PUBLICATIONS ON THE YEASTS FOUND IN LAMANAI

Rosa CA, Lachance MA 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

Lachance MA, Rosa CA, Starmer WT, Schlag-Edler B, Barker JSF, Bowles JM 1998 *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae*, and *Candida lipophila*, five new related yeast species from flowers and associated insects. Int J Syst Bacteriol 48:1431-144

Lachance MA, Rosa CA, Starmer WT, Bowles JM 1998 *Candida ipomoeae*, a new yeast species related to large-spored *Metschnikowia* species. Can J Microbiol 44:718-722

Lachance MA, Bowles JM, Mueller C, Starmer WT 2000 On the biogeography of yeasts in the *Wickerhamiella* clade and description of *Wickerhamiella lipophila* sp. nov., the teleomorph of *Candida lipophila*. Can J Microbiol 46:1145-1148

Lachance MA, Bowles JM, Kwon S, Marinoni G, Starmer WT, Janzen DH 2001 *Metschnikowia lochheadii* and *Metschnikowia drosophilae*, two new yeast species isolated from insects associated with flowers. Can J Microbiol 47:103-109

Teixeira ACP, Marini MM, Nicoli JR, Antonini Y, Martins RP, Lachance MA, Rosa CA 2003 *Starmerella meliponinorum*, a novel ascomycetous yeast species associated with stingless bees. Int J Syst Evol Microbiol 53:339-343

Lachance MA, Bowles JM, Starmer WT 2003 *Metschnikowia santaceciliae*, *Candida hawaiiana*, and *Candida kipukae*, three new yeast species associated with insects of tropical morning glory. FEMS Yeast Res 3:97-103

Marinoni G, Lachance MA 2004 Speciation in the large-spored *Metschnikowia* clade and establishment of a new species, *Metschnikowia borealis* comb. nov. FEMS Yeast Res 4:587-596

Lachance MA, Ewing CP, Bowles JM, Starmer WT 2005 *Metschnikowia hamakuensis* sp. nov., *Metschnikowia kamakouana* sp. nov., and *Metschnikowia mauinuiana* sp. nov., three endemic yeasts from Hawaiian nitidulid beetles. Int J Syst Evol Microbiol 55:1369-1377

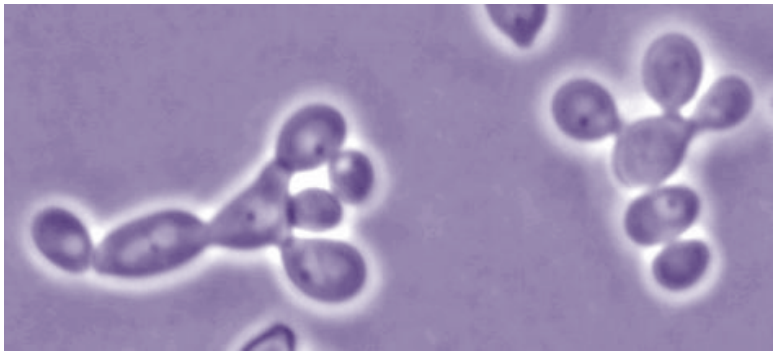
Lachance MA, Lawrie D, Dobson J, Piggott J 2008 Biogeography and population structure of the Neotropical endemic yeast species *Metschnikowia lochheadii*. Antonie van Leeuwenhoek 94:403-414

Wardlaw AM, Berkers TE, Man KC, Lachance MA 2009 Population structure of two beetle-associated yeasts: comparison of a New World asexual and an endemic Nearctic sexual species in the *Metschnikowia* clade. Antonie van Leeuwenhoek 96:1-15.

Lachance MA, Dobson J, Wijayanayaka DN, Smith AME 2010 The use of parsimony network analysis for the formal delineation of phylogenetic species of yeasts: *Candida apicola*, Candi*da azyma*, and *Candida parazyma* sp. nov., cosmopolitan yeasts associated with floricolous insects. Antonie van Leeuwenhoek 97:155-170

Department of Biology, University of Western Ontario
London, Ontario, Canada N6A 5B7
lachance@uwo.ca

THE YEASTS OF LAMANAI



Budding yeast cells

Yeasts are single-celled fungi. They reproduce by bud formation. Many yeasts also undergo a sexual cycle that leads to the formation of spores, each of which contains a single set of chromosomes (haploid). Because of their small size (~0.005 mm), yeasts are usually invisible. To study them, one must culture them on agar media, where single cells multiply and form clones, each containing millions of cells.

Yeast colonies on an agar plate

Yeasts occur in relatively small numbers on plant surfaces. Those found on leaves usually form their sexual spores externally and are classified in the Basidiomycetes. Small numbers of yeasts are also found on the skin and in the guts of animals. When conditions are favorable, yeasts can become abundant and cause infections. However, most of the roughly 1,500 known yeast species are harmless or even beneficial.

Tree sap fluxes and necrotic tissue of succulents such as agave and cactus may contain millions of yeast cells per gram. Nectar and insects that visit flowers contain large populations of very specific kinds of yeast. These form their sexual spores internally and belong to the Ascomycetes.

Beetles of the genus *Conotelus* in a bindweed flower

Two studies conducted in the Lamanai area have demonstrated the presence of seven yeast species in the digestive tract of two insect species. One is a sap beetle of the genus *Conotelus* (family Nitidulidae), which occurs in the corolla of morning glory (Convolvulaceae) flowers across the New World. The other is a stingless bee of the genus *Trigona* (tribe Meliponini). These bees are found in great abundance in water lilies of inlets of the New River Lagoon. Stingless bees are tropical or subtropical and have played a role, historically, in Mayan agriculture.

HOW DO WE KNOW WHAT YEAST IS RELATED TO WHAT YEAST?

Yeasts have very simple shapes and their growth properties are similar. It is therefore difficult to distinguish a species from another or even a genus from another. However, in 1998 and 2000, yeast experts determined and published the DNA sequence of a gene in all known yeast species. Each species has its own unique sequence that can be used as a barcode. Moreover, species of a genus have similar, but not identical sequences. The differences can be used to recognize various species even when they look the same. The sequences of yeasts of different genera have a greater degree of divergence, those of different families even greater and so on as we compare species of different orders or classes. It was DNA sequencing that allowed us, for example, to determine that *Candida ipomoeae* is a relative of large-spored *Metschnikowia* species, that *Candida parazyma* is a cousin of *Wickerhamiella* species, and that certain *Candida* species are related to the genus *Starmerella*.

What does all this have to do with the yeasts used in bread making or in the fermentation of beer or wine? These processes are due entirely to the action of yeasts of the genus *Saccharomyces*, which are able to produce large amounts of alcohol without intoxicating themselves to death. Species of the genera *Metschnikowia*, *Wickerhamiella*, and *Starmerella* belong to other yeast families. They also produce alcohol, but in smaller amounts.

A stingless bee in a water lily
of an inlet of the New River Lagoon

STARMERELLA

Inlets connected to the New River Lagoon of Lamanai are home to all kinds of life forms, including crocodiles, which gave the name "Lamanai" to the area. One frequently encounters large clusters of water lilies that teem with pollinator insects, including solitary bees.

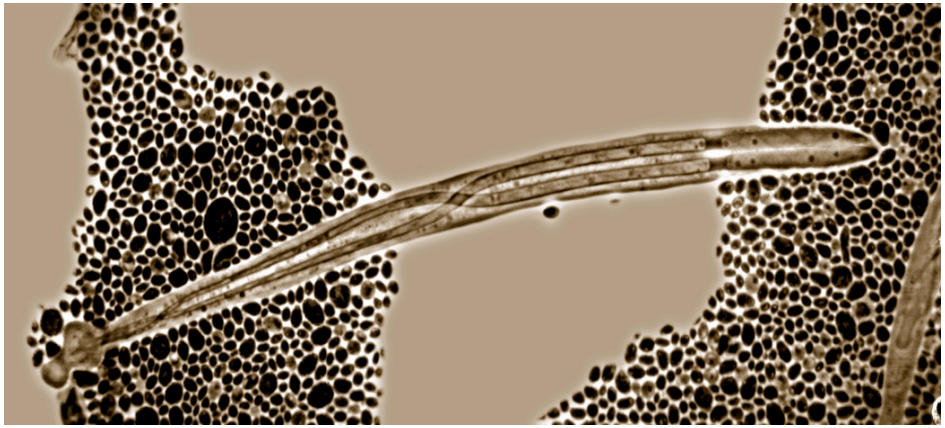
Yeasts of the genus *Starmerella* are intimately associated with bees of all kinds, including bumblebees and solitary bees. Bees collected in water lilies in the inlet opposite Lamanai harbor two yeast species that have affinities with the genus *Starmerella*. These yeasts are new to science and will eventually be named and described as new species that are endemic to Belize and the surrounding area. Their nearest relatives are called *Candida etschellsii* and *Candida versatilis*.

A morning glory flower of Lamanai

THE YEASTS

METSCHNIKOWIA

Metschnikowia santaceciliae is the most abundant yeast species found in morning glory beetles of Lamanai. Named after the village of Santa Cecilia in Costa Rica, this yeast was previously known only from Costa Rican highlands. A close relative, *Metschnikowia lochheadii*, also was found in beetles of a few flowers on the edge of the lagoon. This species occurs from Mexico to Brazil, but has been found also in Hawaii, where it was introduced along with the beetle *Conotelus mexicanus*. The third species, *Candida ipomoeae*, is really another related *Metschnikowia*, but because of some quirk of fungal nomenclature, it bears a different genus name. It is the most broadly distributed member of this group, ranging from Brazil to Tennessee, and like *M. lochheadii*, it was also introduced to Hawaii. Other species in the same group are endemic to the Americas or to Hawaii. They are almost invariably present in large numbers in the digestive tract of beetles of the genus *Conotelus* and relatives, such as species of the Hawaiian genus *Prosopeus*.



Ascus of a *Metschnikowia* species with two large spores, surrounded by budding cells

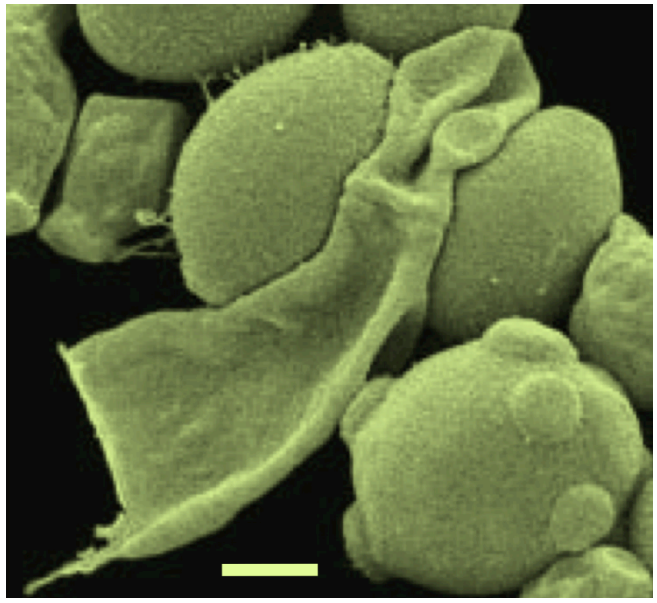
What distinguishes this group of *Metschnikowia* species is the formation of sexual spores that are much larger than the normal cells. The function of the spores is unknown. It is thought that the yeasts are "farmed" by the beetles. Adults carry yeasts to flowers where they feed on nutrients found in the flowers. The beetles lay eggs and the growing larvae feed on the flowers as they decay. The yeasts enhance the food value of the flowers. In the laboratory, the entire cycle of the beetles lasts less than two weeks and larvae can be fed a diet of pure yeast, which they consume with a passion. Other, moderately related *Metschnikowia* species are frequently associated with nectar-feeding bumblebees or with fruit surfaces. Some are pathogens of small crustaceans such as water flees or brine shrimp.

Colonies of *Metschnikowia santaceciliae* (front row) and *Candida ipomoeae* (back row)

WICKERHAMIELLA

Morning glories and their insects often contain yeasts of the genus *Wickerhamiella*. Beetles of Lamanai carry *Wickerhamiella occidentalis*, which has almost the same distribution as *Candida ipomoeae* (Brazil to Tennessee, plus Hawaii). The beetles also carry the related species *Candida parazyma*, which has an even broader range, having been isolated also in Southeast Asia, Australia, and the South Pacific.

Wickerhamiella species rank among the smallest yeasts known, with cells that are as small as 0.002 mm. They form a single sexual spore that is released from the end of a trumpet-shaped ascus. Other species in the genus occur in flower beetles or drosophilid flies across tropical and subtropical regions of the world. Some are indigenous to Australasia or to South America.



The empty trumpet-shaped ascus of a *Wickerhamiella* species