitatio in floribus Ipomoea spp. et in Drosophila juncti in Hawaii. Typus stirpis UWO(PS)91-716.3^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8459^T [= UWO(PS)91-716.3^T] deposita est.

Description of *Candida drosophilae* sp. nov.

Candida drosophilae (dro.so'phi.lae. L. gen. fem. adj. *drosophilae* of *Drosophila*, referring to the source of isolation of some of the strains).

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are spheroidal to ovoid, occur singly or in parent-bud pairs and measure 1–2 × 2–3 µm. A sediment is formed after a month. On malt agar after 2 weeks at 17 °C, colonies are small, convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae are not formed. Asci were not observed in pairwise mixtures of any of the strains. Fermentation is absent. Assimilation of carbon compounds: L-sorbose, D-xylose (weak), ethanol (slow), glycerol (weak), xylitol, succinic acid (weak) and hexadecane (weak) are assimilated; no growth occurs on galactose, inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melzitose, methyl α-D-glucoside, cellobiose, salicin, starch, L-rhamnose, L-arabinose, D-arabinose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, erythritol, ribitol, mannitol, glucitol, galactitol, meso-inositol, lactic acid, citric acid, malic acid, gluconic acid, 2-ketogluconic acid, glucono-δ-lactone, D-glu-

cosamine, *N*-acetylglucosamine, acetone or ethyl acetate. Assimilation of nitrogen compounds: ethylamine (weak), lysine and cadaverine (weak) are utilized; sodium nitrate and sodium nitrite are not utilized. Diazonium Blue B reaction is negative. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 30 °C is positive; at 37 °C it is negative. Acid formation on chalk agar is negative. Urease activity is negative. Gelatin liquefaction is slow. Casein hydrolysis is negative. Lipolytic activity on Tween 80 agar is strong. Production of amyloid compounds is negative. Growth on 50% glucose/yeast extract agar is slow. Growth on YM agar with 15% NaCl is slow. Growth in the presence of 1000 µg cycloheximide ml⁻¹ is slow. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. The habitat is in flowers and associated insects of *Ipomoea* spp. in Hawaii. The type strain of *C. drosophilae* is strain UWO(PS)91-716.3^T. It was isolated from *D. floricola* collected on an *I. acuminata* flower in Hawaii. This strain has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strain CBS 8459^T.

Latin diagnosis of *Candida lipophila* sp. nov.

In medio liquido post dies tres cellulae singulae aut binae; cellulae ovoidae (1–2 × 2–3 µm). Post unum mensem sedimentum formatur. Cultura in agaro malti post dies 14 (17 °C) parva, convexa, glabra, candida et butyrosa. In agaro farinae Zea mays post dies 14 mycelium nec pseudomycelium non formatur. Asci non formantur. Glucosum non fermentatur. L-Sorboseum (exigue), ethanolum (exigue) et hexadecanum (exigue) assimilantur at non inulinum, sucrosam, raffinoseum, melibiosum, galactosum, lactosum, trehalosum, maltosum, melezitoseum, methyl α-D-glucosidum, cellobiosum, salicinum, amyllum solubile, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, 1-propanolum, 2-propanolum, 1-butanolum, glycerolum, erythritolum, ribitolum, xylitolum, mannitolum, glucitolum, galactitolum, meso-inositolum, acidum lacticum, acidum succinicum, acidum citricum, acidum malicum, acidum gluconicum, glucono-δ-lactonum, 2-ketogluconatum, glucosaminum, N-acetylglucosaminum, acetonium, nec ethyl acetat. Lysinum assimilatur at non ethylaminum, cadaverinum, natrium nitricum, nec natrium nitrosam. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 30 °C at non in 37 °C. Habitatio in floribus Ipomoea spp. et insectis junctis in Hawaii. Typus stirps UWO(PS)91-681.3^T. In collectione zymotica Centraalbureau voor Schimmelcultures, Delphi Batavorum, sub no. CBS 8458^T [= UWO(PS)91-681.3^T] deposita est.

Description of *Candida lipophila* sp. nov.

Candida lipophila (li.po'phi.la. L. nom. fem. adj. *lipophila* fat-loving, referring to the strong lipolytic

activity of this yeast as tested by hydrolysis of Tween 80, contrasted to its failure to grow on most carbon compounds tested).

In yeast extract (0.5%)/glucose (2%) broth after 3 d at 25 °C, the cells are spheroidal to ovoid, occur singly or in parent-bud pairs and measure 1–2 × 2–3 µm. A sediment is formed after a month. On malt agar after 2 weeks at 17 °C, colonies are small, convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on cornmeal agar after 2 weeks, hyphae are not formed. Asci were not observed in pairwise mixtures of any of the strains. Fermentation is absent. Assimilation of carbon compounds: L-sorbose (weak), ethanol (weak) and hexadecane (weak) are assimilated; no growth occurs on galactose, inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, methyl α-D-glucoside, cellobiose, salicin, starch, L-rhamnose, L-arabinose, D-arabinose, D-xylose, D-ribose, methanol, 1-propanol, 2-propanol, 1-butanol, glycerol, erythritol, ribitol, xylitol, mannitol, glucitol, galactitol, meso-inositol, lactic acid, succinic acid, citric acid, malic acid, gluconic acid, 2-ketogluconic acid, glucono-δ-lactone, D-glucosamine, *N*-acetylglucosamine, acetone or ethyl acetate. Assimilation of nitrogen compounds: lysine is utilized; sodium nitrate, sodium nitrite, ethylamine and cadaverine are not utilized. Diazonium Blue B reaction is negative. Growth in vitamin-free medium is negative. Growth in amino-acid-free medium is positive. Growth at 30 °C is positive; at 37 °C it is negative. Acid formation on chalk agar is negative. Urease activity is negative. Gelatin liquefaction is weak or negative. Casein hydrolysis is negative. Lipolytic activity on Tween 80 agar is strong. Production of amyloid compounds is negative. Growth on 50% glucose/yeast extract agar is weak. Growth on YM agar with 10% NaCl is positive; with 15% NaCl it is negative. Growth in the presence of 1000 µg cycloheximide ml⁻¹ is weak. Growth in the presence of 8 µg digitonin ml⁻¹ at 25 °C is negative. The habitat is in flowers and associated insects of *Ipomoea* spp. in Hawaii. The type strain of *C. lipophila* is strain UWO(PS)91-681.3^T. It was isolated from an *I. acuminata* flower in Hawaii. This strain has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures, Delft, the Netherlands, as strain CBS 8458^T.

Identification

Existing keys (e.g. Barnett *et al.*, 1990) did not lead to any satisfactory matches for any of the new species described here. A computer-based comparison with the physiological profiles of all described species suggested a vague resemblance with *W. domercqiae*. As shown in Table 2, although these yeasts generally assimilate few compounds, most have sufficiently unique attributes to be distinguished from the others. All *Wickerhamiella* species share the ability to utilize nitrate. *W. domercqiae* differs from the others by a few traits, the most noteworthy being growth on citric acid and sensitivity to cycloheximide (100 p.p.m.). The

three new *Wickerhamiella* species are less easily sorted from one another by conventional attributes, and the key presented below resorts to rather minor differences. Ultimately, sexual compatibility would be the clearest criterion for species determination. The two *Candida* species are nitrate-negative and easily separated from each other by assimilation of xylitol.

Key to *Wickerhamiella* and related *Candida* species

1. a. Assimilation of nitrate positive2
 - b. Nitrate not assimilated5
2. a. Growth in the presence of 100 p.p.m. cycloheximide3
 - b. No growth in the presence of 100 p.p.m. cycloheximide
 - *W. domercqiae*
3. a. Growth in the presence of 1000 p.p.m. cycloheximide4
 - b. No growth in the presence of 1000 p.p.m. cycloheximide
 - *W. cacticola*
4. a. Ascus forms and protuberance 10 µm or more in length
 - *W. occidentalis*
 - b. Ascus different
 - *W. australiensis*
5. a. Assimilation of xylitol positive
 - *C. drosophilae*
 - b. Assimilation of xylitol negative
 - *C. lipophila*

Evolutionary considerations

van der Walt & Liebenberg (1973) noted that the genus *Wickerhamiella* exhibits a number of presumed primitive characteristics such as lack of fermentation and a narrow range of carbon utilization, and on this basis, regarded this yeast as ancestral. The new species added to the *Wickerhamiella* clade lend support to some of these views. Indeed, the very small cell size, the unusual shape of the bud scars (Figs 4, 5 and 6), and the low chromosome numbers (Fig. 2) could be regarded as symplesiomorphic traits if they are shared by less related members of an ancient clade. The very basal position of *Wickerhamiella* (and its asexual relatives) with respect to other ascomycetous genera (Kurtzman

& Robnett, 1997) and the high genetic distances unravelled within the clade as it now stands suggest that this taxon is ancient. However, whether their nutritional specialization should be viewed as an ancestral characteristic or instead as the result of a relatively recent adaptation to the ephemeral flower-insect ecosystem remains to be seen. Clearly, lipase production and nitrate utilization are good examples of plesiomorphic attributes in the present case, as they are shared by many species in the clade. The numerous physiological traits for which the responses were slow, weak or variable also would indicate that these functions are in the process of being lost. According to this view, the most derived species in the clade would be *C. lipophila*, which is the most nutritionally specialized of the lot.

ACKNOWLEDGEMENTS

We acknowledge financial support from the Natural Science and Engineering Research Council of Canada (M.-A.L.), the Conselho Nacional de Desenvolvimento Científico e Tecnológico of Brazil (Postdoctoral Fellowship to C.A.R. and Visiting Professor Fellowship to M.-A.L.), the National Science Foundation International Programs (W.T.S.) and the Australian Research Council (J.S.F.B.). Permission to collect was obtained from the Queensland National Parks and Wildlife Service and the Queensland Forest Service. We thank C. P. Kurtzman for the gift of a strain and for sharing sequence data prior to publication, and L. L. Wolf for collection assistance.

REFERENCES

- Barnett, J. A., Payne, R. W. & Yarrow, D. (1990). *Yeasts: Characteristics and Identification*. Cambridge: Cambridge University Press.
- Gardner, D. C., Heale, S. M., Stateva, L. I. & Oliver, S. G. (1993). Treatment of yeast cells with wall lytic enzymes is not required to prepare chromosomes for pulsed-field gel analysis. *Yeast* **9**, 1053–1055.
- Kurtzman, C. P. & Robnett, C. J. (1997). Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal gene. *J Clin Microbiol* **35**, 1216–1223.
- Lachance, M. A. (1990). Ribosomal DNA spacer variation in the cactophilic yeast *Clavispora opuntiae*. *Mol Biol Evol* **7**, 178–193.
- Lachance, M. A., Starmer, W. T. & Phaff, H. J. (1988). Identification of yeasts found in decaying cactus tissue. *Can J Microbiol* **34**, 1025–1036.
- Lachance, M. A., Starmer, W. T. & Bowles, J. M. (1989). The yeast community of morning glory and associated drosophilids in a Hawaiian Kipuka. *Yeast* **5**, S501–S504.
- O'Donnell, K. (1993). *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*, pp. 225–233. Edited by D. R. Reynolds & J. W. Taylor. Wallingford: CAB International.
- Rosa, C. A. & Lachance, M.-A. (1998). The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. *Int J Syst Bacteriol* **48**, 1413–1417.

- Rosa, C. A., Morais, P. B., Hagler, A. N., Mendonça-Hagler, L. C. & Monteiro, R. F. (1994). Yeast communities of the cactus *Pilosocereus arrabidaei* and associated insects in the sandy coastal plains of southeastern Brazil. *Antonie Leeuwenhoek* **65**, 55–62.
- Rosa, C. A., Morais, P. B., Santos, S. R., Peres Neto, P. R., Mendonça-Hagler, L. C. & Hagler, A. N. (1995). Yeast communities associated with different plant resources in sandy coastal plains of southeastern Brazil. *Mycol Res* **99**, 1047–1054.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- van der Walt, J. P. & Liebenberg, N. V. D. W. (1973). The yeast genus *Wickerhamiella* gen. nov. (Ascomycetes). *Antonie Leeuwenhoek* **39**, 121–128.
- van der Walt, J. P. & Yarrow, D. (1984). Methods for the isolation, maintenance, classification and identification of yeasts. In *The Yeasts: a Taxonomic Study*, 3rd edn, pp. 45–104. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.