Hagleromyces gen. nov., a yeast genus in the Saccharomycetaceae, and description of Hagleromyces aurorensis sp. nov., isolated from water tanks of bromeliads

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Three strains of a novel yeast species were isolated from water tanks (phytotelmata) of a bromeliad species collected in the state of Tocantins, Brazil. Analysis of sequences for the region spanning the SSU rRNA gene, the internal transcribed spacer, the 5.8S rRNA gene and the D1/D2 domains of the LSU rRNA gene and RNA polymerase II gene showed that these novel yeasts belong to a species that is distinct from all recognized ascomycetous yeast species. Based on the results of gene sequence analyses, a novel species representing a new genus in the Saccharomycetaceae is proposed. The novel species is assigned to the genus Hagleromyces gen. nov. The three isolates of the novel yeast species failed to form sexual spores alone or in mixtures. The name Hagleromyces aurorensis sp. nov. is proposed to accommodate these isolates. The type strain of H. aurorensis sp. nov. is UFMG-CM-Y311T (=CBS 13264T).

Separation of yeast species using gene sequences is now rapid and accurate. Several DNA sequences have been used for species recognition, including the D1/D2 domains of the LSU rRNA gene, the internal transcribed spacer (ITS) region, the SSU rRNA gene, and the coding regions of the cytochrome oxidase II, actin-1, elongation factor 1-α and RNA polymerase II genes (Kurtzman & Fell, 2006; Kurtzman & Robnett, 2013). However, the delineation of genera and taxa of higher ranks remains problematic. Yeast genera that were defined on the basis of unique morphological and physiological traits or sexual cycles usually receive corroboration from DNA gene sequence analyses. As a substantial proportion of ascomycetous yeasts are known only from their asexual stage, the delineation of genera may in some cases depend entirely on phylogenetic considerations derived from sequence analyses. The current International Code of Nomenclature for Algae, Fungi and Plants allows the inclusion in the same yeast genera of species both with and without known sexual forms (McNeill et al., 2012; Lachance & Kurtzman, 2013; Kurtzman & Robnett, 2013).

Kurtzman & Robnett (2013) examined the sequence divergence of the SSU and LSU rRNA genes, and those of the translation elongation factor-1α and RNA polymerase II, subunits 1 and 2, among the type strains of 70 recognized genera of ascomycetous yeasts. Their study provides a foundation for our understanding of sequence divergence among genera in the Saccharomycotina.

During a survey of yeast species associated with the water tanks (phytotelmata) of bromeliads in the state of Tocantins, Brazil, three isolates (UFMG-CM-Y311T, Y354 and UFMG-CM-Y355) of a possible novel species were found. Analysis of the sequence spanning the SSU gene to the D1/D2 domains of the LSU rRNA gene failed to establish a close relationship with any described species in the Saccharomycotina, but suggested membership in the Saccharomycetaceae, equivalent to clade 1 in the type strain phylogeny of Kurtzman & Robnett (2013). We therefore propose the monotypic genus Hagleromyces gen. nov., with the species Hagleromyces aurorensis sp. nov. to accommodate these yeast isolates.

Abbreviation: ITS, internal transcribed spacer.
The GenBank/EMBL/DDBJ accession numbers for the SSU, internal transcribed spacer, 5.8 and D1/D2 sequences of the rRNA gene and RNA polymerase II gene of strain UFMG-CM-Y311T are KF898353 and KJ863400, respectively.
Yeast isolation and identification

Yeasts were collected from a tableland site (known in Brazil as ‘campos rupestres’) of the cerrado ecosystem in Aurora of Tocantins, south-east Tocantins State (12° 42’ 07” S 46° 25’ 04” W). The campos rupestre vegetation is characterized by high levels of species richness and endemism. The high diversity in this ecosystem has been attributed to the mosaic of environments formed by several soil classes, rugged relief and microclimatic variation (Carvalho et al., 2012). The Bromeliaceae stand out as one of the most important plant families in these ecosystems (Alves & Kolbek, 2010; Safar et al., 2013). Collections were made in December 2011 and February 2012. Water samples from phytotel mata were collected from 30 individuals of Bromelia karatas (subfamily Bromelioideae, Bromeliaceae). Water samples were collected aseptically with a sterile pipette and transferred to sterile flasks that were transported to the laboratory on ice for processing within 24 h. Aliquots of 0.2 ml of appropriate decimal dilutions were spread on YM agar (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1 % glucose and 2 % agar) supplemented with 0.02 % chloramphenicol. The plates were incubated at 25 °C for 3–8 days and selected colonies of the different yeast morphotypes were purified by repeated streak inoculation of YM agar and preserved at −80 °C for later identification. The yeasts were physiologically characterized by standard methods (Kurtzman et al., 2011).

The region spanning the SSU rRNA gene, the ITS region, including the 5.8S rRNA gene, and the D1/D2 domains of the LSU rRNA gene was amplified and sequenced with combinations of primers listed in Rosa et al. (2007) as well as primers NS7a and NL5a of Kurtzman & Robnett (2013). Amplification was performed directly from whole yeast cells as described by Lachance et al. (1999). Sequencing was performed by the London Regional Genomics Centre of the Robarts Research Institute, London, Ontario. Sequencing of RNA polymerase II subunit 1 (RPB1) was performed as described by Liu et al. (1999). Sequencing was performed using an ABI 3130 automated DNA gene analyser according to the manufacturer’s instructions. The sequences were assembled, edited and aligned with the program MEGA5 (Tamura et al., 2011). Trees were reconstructed using the programs provided within MEGA5 or the plugins of the Geneious R7.06 platform.

Species delineation and phylogenetic placement

Searches of various components of the ribosomal gene cluster using the BLAST program failed to identify any sister species among those represented in the NCBI database. The highest levels of similarity obtained for full-coverage hits were 94–96 % for the 5.8S rRNA gene, 90–91 % for the SSU rRNA gene and 86–88 % for the D1/D2 domains of the LSU rRNA gene. The species showing these levels of similarity belong to genera assigned to clade 1 of Kurtzman & Robnett (2013), which corresponds to the novel genus Saccharomycetaceae. Lower values were obtained for species assigned to clades 2–6, and lower still values for other clades. The two ITS regions yielded no significant hits. From this exclusively phenetic approach, we conclude that strain UFMG-CM-Y311T represents a novel species of a new genus in the Saccharomycetaceae. Available sequences for clades 1–4 were aligned with the sequence of strain UFMG-CM-Y311T. Not surprisingly, alignments of ITS regions were generally not credible. Moreover, as sequences for that region were not available for all species, phylogenetic analyses were performed on concatenated SSU and D1/D2 sequences. Phylogenetic trees reconstructed with various programs from a MUSCLE alignment identified Cynciomyces guttulatus as a possible sister taxon. The high bootstrap value shown in Fig. 1 in support of sisterhood of the novel species with Cynciomyces should be interpreted with caution in view of the long terminal branches subtending the two taxa. We feel justified in assigning the species to a new genus in the Saccharomycetaceae, but not to speculate too boldly as to its exact position relative to other members of the family. On the recommendation of an anonymous reviewer, we determined the sequence for 650 positions of the RNA polymerase II gene (GenBank accession number KJ863400). Analyses of this sequence in comparison with those deposited by Kurtzman & Robnett (2013, data not shown) confirmed the position of the novel species as a member of the Saccharomycetaceae, but whether this species joined a clade that contained Cynciomyces guttulatus depended on the method used to infer the tree and the exact placement in the family remained problematic.

We propose the novel genus Hagleromyces gen. nov. in honour of Allen N. Hagler and Leda C. Mendonça-Hagler, in recognition of their many contributions to the study of yeast diversity in Brazil and their mentorship of many Brazilian microbiologists. The single species proposed for the novel genus is Hagleromyces aurorensis sp. nov.

Habitat

Hagleromyces aurorensis sp. nov. was isolated from three different samples of phytotel mata of B. karatas, with counts of approximately 2.28 log c.f.u. ml⁻¹. The entire yeast population in the phytotel mata samples ranged from 2.49 to 3.45 log c.f.u. ml⁻¹. Isolates UFMG-CM-Y311T and Y354 were obtained during the first field collection and isolate UFMG-CM-Y355 during the second collection. This result suggests that the novel species is numerically a minor component of the yeast community associated with this bromeliad. The prevalent species in this community were Candida orthopsilosis, Candida pseudointermedia, Cryptococcus heveanensis and Meyerozyma guilliermondii. The phytotel mata of bromeliads are a rich source of novel yeast species, and several species have been described from this microhabitat in Brazil, namely Candida aechmeae (Metschnikowia clade), Candida bromeliacearum (Phaffomyces/Komataella clade), Candida ubatubensis (Metschnikowia clade), Candida vrisaea (Yamadazyma clade), Cryptococcus bromeliarum (Tremellales clade), Kazachstania
The three isolates of a feeding site. tanks of responsible for vectoring organic matter in the phytelmata. Insects are probably including the yeasts, may contribute to the degradation of accumulated in phytotelmata. The microbial communities, probably survive using the water and organic detritus agars incubated at 20 or 28 °C. 0.01 % ammonium sulphate (YCBAS) and Gorodkowa 5 % malt extract, yeast carbon base supplemented with individually or mixed in pairs on cornmeal, V8, dilute V8, Araujo et al., 2012; Kazachstania rupicola and Hannaella pogonnaceae (Ruivo et al., 2005; Landell et al., 2009, 2010, 2014; Araújo et al., 2012; Safar et al., 2013). The yeast communities probably survive using the water and organic detritus accumulated in phytotelmata. The microbial communities, including the yeasts, may contribute to the degradation of organic matter in the phytotelmata. Insects are probably responsible for vectoring H. aurorensis sp. nov. to the water tanks of B. karatas. Several insect species use this substrate as a feeding site.

The three isolates of H. aurorensis sp. nov. were examined individually or mixed in pairs on cornmeal, V8, dilute V8, 5 % malt extract, yeast carbon base supplemented with 0.01 % ammonium sulphate (YCBAS) and Gorodkowa agars incubated at 20 or 28 °C for up to 30 days, but asc or signs of conjugation were not seen. The novel species has a restricted physiological profile, assimilating only seven carbon sources out of 41 tested, which is typical of many Saccharomycetaceae. This result suggests that the novel species is probably copiotrophic and resides in habitats with high concentrations of simple carbon sources. Differentiation of the novel species from other ascomycetous yeasts is preferably achieved by gene sequencing.

### Description of Hagleromyces Sousa, Morais, Lachance & Rosa gen. nov.

Hagleromyces (Hagler.o.my’ces. N.L. masc. n. Hagleromyces pertaining to Hagler, in honour of Allen N. Hagler and Leda C. Mendonça-Hagler, in recognition of their contribution to the studies on yeast diversity in Brazil).

Growth is by multilateral budding. Neither pseudohyphae nor true hyphae are produced. Colonies are butyrous. An ascosporic state is unknown. Glucose is fermented. Relatively few sugars are assimilated and nitrate is not utilized as a sole source of nitrogen.

Phylogenetic placement: Saccharomyctales, Saccharomycotina, Ascomycota.

The type species is Hagleromyces aurorensis Sousa, Morais, Lachance & Rosa.

MycoBank number: MB 807876.

### Description of Hagleromyces aurorensis Sousa, Morais, Lachance & Rosa sp. nov.

Hagleromyces aurorensis (au.ro.ren’sias. N.L. masc. adj. aurorensis pertaining to Aurora, the location from where the type strain was isolated).

Has the following characteristics in addition to those for the genus. After 3 days on YM agar at 25 °C, cells are ovoid to ellipsoid (2–4 × 2–3.5 μm) and occur singly or in pairs (Fig. 2). Colonies are white, convex, smooth and with a glistening surface. Assimilates glucose, galactose, trehalose, ethanol, glycerol, DL-lactate and gluconate, but not L-sorbose, maltose, sucrose, cellobiose, D-ribose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch,
The type strain is UFMG-CM-Y311T (＝CBS 13264T), which was isolated from water tanks (phytotelmata) of B. karatas in the cerrado ecosystem of Aurora do Tocantins, Tocantins, Brazil. The Mycobank number is MB 807877. Y354 and UFMG-CM-Y355, isolated from similar sources, are other strains of the species.

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