**Metschnikowia orientalis** sp. nov., an Australasian yeast from nitidulid beetles

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A novel species, *Metschnikowia orientalis* sp. nov., is described for haploid, heterothallic yeasts isolated from nitidulid beetles sampled in flowers in Rarotonga in the Cook Islands, and the Cameron Highlands of Malaysia. As evidenced by analysis of D1/D2 large subunit rDNA sequences, the species is related to *Candida hawaiiiana*, to which it is similar in growth responses. Cylindrical, conjugated asci and acicular ascospores of moderate size are formed. Rudimentary mating reactions were observed with *Metschnikowia aberdeenae* and *Metschnikowia continentalis*, but not with *C. hawaiiiana*. The type strain of *M. orientalis* is UWOPS 99-745.6¹ (h⁺) (≡ CBS 10331¹ = NRRL Y-27991¹) and the designated allotype is UWOPS 05-269.1 (h⁻) (≡ CBS 10330 = NRRL Y-27992).

Insect-borne yeasts in the Metschnikowiaceae have mostly regional distributions, but are isolated at high frequencies in their habitats, suggesting that they engage in strong associations with endemic insects (Lachance et al., 2003b). On occasion, a species may be isolated only sporadically or in widely distinct localities. For example, *Candida hawaiiiana* was recovered from nitidulid beetles in three localities on the island of Hawai’i and one locality in Costa Rica (Lachance et al., 2003a; unpublished data). In both regions, the isolates were part of multiple collections involving hundreds of isolates. Interestingly, two of the Hawaiian isolates came from beetles collected in the same flower patch but 8 months apart (Lachance et al., 2003b). The species was not isolated elsewhere in the same locality, in spite of years of extensive sampling in the area. The recovery of an apparently rare species in sites separated by thousands of kilometres of ocean is problematic. One explanation is that the species is an allochthonous member of the habitat studied and in fact represents a contaminant from a more abundant population in a neighbouring community. In the case of *C. hawaiiiana*, it is possible that the species was carried as a contaminant of the nitidulid beetle *Conotelus mexicanus*, which is known to have been introduced to the Hawaiian archipelago in the 20th century. This would also imply that the species occurs abundantly in other localities on the American continent. An alternative, to which we do not subscribe, is the notion that all microbes are ubiquitous by virtue of their small size and that their recovery is mostly a matter of sampling intensity (Fenchel & Finlay, 2004).

Although a case can be made for the massive intercontinental dispersal of certain soil micro-organisms via dust storms (Griffin et al., 2002), the generalization of such phenomena to specialized, insect-vectored yeasts (or, for that matter, aquatic protozoa) is difficult to conceive.

In 1999, two isolates of an apparently asexual yeast with an affinity in the Metschnikowiaceae were recovered in two nitidulid beetle specimens collected on the island of Rarotonga in the southern Pacific Ocean. These were the only representatives of that species in very large collections not only in Rarotonga, but also in New Caledonia, two localities in the Fiji islands and a wide array of sites in eastern and northern Australia (Lachance et al., 2001). In June 2005, we collected a small number of insects from roadside morning glory flowers in the Cameron Highlands, a relatively cool locality in peninsular Malaysia. A wide variety of yeasts (e.g. *Kodamaea*, *Wickerhamiella* and related *Candida* species) typical of those found in such insects were recovered. Among these was a single isolate whose growth characteristics and partial rDNA sequence were similar to those of the Rarotongan isolates. When mixed in pairs, the Malaysian and Rarotongan isolates gave rise to abundant mature ascii. We now describe them as a novel species.

**Isolation and characterization**

The origin of the strains is given in Table 1. Beetles were collected from roadside ornamentals near sea level in Rarotonga (21° 20' S 160° 16' W), where the climate is stable with an annual temperature range of 18–29 °C. The plants included an apparently native *Hibiscus* species and a laticiferous shrub with purple flowers, neither of which could be identified precisely. The Malaysian plants were...
Table 1. Selected properties of strains of Metschnikowia orientalis sp. nov.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mating type</th>
<th>Source</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>UWOPS 99-733.2</td>
<td>h⁺</td>
<td>Aethina concolor in flower of Hibiscus sp.</td>
<td>Titikaveka, Rarotonga, Cook Islands</td>
</tr>
<tr>
<td>UWOPS 99-745.6</td>
<td>h⁺</td>
<td>Aethina concolor in flower of unidentified</td>
<td>Avarua, Rarotonga, Cook Islands</td>
</tr>
<tr>
<td>(= CBS 10331⁵=NRRRL Y-27991⁵)</td>
<td></td>
<td>member of Rubiaceae</td>
<td></td>
</tr>
<tr>
<td>UWOPS 05-269.1</td>
<td>h⁻</td>
<td>Euporea (Haptocornnina) motschulskyi in</td>
<td>Road between Berincang and Teringkap,</td>
</tr>
<tr>
<td>(= CBS 10330⁴=NRRRL Y-27992⁴)</td>
<td></td>
<td>flower of Ipomea cairica</td>
<td>Cameron Highlands, Malaysia</td>
</tr>
</tbody>
</table>

roadside weeds known in Malay as tatampajan. Although the biotype sampled in this study is probably native to South-East Asia, Ipomea carnea is pleomorphic and pantropical. The collection sites were located at an altitude of about 1500 m in the Cameron Highlands (4° 30’ N 101° 24’ E), where the climate is cool and humid year round with a temperature range of 14–23 °C. Insects were collected in sterile WhirlPaks and later allowed to walk on the surface of YM-chloramphenicol agar plates (2 % agar, 1 % glucose, sterile WhirlPaks and later allowed to walk on the surface of YM-chloramphenicol agar plates (2 % agar, 1 % glucose, 0.5 % peptone, 0.3 % malt extract, 0.3 % yeast extract, 0.01 % chloramphenicol). Yeasts are normally deposited along with faecal material, which is then spread on the plate with a loop. Representative colonies were picked and stored on small YM agar slants pending identification and characterization following standard methods (Yarrow, 1998). Any isolate whose identification was doubtful was subjected to DNA sequence analysis. We amplified the rDNA region covering the internal transcribed spacers and the D1/D2 divergent domains of the large subunit as described elsewhere (Marinoni & Lachance, 2004). For tree construction, this and other sequences retrieved from GenBank were aligned using the program DNAMAN (version 4.1). A satisfactory alignment of the D2 variable region could not be obtained due to excessive indels, so the tree was constructed from the D1 region only. The neighbour-joining algorithm included in DNAMAN was used, with 1000 pseudoreplicates for bootstrapping. Ascus formation was observed in mixtures of cells prepared on yeast carbon base agar supplemented with 0.01 % ammonium sulphate. Beetles and plants were identified morphologically using available keys.

Latin diagnosis of Metschnikowia orientalis sp. nov.


Description of Metschnikowia orientalis sp. nov. Lachance and Bowles

Metschnikowia orientalis (o.ri.en.ta’lis. L. nom. fem. adj. orientalis from the east, referring to the Asia-Pacific biogeographical region, where all known strains were recovered).

After 3 days in yeast extract-glucose broth at 25 °C, the cells are ovoid to elongate, occur singly or in parent-bud pairs and measure 2–3 by 3–5 μm. On malt agar after 2 weeks at 17 °C, colonies are low-convex, glossy, smooth, white and butyrous. In Dalmau plate cultures on GY agar after 2 weeks, pseudomycelium or true mycelium are not formed. After 6 h on yeast carbon base supplemented with 0.01 % ammonium sulphate, mixtures of compatible cells conjugate and develop into ascii. Some ascospores are formed overnight (Fig. 1b). After 2 days, most ascii are two-spored (Fig. 1c). The mature asci are cylindrical, measure 2–4 × 30–60 μm and contain two aciculate ascospores. Early deliquescence is not observed. Apical rupture of asci is known to occur in older cultures of other large-spored species under certain conditions. Of the sugars utilized, glucose is fermented within 1 week and trehalose weakly and slowly. Glucose, sucrose, galactose, trehalose, maltose, melezitose, cellobiose, salicin, sorbose, xylose, ribose (variable), ethanol (slow), glycerol, ribitol (slow), xylitol, mannitol (sometimes weak), glucitol, sucrose (slow), citric acid (slow and variable), gluconic acid (slow), glucose-δ-lactone (slow or weak), 2-ketoglucaric acid, glucosamine (slow) and N-acetylglucosamine are assimilated. Inulin,
raffinose, melibiose, lactose, methyl z-D-glucoside, starch, rhamnose, L- and D-arabinose, methanol, 1- and 2-propanol, erythritol, galactitol, inositol, lactic acid, acetone, ethyl acetate and hexadecane are not assimilated. Ethylamine, lysine and cadaverine are utilized as sole nitrogen sources, but not sodium nitrate or nitrite. Vitamins are required for growth, but not amino acids. Growth is positive at 30 °C, weak and variable at 31 °C and negative at 32 °C. Hydrolysis of gelatin, casein and Tween 80 and acid production on chalk medium are negative. Growth is positive in the presence of 5 % NaCl, positive or slow at 10 % and variable at 15 %. Growth in the presence of 50 % glucose is positive or slow. Growth in the presence of 0-001 % cycloheximide is negative. Growth in the presence of 10 μg CTAB ml⁻¹ is positive; variable with 75 μg ml⁻¹. Starch production and Diazonium blue B reaction are negative. The habitat is in nitidulid beetles associated with flowers in Malaysia and Rarotonga.

The type strain is strain UWOPS 99-745.6T (h⁺) (= CBS 10331T = NRRL Y-27991T), isolated from Aethina concolor (Coleoptera: Nitidulidae) in a flower of an unidentified species of the Rubiaceae, in Avarua, Rarotonga, Cook Islands. The designated allotype is strain UWOPS 05-269.1 (h⁻) (= CBS 10330 = NRRL Y-27992), isolated from Epuraea motschulskyi (Coleoptera: Nitidulidae) in a flower of Ipomoea cairica in the Cameron Highlands, Malaysia.

Species delineation and phylogenetic affinities

Strains of the novel species were mixed in pairs with one another and with mating types of other species. Large protuberances reminiscent of developing asci (Fig. 1a) were observed in a mixture of the types of the novel species (UWOPS 99-745.6T) and Metschnikowia aberdeeniae (SUB 05-213.1T h⁻), suggesting that the former has the mating type h⁺. Short conjugation tubes were seen in mixtures of the allotype (UWOPS 05-269.1) of the novel species and the type of Metschnikowia continentalis (h⁺, UFMG 96-173T) or the allototype of M. aberdeeniae (h⁺, SUB 05-213.2), confirming the concordance of mating types between species. Conjugation tubes were not formed in the remaining interspecific crosses. The observation of mating reactions between these species is all the more astonishing given the 17–18 % D1/D2 sequence divergence observed between the novel species and M. continentalis or M. aberdeeniae. The tree presented in Fig. 2 shows that the novel species is a distant sister of C. hawaiiana, which incited us also to mix cells of all available strains of the two species in all possible pairs to see if mating reactions could be elicited. The results were negative.

Also suggested by Fig. 2 is the highly divergent nature of the species between which some form of mating reaction was observed, arguing against the subdivision of Metschnikowia into smaller genera on the basis of rDNA sequence data, as is sometimes suggested (Mendonça-Hagler et al., 1993). An additional enticement for a nomenclatural revision might be the apparent paraphyly of the genus Metschnikowia with

![Fig. 1. Phase-contrast micrographs of Metschnikowia orientalis. (a) Mixed culture of strain UWOPS 99-745.6T and M. aberdeeniae SUB 05-213.1T showing the formation of protuberances suggestive of a rudimentary mating reaction. (b, c) Asci obtained from mixing strains UWOPS 99-733.2 and UWOPS 05-269.1. Immature asci after overnight incubation (b) and a mature ascus after 2-day incubation (c) are shown. Bar, 5 μm.](http://ijs.sgmjournals.org)
respect to the genus *Clavispora*. Several reasons exist for avoiding a precipitous rearrangement of taxa in order to restore a presumed monophyly. First and foremost are the unusually high rates of ribosomal RNA gene sequence divergence observed in the clade. This is in sharp contrast to protein-coding gene sequences, which are only beginning to be available, and which indicate that *Metschnikowia* and allies may not be as isolated phylogenetically as previously thought (S. O. Suh, M. Blackwell, C. P. Kurtzman and M.-A. Lachance, unpublished data). Another factor is the formation, by *Metschnikowia lachancei*, of ascospores that are intermediate in morphology between typical *Metschnikowia* and *Clavispora* ascospores (Giménez-Jurado *et al.*, 2003). Yet another reason for caution is the absence of a mating reaction between the two *Clavispora* species combined with the presence of major polymorphisms in the D2 domain of the large subunit rDNA in *Clavispora lusitaniae* (Lachance *et al.*, 2003).

The tree in Fig. 2 is somewhat different from that given in the recent description of *M. aberdeeniae* (Lachance *et al.*, 2006). The difference is due to a large extent to taxon sampling effects, as the present tree contains only one representative of the *M. aberdeeniae* subclade and of course, the added sequence of *M. orientalis*. The addition of the novel species further exacerbated the difficulty of constructing a satisfactory alignment, which prompted us to retain only the more conserved D1 region in the analysis.

**Ecology and biogeography**

The nutritional profile of the novel species is typical of many members of the *Metschnikowiaceae* and is practically indistinguishable not only from that of the sister species, *C. hawaiiana*, but also from those of such diverse species as *Metschnikowia reukaufii, Metschnikowia hibisci* or *Candida kipukae*, all of which are distant relatives, at least as suggested by rDNA sequences. The isolation of *M. orientalis* in two localities separated not only by Wallace’s line, but also by 11 000 km of land and sea, is all the more remarkable, since extensive collections of similar habitats in Australia, New Caledonia and Fiji did not yield that species. Other yeasts recovered from the same substrates in Rarotonga consisted mostly of species typically recovered in floricolous beetles of other Australasian localities (*Candida azyma*, *Wickerhamiella australiensis, Kodamaea anthophila*) as well as a few unique species. The Malaysian samples yielded a mixture of the same Australasian species (but not *K. anthophila*) and species previously collected in the Neotropics (e.g. *Candida restigiae, Candida quercitrusa*) as well as a few unique ones.

The presence of species that are endemic but locally common or species that are cosmopolitan and globally common can be explained in terms of insect host specificity or vagility. The presence of rare species in disparate localities, as is the case here, is more difficult to explain in terms of both vicariance or dispersal. The relatively low maximum growth temperature (30–31 °C) of *M. orientalis* may account in part for its narrow distribution in collection sites that are located in cool habitats within tropical regions. Similar sites in Australia and other South Pacific islands exhibit higher temperature extremes. The isolation of *M. orientalis* from *Aethina concolor* on Rarotonga may have been accidental. Other beetle species were present in the same flowers, although only a few specimens were examined for yeasts. These included an inconspicuous niptidulid identified as *Eurarea* (*Haptoncus*) *oculatis*. This and a number of other *Eurarea* species, including *Eurarea motschulskyi*, occur over a large geographical range that includes much of the Australasian region.

**Identification**

The identification of *M. orientalis* based on morphology or nutritional characters is nearly impossible, due to strong similarities to many other clade members, which tend to be phenotypically homogeneous. Ascospore size and appearance are comparable to those seen in *M. aberdeeniae*, an African endemic (Lachance *et al.*, 2006), and *Metschnikowia hamakuensis*, a Hawaiian endemic (Lachance *et al.*, 2005). The maximum growth temperature is also comparable to that of other species including *M. aberdeeniae* (32–33 °C) and several Hawaiian endemics (26–33 °C). The formation of ascospores in mixtures with authentic strains or DNA sequence-based methods are the only practical means available for correct identification.

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**References**


