Saccharomycopsis fodiens sp. nov., a rare predacious yeast from three distant localities

Marc-André Lachance,[1] Carlos A. Rosa,[2] Enrique Javier Carvajal,[3] Larissa F. D. Freitas[2] and Jane M. Bowles[1]

Correspondence
Marc-André Lachance
lachance@uwo.ca

[1]Department of Biology, University of Western Ontario, London, ON N6A 5B7, Canada

[2]Department of Microbiology, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270-901, Brazil

[3]Colección de Levaduras Quito Católica, Centro Neotropical para Investigación de la Biomasa, Pontificia Universidad Católica del Ecuador, Escuela de Ciencias Biológicas, CP 17-01-2184, Quito, Ecuador

Three strains representing a novel yeast species were recovered as part of independent collections from flower-associated nitidulid beetles in Australia, Costa Rica and the Galapagos Islands, Ecuador. Analysis of the D1/D2 domains of the large subunit rRNA gene indicated that the species belongs to the genus *Saccharomycopsis*, although the formation of ascospores was not observed. The yeast is capable of necrotrophic parasitism by means of infection pegs when mixed with other yeasts or filamentous fungi. Of particular interest is the fact that despite the large distances separating the isolation sites of the three strains, other strains of the species have not been recovered in other samples of flower-associated nitidulids even though these habitats have been sampled extensively. It is suggested that the dispersal of the yeast may be linked to human historical factors. The name *Saccharomycopsis fodiens* sp. nov. is proposed for the yeast. The type strain is UWOPS 95-697.4^T (=CBS 8332^T=NRRL Y-48786^T).

The global dispersal of micro-organisms remains poorly understood in spite of attempts (Fenchel & Finlay, 2004) to fit the problem to a simplistic model known as 'Everything is Everywhere'. Our ability to understand yeast dispersal has been hampered by the fact that a significant proportion of what we know of yeast diversity comes from the description of poorly represented species whose distributions are not properly characterized. Although such an acontextual approach to the study of yeast diversity is not wholly encouraged, it is still defended (Kurtzman, 2010; Kurtzman *et al.*, 2011). Fortunately, many contemporary surveys attempt to characterize whole yeast communities within a broader biological framework. As a result, systematic ecological studies of specific yeast communities have in some cases demonstrated strong endemism, such as is often the case for yeasts isolated from flower-inhabiting nitidulid beetles. Strong endemism is apparent in several ascomycetous yeast species of the genera *Kodamaea* (Rosa *et al.*, 1999), *Metschnikowia* (Lachance *et al.*, 1998a, 2005) and *Wickerhamiella* (Lachance *et al.*, 1998b). Examples of nitidulid-associated species with a more widespread, even possibly global distribution have also been identified for these clades, as seen for example in *Candida* (*iter. nom. Wickerhamiella*) *parazyma* (Lachance *et al.*, 2010). Most perplexing among these are instances where a yeast species is isolated only rarely, as a very minor component of the community, but in remote localities. *Candida* (*iter. nom. Metschnikowia*) *hawaiiana* (Lachance *et al.*, 2003) and *Metschnikowia orientalis* (Lachance *et al.*, 2006) are such examples, as is the species to be described here as *Saccharomycopsis fodiens* sp. nov.

Some brief remarks about nomenclature are appropriate. The 18th International Botanical Congress, held in July 2011, adopted an amendment to Article 59 of the International Code of Botanical Nomenclature which effectively does away with the dual nomenclature traditionally used in mycology to designate the sexual and asexual forms of a fungal species (Norvell, 2011). As a result, asexual species that are clearly part of the monophyletic assemblage typified by the genus *Saccharomycopsis* must now be given that name. Existing *Candida* species belonging to the clade will be reassigned, as appropriate, by a special subcommittee. In anticipation of this exercise, we are designating species of the genus *Candida* with a clear teleomorphic affiliation as *iter. nom.* (*iterum nominanda*, meaning 'to be renamed').

The GenBank/EMBL/DDBJ accession number for the D1/D2 LSU rRNA gene sequence of strain UWOPS 95-697.4^T is AF203418.

The MycoBank number for *Saccharomycopsis fodiens* sp. nov. UWOPS 95-697.4^T is MB564897.

Origin of strains

The first isolate, strain UWOPS 95-697.4^T, came from a specimen of *Aethina concolor* (Coleoptera: Nitidulidae) found in a flower of *Hibiscus heterophyllus*, in Cooloola National Park, Queensland, Australia, in 1995. A bee was also present in the flower at the time of collection, but other yeasts isolated from that specimen were typical of this nitidulid–flower ecosystem, consisting of several morphotypes of *Kodamaea anthophila*. *A. concolor* is almost always present in flowers of *Hibiscus* and *Ipomoea* species in Eastern Australia, is widespread in the South Pacific and has been introduced to Hawaii in the recent past (Kirejtshuk & Lawrence, 1999). This beetle carries a highly specific yeast community and coexists with other beetles as well as with drosophilids that also carry yeasts. Despite extensive sampling of nearly a thousand yeasts from *A. concolor* and sympatric insects in many localities, only a single strain of *S. fodiens* sp. nov., UWOPS 95-697.4^T, has been found in that community. This isolate was the first among the Saccharomycetes to be identified as capable of necrotrophic mycoparasitism involving penetration of yeasts or other fungi with infection pegs, causing death of the penetrated cell (Lachance & Pang 1997). Strain UWOPS 95-697.4^T was also found to grow poorly in the absence of organic sulfur, which led to the discovery that many natural sulfur auxotrophs are also predaceous. This observation led to the generalization of predation and sulfate uptake deficiency across the genus *Saccharomycopsis* (Lachance *et al.*, 2000).

Strain UWOPS 03-190.3 originated from a specimen of *Conotelus* sp. (Coleoptera: Nitidulidae) found in a flower of *Ipomoea batatas*, near El Gavilán, on El Rincón de la Vieja volcano, Guanacaste Province, Costa Rica, in 2003. Again, these communities have been subject to extensive sampling, totalling hundreds of yeast isolates, but hitherto have yielded only a single strain of *S. fodiens* sp. nov. The sample was unusual in that it contained, in addition to *Metschnikowia similis*, which is apparently endemic to that region, a strain of *Candida* (*iter. nom. Kurtzmaniella*) *quercitrusa*. The latter was described on the basis of an oak frass isolate, but has rarely been reported subsequently from that substrate (Lachance *et al.*, 2011). According to our records, *C. quercitrusa* occurs sporadically in beetles or flowers in Australia, Belize, Hawaii and Malaysia, and the CBS database (Robert & Groenewald, 2012) mentions an isolation from flowers in French Guiana.

Strain CLQCA-24ST-010 came from an unidentified nitidulid beetle found in a flower of *Ipomoea* sp. on the Island of San Cristóbal, Galapagos Islands, Ecuador, in October 2010. Other yeasts in the sample included *Meyerozyma guilliermondii* and an isolate tentatively assigned to the genus *Kodamaea*. In this collection, 12 flowers were sampled, and the most frequent yeasts were a novel species of the genus *Kodamaea* as well as *Candida parazyma*, *C. quercitrusa* and *Hanseniaspora uvarum*. Strain CLQCA 24ST-010 was isolated only from the nitidulid beetle collected from this *Ipomoea* sp. in San Cristóbal. Two other *Ipomoea* species collected on the Island of Isabela had as most frequent yeasts *C. parazyma*, *Candida* (*iter. nom. Metschnikowia*) *kipukae*, the novel species of the genus *Kodamaea* and *H. uvarum*.

The patterns described above suggest that *S. fodiens* sp. nov. may not be an autochthonous member of the flower–nitidulid community, but instead that it may be vectored by other insects that share the same flowers.

Species delineation and phylogeny

The isolates were characterized by the standard methods of Yarrow (1998) unless otherwise indicated. Preparation of cells for scanning electron microscopy was as described by Lachance *et al.* (1998a). The rDNA gene region spanning the ITS rDNA gene, the 5.8S rRNA gene, and the D1/D2 domains of the large subunit rRNA gene was amplified from whole cells and sequenced as reported previously (Marinoni & Lachance, 2004). The sequences of the three strains were identical, supporting the existence of a single species. Note that the sequence deposited as GenBank no. AF203418, originally deposited in 1999, was updated as a result of this work. The original deposit was limited to the D1/D2 segment and contained several artefacts. Alignment with other published sequences and phylogenetic analysis was accomplished with MEGA5 (Tamura *et al.*, 2011). The analysis in Fig. 1 clearly indicates that *S. fodiens* sp. nov. occupies a basal position within the inner *Saccharomycopsis* clade and is well differentiated from any other species.

Description of *Saccharomycopsis fodiens* sp. nov. Lachance, Rosa & Carvajal

Saccharomycopsis fodiens (fo′di.ens. L. nom. fem. sing. adj. *fodiens* present participle of *fodere*, to transpierce, referring to the process of penetration of cells with infection pegs formed by this species).

After 3 days on YM agar at 25 °C, cells are ellipsoid (1.3–1.7 × 2.1–3.3 μm) and occur singly or in mother-bud pairs. The colonies are off-white, dull, flat-umbonate, with a slightly undulating margin. A faint ring is formed after 10 days in liquid media. On YCBY agar (yeast carbon base supplemented with 0.01 % yeast extract), the slide culture consists primarily of budded cells (Fig. 2b). Short chains of elongated cells may be formed after 3 days. After 2 weeks, a few pseudohyphae consisting of elongated cells with lateral buds are formed (Fig. 2c). Larger (up to 5 μm) spheroid cells with aseptate protuberances up to 30 μm long also occur (Fig. 2d). Asci are not formed on common sporulation media even after mixing the strains in pairs. In the presence of other micro-organisms such as yeasts and other fungi, short protuberances are formed (Fig. 2a) that can penetrate foreign cells and cause their death. Glucose fermentation is weak and variable. Trehalose, cellobiose, salicin, sorbose, xylose, L-arabinose, D-arabinose, ribose (slow), ethanol (sometimes weak), 1-propanol

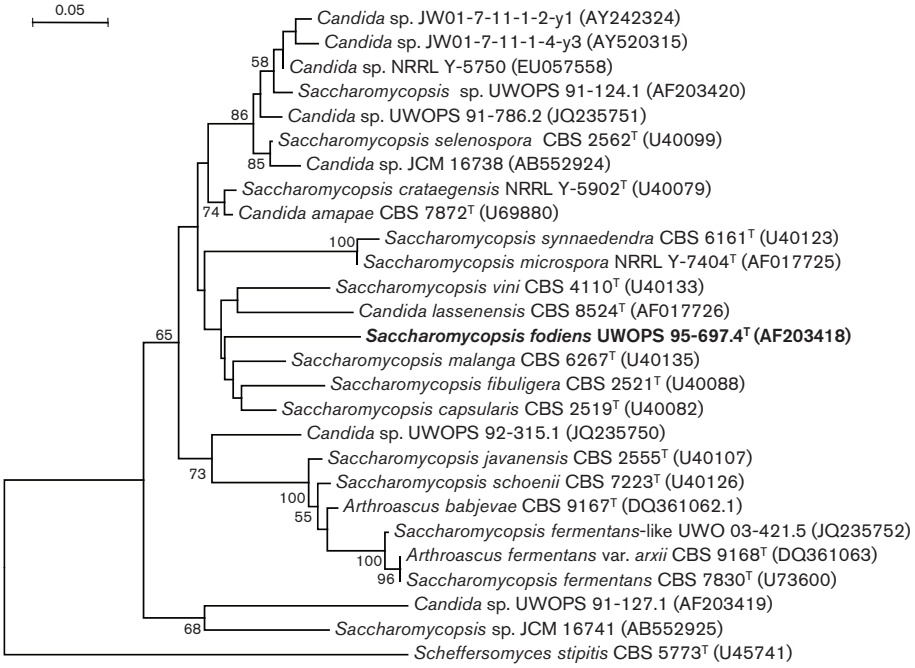

Fig. 1. Phylogenetic placement of *Saccharomycopsis fodiens* sp. nov. based on a maximum-likelihood analysis of D1/D2 LSU rRNA gene sequences, using MEGA5. Distances were Kimura two-parameter transformed. Log-likelihood=−3320.2. Evolutionary rates were fitted to a gamma distribution with 5 categories (0.4055). A total of 533 positions were used in the analysis. Bootstrap values >50 % are shown for 100 iterations. Bar, 0.05 K_{nuc}.

(weak), glycerol, erythritol, ribitol, xylitol, mannitol, glucitol, inositol, D-glucuronic acid, lactic acid, succinic acid, citric acid (variable), malic acid (slow), D-gluconic acid, D-glucono-δ-lactone and ethyl acetate are assimilated. Inulin, sucrose, raffinose, melibiose, galactose, lactose, maltose, melezitose, methyl α-D-glucoside, starch, rhamnose, methanol, 2-propanol, 1-butanol, galactitol, 2-keto-D-gluconic acid, D-glucosamine, *N*-acetyl-D-glucosamine, acetone and hexadecane are not assimilated. Lysine and cadaverine are utilized as sole nitrogen sources, but not nitrate, nitrite or ethylamine. Growth on vitamin-free medium is absent. Growth on amino acid-free medium is slow and weak. Growth at 32 °C is positive or weak; growth at 33 °C is negative or weak; growth at 34 °C is negative. Hydrolysis of gelatin and casein are slow. Growth in the presence of 0.1 % cycloheximide is positive. Growth in the presence of 5 % NaCl (w/v) is absent. Growth in the presence of 50 % glucose is weak and variable. Tween 80 hydrolysis is negative. Starch-like compounds are not produced. The Diazonium blue B reaction is negative.

The type culture, strain UWOPS 95-697.4^T, was isolated from *Aethina concolor* (Coleoptera: Nitidulidae) collected in a flower of *Hibiscus heterophyllus*, in Cooloola National Park, Queensland, Australia. It has been deposited in the Centraalbureau voor Schimmelcultures collection under the number CBS 8332^T (=NRRL-Y 48786^T). The name is registered in MycoBank under the number MB 564897.

Latin diagnosis of *Saccharomycopsis fodiens* sp. nov. Lachance, Rosa & Carvajal

In agaro YM post dies tres ad 25 °C, *cellulae ellipsoidae* (1.3–1.7 × 2.1–3.3 μm), *singulae at binae. In medio liquido annulus formatur post dies* 10. *Colonia candida*, *hebes*, *plana et umbonata*, *cum margine undulata. In agaro YCBY*, *pseudohyphae formantur. Ascosporae nullae. Glucosum fermentatur*, *exigue et variabile. Trehalosum*, *cellobiosum*, *salicinum*, *sorbosum*, *xylosum*, L-*arabinosum*, D-*arabinosum*, *ribosum* (*lente*), *ethanolum* (*aliquando exigue*), 1-*propanolum* (*exigue*), *glycerolum*, *erythritolum*, *ribitolum*, *xylitolum*, *mannitolum*, *glucitolum*, *inositolum*, D-*glucuronatum*, *lactatum*, *succinatum*, *citratum* (*variabile*), *malatum* (*lente*), D-*gluconatum*, D-*glucono-δ-lactonum et ethyl acetas assimilantur, at non inulinum*, *sucrosum*, *raffinosum*, *melibiosum*, *galactosum*, *lactosum*, *maltosum*, *melezitosum*, *methyl* α-D-*glucosidum, amylum, rhamnosum, methanolum,* 2-*propanolum*, 1-*butanolum*, *galactitolum*, 2-*keto*-D-*gluconatum*, D-*glucosaminum*, *N-acetyl*-D-*glucosaminum*, *acetonum*, *nec hexadecanum. Lysinum et cadaverinum assimilantur at non nitratum*, *nitritum et ethylaminum. Vitamina externa necessaria sunt. Incrementum sine acidis aminatis exiguum et lentum. Crescit ad* 32 °C *at non* 34 °C. *Fodet alios fungos cum fistula interfectoria. Typus* UWOPS 95-697.4^T, *isolatus ex Aethina concolor* (Coleoptera: Nitidulidae) *in flore Hibiscus heterophyllus*, *in* Cooloola National Park, Queensland, Australia. *Depositus est in Centraalbureau voor*

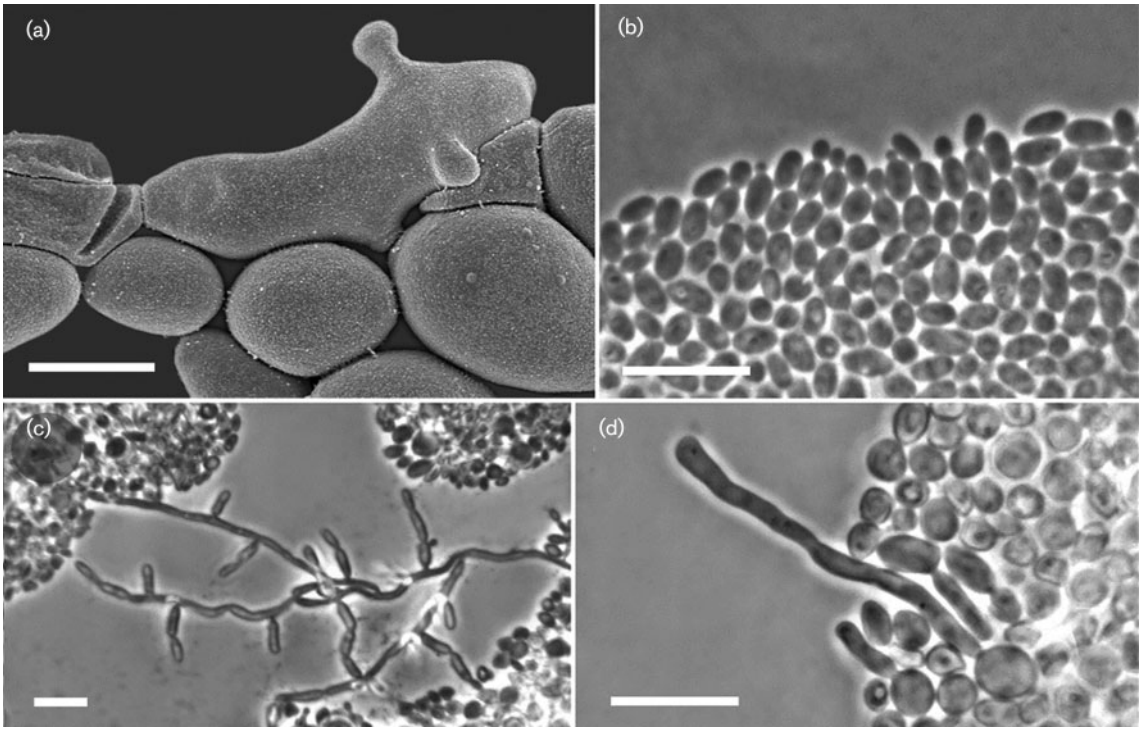

Fig. 2. Growth forms of *Saccharomycopsis fodiens* sp. nov. Strains UWOPS 95-697.4^T (a, c) and UWOPS 03-190.3 (b, d). Scanning electron micrograph of cell with infection pegs formed in the presence of *Metschnikowia hibisci* UWOPS 95-747.4 (a); bar, 2 μm. Phase-contrast micrographs of budding cells on YCBY agar, 3 days (b), pseudohyphae on YCBY agar, 2 weeks (c) and swollen cells with short pseudohyphae on YCBY agar, 2 weeks (d); bars, 10 μm (b–d).

Schimmelcultures collection sub numero CBS 8332^T (=NRRL-Y 48786^T).

Expansion of the genus *Saccharomycopsis*

The addition of *S. fodiens* sp. nov. broadens the definition of the genus (Kurtzman & Smith, 2011), which is hereby emended to include: 'Asexual reproduction: True hyphae, often with blastoconidia are abundant in some species and absent in others. Pseudohyphae may be formed'. The rest of the diagnosis remains unchanged.

Sulfur uptake

Known species of the *Saccharomycopsis* clade are deficient in sulfate uptake and require supplementation with one of a variety of organic sulfur sources (Lachance *et al.*, 2000). In *S. fodiens* sp. nov. weak growth may occur at the expense of sulfate over extended incubation times. When tested by auxanography on yeast nitrogen base without amino acids (0.67 %), glucose (1 %) agar with the addition of a few crystals of DL-methionine near the edge of the plate (M, Fig. 3), growth was confined to the diffusion zone after 2 days at room temperature, and no background growth was detectable for 5 days, after which weak growth became perceptible across the entire plate. Interestingly, the highest yield occurred near the edge of the diffusion zone.

Growth habit

Members of the *Saccharomycopsis* clade exhibit considerable variation in the extent of hyphal and pseudohyphal growth (Kurtzman & Smith, 2011). In our experience, some isolates of *S. fibuligera* or *S. selenospora* grow almost exclusively as filamentous forms, whereas species such as *S. fermentans* or *S. schoenii* grow in a predominantly unicellular fashion, at least in rich media. *S. fodiens* sp. nov. may represent a new extreme in this continuum, where growth is almost entirely unicellular even on nitrogen-poor media such as YCBY used to stimulate filamentous growth. Some variation was observed among the three strains, where strain CLQCA-24ST-010 exhibited the highest propensity to form short chains of elongated cells even in young cultures. The cell size in *S. fodiens* sp. nov. appears to be at the lower end of the range for the clade. Lachance *et al.* (2000) observed cannibalism in species formerly assigned to the genus *Arthroascus* (*S. fermentans*, *S. javanensis*, *S. schoenii*) whereby a mixture of freshly transferred cells with older cells of the same strain engaged in predation. Analogous mixtures of the different isolates

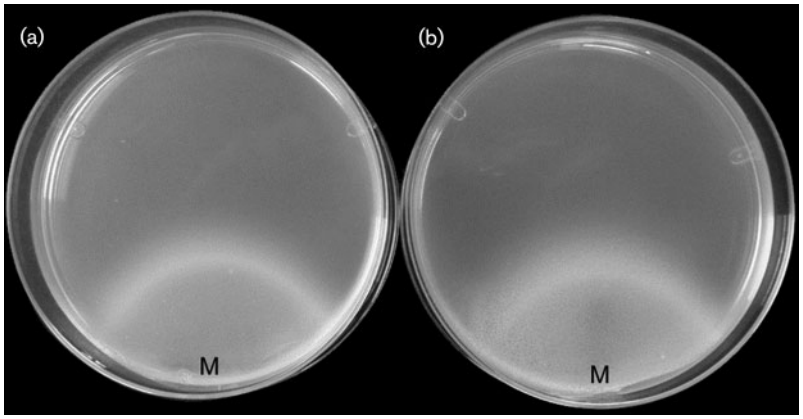

Fig. 3. Auxanograms of strains UWOPS 95-697.4^T (a) and CLQCA-24ST-010 (b) after 3 days at room temperature (~20 °C). The amino acid-free YNB-glucose agar plates were inoculated with lawns of cells and briefly air-dried. Crystals of DL-methionine (~1 mg) were deposited near the edge of the plates (M).

of *S. fodiens* sp. nov. did not exhibit any such behaviour, necrotrophy being targeted exclusively to members of other species.

Biogeography

In the beginning, we noted that *S. fodiens* sp. nov. appears to join a growing list of species where it may indeed be appropriate to invoke the ubiquity model of microbial biogeography, at least at first glance. Most of the data presented in this paper, including identical barcode DNA sequences, point to a high degree of homogeneity among the three isolates. We suggest that a possible explanation is that species such as *S. fodiens* sp. nov. may in fact be allochthonous members of the flower nitidulid yeast community and that they properly belong to another community whose distribution overlaps with the known sources of isolation. An intriguing possibility is that human migrations, along with the displacement of domesticated or commensal plants or animals, could account for the rapid dispersal of very specialized micro-organisms. Further evidence for this proposition would need to come from the identification of a biogeographic centre of origin for *S. fodiens* sp. nov. or other species with a rare record of occurrence, but widespread distributions. The centre of origin should match the purported points of departure or passage of human migrations. The three collection sites for *S. fodiens* sp. nov. are not incompatible with the purported range of Polynesian migrations over the last few thousand years. Anthropologists describe a remarkable migration departing from Taiwan and Southern China around 6000 years ago in the direction of Indonesia, Melanesia and Micronesia. This event is termed the 'out of Taiwan' model and is popularly referred to as the 'Express train to Polynesia' (Oppenheimer & Richards, 2001). Polynesians were remarkable sailors who saw the ocean not as a barrier, but as a highway. An analysis of human genes (Kayser *et al.*, 2006) supports a dual origin in Taiwan and Melanesia. Trans-Pacific contact during these migrations has received support from the finding of the Andean sweet potato, *Ipomaea batatas*, in excavations at Tangatatau, a large rock shelter in the Cook Islands (Polynesia). Several specimens of carbonized sweet potato tubers in prehistoric contexts unequivocally establishes the presence of *I. batatas* in central eastern Polynesia by the year 1000 CE. Moreover, the similarity of the word *cumar* (meaning sweet potato) in the Quichua language of the Ecuadorian and Peruvian high Andes and the word *kumara* and its variants in Polynesia provide additional evidence of ancient contacts between Polynesians and Andean populations (Scaglion & Cordero, 2011). Recently a novel yeast species (*Candida theae*) belonging to the *Lodderomyces* clade was described from two isolates, one from an Indonesian tea bottle collected in 2009 and the other from an ancient chicha fermentation vessel (680 CE; Chang *et al.*, 2012) uncovered during a microbial archaeology survey in Quito, Ecuador (Gomes *et al.*, 2009; Carvajal *et al.*, 2011). The relatedness of *C. theae* to other species such as *Candida parapsilosis*, *Candida metapsilosis*, *Candida orthopsilosis*, *Candida tropicalis* and *Candida albicans* suggested that the newly discovered species may have been a member of the microbiota of ancient human populations. One can further predict that other isolates of *C. theae* will eventually be recovered in association with human materials in South-East Asian localities and likewise that *S. fodiens* sp. nov. will be identified in a plant–insect habitat of the same region.

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References

Carvajal, E. J., Libkind, D., Briones, A., Úbeda, J., Portero, P., Roberts, I., James, S. A., Morais, P. & Rosa, C. A. (2011). Yeast biodiversity and its significance: case studies in natural and human-related

environments, ex situ preservation, applications and challenges. In *Changing Diversity in Changing Environments*, pp. 55–86. Edited by O. Grillo & G. Venora. Rijeka, Croatia: InTech Publishers.

Chang, C.-F., Lin, Y.-C., Chen, S.-F., Carvajal Barriga, E. J., Barahona, P. P., James, S. A., Bond, C. J., Roberts, I. N. & Lee, C.-F. (2012). *Candida theae* sp. nov., a new anamorphic beverage-associated member of the *Lodderomyces* clade. *Int J Food Microbiol* **153**, 10–14.

Fenchel, T. & Finlay, B. J. (2004). The ubiquity of small species: patterns of local and global diversity. *Bioscience* **54**, 777.

Gomes, F. C. O., Lacerda, I. C. A., Libkind, D., Lopes, C. A., Carvajal, J. & Rosa, C. A. (2009). Traditional foods and beverages from South America: microbial communities and production strategies. In *Industrial Fermentation: Food Processes, Nutrient Sources and Production Strategies*, pp. 2–27. Edited by J. Krause & O. Fleischer. Hauppauge, NY, USA: Nova Science Publishers Inc.

Kayser, M., Brauer, S., Cordaux, R., Casto, A., Lao, O., Zhivotovsky, L. A., Moyse-Faurie, C., Rutledge, R. B., Schiefenhoevel, W. & other authors (2006). Melanesian and Asian origins of Polynesians: mtDNA and Y chromosome gradients across the Pacific. *Mol Biol Evol* **23**, 2234–2244.

Kirejtshuk, A. G. & Lawrence, J. F. (1999). Notes on the *Aethina* complex (Coleoptera: Nitidulidae: Nitidulinae), with a review of *Aethina* (*Cleidorura*) subgen. nov. and *Aethina* (*Idaethina*) Gemminger et Harold. *Annales Zoologici* **49**, 233–254.

Kurtzman, C. P. (2010). Description of new yeast species – is one strain enough? *Bull BISMiS* **1**, 17–24.

Kurtzman, C. P. & Smith, M. Th. (2011). *Saccharomycopsis* Schiönning (1903). In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 751–763. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

Kurtzman, C. P., Fell, J. W. & Boekhout, T. (2011). Definition, classification and nomenclature of the yeasts. In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 3–5. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

Lachance, M. A. & Pang, W. M. (1997). Predacious yeasts. *Yeast* **13**, 225–232.

Lachance, M.-A., Rosa, C. A., Starmer, W. T., Schlag-Edler, B., Barker, J. S. F. & Bowles, J. M. (1998a). *Metschnikowia continentalis* var. *borealis*, *Metschnikowia continentalis* var. *continentalis*, and *Metschnikowia hibisci*, new heterothallic haploid yeasts from ephemeral flowers and associated insects. *Can J Microbiol* **44**, 279–288.

Lachance, M. A., Rosa, C. A., Starmer, W. T., Schlag-Edler, B., Barker, J. S. F. & Bowles, J. M. (1998b). *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**, 1431–1443.

Lachance, M. A., Pupovac-Velikonja, A., Natarajan, S. & Schlag-Edler, B. (2000). Nutrition and phylogeny of predacious yeasts. *Can J Microbiol* **46**, 495–505.

Lachance, M. A., Bowles, J. M. & Starmer, W. T. (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.

Lachance, M. A., Ewing, C. P., Bowles, J. M. & Starmer, W. T. (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, M. A., Bowles, J. M., Wiens, F., Dobson, J. & Ewing, C. P. (2006). *Metschnikowia orientalis* sp. nov., an Australasian yeast from nitidulid beetles. *Int J Syst Evol Microbiol* **56**, 2489–2493.

Lachance, M. A., Dobson, J., Wijayanayaka, D. N. & Smith, A. M. E. (2010). The use of parsimony network analysis for the formal delineation of phylogenetic species of yeasts: *Candida apicola*, *Candida azyma*, and *Candida parazyma* sp. nov., cosmopolitan yeasts associated with floricolous insects. *Antonie van Leeuwenhoek* **97**, 155–170.

Lachance, M. A., Boekhout, T., Scorzetti, G., Fell, J. W. & Kurtzman, C. P. (2011). *Candida* Berkhout (1923). In *The Yeasts, a Taxonomic Study*, 5th edn, pp. 987–1278. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

Marinoni, G. & Lachance, M. A. (2004). Speciation in the large-spored *Metschnikowia* clade and establishment of a new species, *Metschnikowia borealis* comb. nov. *FEMS Yeast Res* **4**, 587–596.

Norvell, L. L. (2011). Fungal nomenclature. 1. Melbourne approves a new code. *Mycotaxon* **116**, 481–490.

Oppenheimer, S. & Richards, M. (2001). Fast trains, slow boats, and the ancestry of the Polynesian islanders. *Sci Prog* **84**, 157–181.

Robert, V. & Groenewald, M. (2012). CBS Database (yeasts) [updated 2012]. http://www.cbs.knaw.nl/collections/Biolomics.aspx?Table=CBS%20strain%20database.

Rosa, C. A., Lachance, M. A., Starmer, W. T., Barker, J. S. F., Bowles, J. M. & Schlag-Edler, B. (1999). *Kodamaea nitidulidarum*, *Candida restingae* and *Kodamaea anthophila*, three new related yeast species from ephemeral flowers. *Int J Syst Bacteriol* **49**, 309–318.

Scaglion, R. & Cordero, M. A. (2011). Did ancient Polynesians reach the new world? Evaluating evidence from the Ecuadorian Gulf of Guayaquil. In *Polynesians in America: Pre-Columbian Contacts with the New World*, pp. 171–193. Edited by T. Jones, A. A. Storey, E. A. Matisoo-Smith & J. M. Ramírez-Aliaga. Lanham, MD: Altamira Press.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.