Saturnispora quitensis sp. nov., a yeast species isolated from the Maquipucuna cloud forest reserve in Ecuador

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A single strain, CLQCA-10-114^T, representing a novel yeast species belonging to the genus *Saturnispora* was isolated from the fruit of an unidentified species of bramble (*Rubus* sp.), collected from the Maquipucuna cloud forest reserve, near Quito, in Ecuador. Sequence analyses of the D1/D2 domains of the large-subunit rRNA gene and ribosomal internal transcribed spacer region indicated that the novel species is most closely related to the recently described species *Saturnispora gosingensis*, isolated from the fruiting body of a mushroom collected in Taiwan, and *Saturnispora hagleri*, a *Drosophila*-associated yeast found in Brazil. The name *Saturnispora quitensis* sp. nov. is proposed to accommodate this strain; the type strain is CLQCA-10-114^T (=CBS 12184^T=NCYC 3744^T).

The yeast genus *Saturnispora* is characterized by teleomorphic species that typically produce one to four spheroidal ascospores ornamented with an equatorial ledge (i.e. saturn-shaped) and have a fairly restricted physiological profile (Kurtzman, 1998). The genus is well-supported by phylogenetic analyses based on multigene sequence analysis of the small-subunit and large-subunit (LSU) rRNA genes, and translation elongation factor-1α gene (Kurtzman *et al.*, 2008). At present, the genus comprises nine teleomorphic species: *Saturnispora ahearnii*, *Saturnispora besseyi*, *Saturnispora dispora*, *Saturnispora gosingensis*, *Saturnispora hagleri*, *Saturnispora mendoncae*, *Saturnispora saitoi*, *Saturnispora serradocipensis* and *Saturnispora zaruensis* (Morais *et al.*, 2005; Kurtzman, 2006; Kurtzman *et al.*, 2008; Canelhas *et al.*, 2011). Six anamorphic species, *Candida diversa*, *Candida sanitii*, *Candida sekii*, *Candida siamensis*, *Candida silvae* and *Candida suwanaritii*, are also accommodated within the genus (Kurtzman *et al.*, 2008; Boonmak *et al.*, 2009; Limtong *et al.*, 2010). Collectively, these yeasts have been isolated from a wide variety of different sources and habitats including *Drosophila* flies (*Drosophila cardinae* and *Drosophila fascioloides*), estuarine water from mangrove forest, flowers, forest soil, insect frass, leaf detritus, marsh water, rhizosphere of oyster grass, sauerkraut, tree bark and exudate (*Quercus* spp.), and wild mushrooms (*Coprinus* and *Hygrophorus* spp.) (Liu & Kurtzman, 1991; Kurtzman, 1998; Morais *et al.*, 2005; Boonmak *et al.*, 2009; Canelhas *et al.*, 2011; Limtong *et al.*, 2010).

During a pilot study to survey yeast diversity found in the Maquipucuna cloud forest nature reserve, located 50 miles north-west of Quito, Ecuador (0° 03′ 09″ N 78° 41′ 06″ W; 1668 m above sea-level), CLQCA-10-114^T was isolated together with more than 70 other yeast strains. Sequence analysis of the D1/D2 domain of the LSU rRNA gene identified the isolates as belonging to 25 different species of the genera *Barnettozyma* (1 isolate), *Candida* (6), *Hanseniaspora*

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Abbreviations: ITS, internal transcribed spacer; LSU, large-subunit.

The GenBank/EMBL/DDBJ accession numbers for the LSU D1/D2 and ITS sequences of CLQCA-10-114^T are FN908197 and FN985101, respectively.

A supplementary figure is available with the online version of this paper.

(2), *Lachancea* (1), *Lodderomyces* (1), *Metschnikowia* (2), *Pichia* (3), *Rhodotorula* (1), *Saccharomyces* (1), *Saturnispora* (1), *Trichosporon* (2), *Wickerhamomyces* (3) and *Yarrowia* (1). Strain CLQCA-10-114^T was isolated from the fruit of an unidentified species of bramble (*Rubus* sp.) and, based on its physiology and ability to produce saturn-shaped ascospores, was identified as a representative of the genus *Saturnispora* (Kurtzman, 1998). Subsequent sequence analyses of the LSU D1/D2 domain and ribosomal internal transcribed spacer (ITS) region established that this strain belongs to a genetically distinct and hitherto undescribed species closely related to *S. hagleri* and the recently described *S. gosingensis* (isolated in Taiwan) and *S. serradocipensis* (isolated in south-eastern Brazil) (Canelhas *et al.*, 2011). The name *Saturnispora quitensis* sp. nov. is proposed in recognition of the location in Ecuador where it was first found.

The variable D1/D2 domain of the LSU rRNA gene and ribosomal ITS region were amplified by PCR directly from whole yeast cell suspensions as described previously by James *et al.* (1996). The LSU D1/D2 domain was amplified and sequenced using primers NL1 and NL4 (O'Donnell, 1993). The ITS region was amplified using primers ITS5 and ITS4, and sequenced using these primers as well as internal primers ITS2 and ITS3 (White *et al.*, 1990). PCR products were checked by agarose gel electrophoresis, purified and concentrated using QIAquick PCR purification columns (Qiagen), and sequenced using a Life Technologies 3730XL sequencer at The Genome Analysis Centre, Norwich, UK. Sequence traces were edited and consensus sequences were generated using the program SEQMAN version 7 (DNASTAR). The LSU D1/D2 sequences were compared pairwise using a FASTA similarity search (Pearson & Lipman, 1988) and were aligned with sequences of closely related taxa using the multiple alignment program CLUSTAL W (Thompson *et al.*, 1994), included in the software package DNAMAN version 5.1.5 (Lynnon BioSoft). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes–Cantor distance measure. Confidence limit values were estimated from bootstrap analyses of 1000 replicates (Felsenstein, 1985).

Phylogenetic analysis based on LSU D1/D2 sequences showed that CLQCA-10-114^T was placed on a separate branch within the *Saturnispora* clade (Fig. 1). In terms of pairwise sequence similarity, CLQCA-10-114^T displayed 0.8 % divergence (4 nt substitutions) with *S. gosingensis*, 1.1 % divergence (3 nt substitutions and two indels) with *S. hagleri* and 1.3 % divergence (5 nt substitutions and one indel) with *S. serradocipensis*. Indeed, along with *S. dispora* (2.6 % divergence; 12 nt substitutions and two indels), *S. zaruensis* (3.9 % divergence; 18 nt substitutions), *Candida agrestis* ES3M03 (3.3 % divergence; 16 nt substitutions and one indel) and *Saturnispora* sp. GJ5M11 (3.3 % divergence;

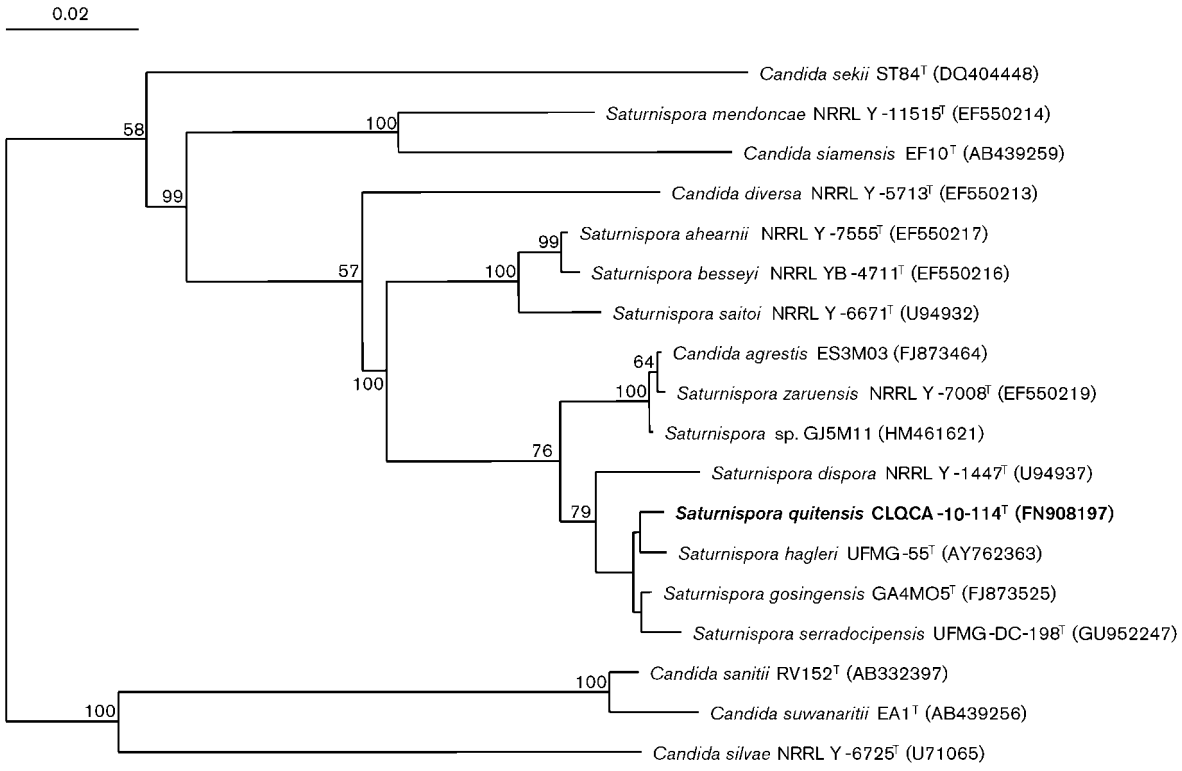

Fig. 1. Neighbour-joining dendrogram based on sequences of the D1/D2 domain of the LSU rRNA gene of *Saturnispora quitensis* sp. nov. and its closest relatives. Bootstrap values $\geqslant$50 %, determined from 1000 replicates, are shown at branch nodes. Bar, 2 base substitutions per 100 nt.

17 nt substitutions), these taxa form a distinct and separate species subgroup within the *Saturnispora* clade (Fig. 1). Despite the close phylogenetic relationship based on LSU D1/D2 sequences (0.8–1.3 % sequence divergence), strain CLQCA-10-114^T, *S. gosingensis* GA4M05^T (=MRC-2010a^T), *S. hagleri* UFMG-55^T and *S. serradocipensis* UFMG-DC-198^T (=MRC-2010b^T) can be readily distinguished from one another by ITS sequencing.

Although only partial ITS1 sequences currently exist for *S. gosingensis* GA4M05^T, *S. hagleri* UFMG-55^T and *S. serradocipensis* UFMG-DC-198^T, all three strains still differ from CLQCA-10-114^T by 6 nt substitutions and two indels, 3 nt substitutions and two indels, and by 1 nt substitution and one indel, respectively. In the ITS2 region, for which there are complete sequences available, the variation is far more pronounced: *S. gosingensis* GA4M05^T differs by 17 nt substitutions and one indel; *S. hagleri* UFMG-55^T by 28 nt substitutions and four indels; and *S. serradocipensis* UFMG-DC-198^T by 35 nt substitutions and five indels. In fact, the overall size of the ITS2 region varies quite markedly between the four *Saturnispora* strains ranging from 110 bp (UFMG-55^T) to 133 bp (CLQCA-10-114^T). Separate pairwise ITS alignments between *S. quitensis* CLQCA-10-114^T and the other three strains are shown in Supplementary Fig. S1 (available in IJSEM Online).

A similar situation exists between the closely related species pair of *S. ahearnii* and *S. besseyi* (formerly *Pichia besseyi*) (Fig. 1). On the basis of LSU D1/D2 sequence divergence, the two taxa appear to be conspecific, as they differ from one another by only two (single nucleotide) indels. However, when Kurtzman (2006) compared the ITS sequences of the type strains (*S. ahearnii* NRRL Y-7555^T and *S. besseyi* NRRL YB-4711^T), currently the only known representatives of these species, far greater sequence diversity was detected. In the ITS1 region, the two strains differ by four substitutions and eight indels, and in the ITS2 region, by five substitutions and seven indels (Kurtzman, 2006). When compared against the level of ITS sequence variation observed between three different strains of *S. saitoi*, Kurtzman (2006) reported that *S. ahearnii* and *S. besseyi* (*P. besseyi*) had three times as many ITS substitutions relative to the number found among the three *S. saitoi* strains. Based on these results, he concluded that *S. ahearnii* and *S. besseyi* represent closely related species rather than divergent members of the same species (Kurtzman, 2006).

At present, it is difficult to generalize on the ecological niches of these four closely related species, as only a small number of strains currently exist (*S. gosingensis*, 1 isolate; *S. hagleri*, 6, *S. quitensis*, 1; *S. serradocipensis*, 2) (Morais *et al.*, 2005; Canelhas *et al.*, 2011; data from this study). However, based on those isolates that have been identified, *S. quitensis* appears to be most similar to *S. hagleri* and *S. serradocipensis* as all three species have been found in the neotropics. *S. hagleri* has been isolated from two different species of *Drosophila* (*D. cardinae* and *D. fascioloides*) collected in an Atlantic rainforest site in Brazil (Morais *et al.*, 2005), *S. quitensis* from a bramble fruit collected in a cloud forest site in Ecuador, and *S. serradocipensis* from leaf detritus in a tropical stream in south-eastern Brazil (Canelhas *et al.*, 2011). In contrast, the solitary strain of *S. gosingensis* so far identified was isolated from the fruiting body of a mushroom (*Coprinus* sp.) collected in Taiwan (Canelhas *et al.*, 2011). In their species description of *S. hagleri*, Morais *et al.* (2005) noted that of the six identified strains, four were recovered from the crops of *D. cardinae*. This led the authors to suggest that this yeast may colonize tropical fruits and substrates regularly visited by these flies and utilized as a food source. Although only a single strain of *S. quitensis* has so far been isolated, it seems plausible to speculate that, like *S. hagleri*, additional strains of *S. quitensis* could, in future, be isolated from *Drosophila* flies and other insects that visit and feed upon tropical fruits found in Maquipucuna and other neotropical regions.

Physiologically, *Saturnispora* yeasts are almost indistinguishable from one another (Kurtzman, 1998, 2006). As reported previously, species separation is restricted to a small number of growth characteristics, specifically the assimilation of glycerol, ribitol and trehalose, as well as the ability to form a pellicle on the surface of liquid media (Morais *et al.*, 2005). *S. quitensis*, like *S. gosingensis*, *S. hagleri* and *S. serradocipensis*, assimilates both ribitol and trehalose, and is unable to form a pellicle (Morais *et al.*, 2005; Canelhas *et al.*, 2011). The only growth characteristic that appears to be discriminatory is the assimilation of glycerol. *S. hagleri* and *S. serradocipensis* both assimilate this carbon source, but this is a variable characteristic for *S. gosingensis* (Morais *et al.*, 2005; Canelhas *et al.*, 2011). In the case of *S. quitensis*, no growth was observed, even after prolonged incubation (4+ weeks). Thus, in view of the fact that these four species are physiologically almost indistinguishable from one another, we strongly recommend that LSU D1/D2 and, in particular, ITS sequencing are used to reliably determine species identity.

Latin diagnosis of *Saturnispora quitensis* James, Cadet, Bond, Carvajal *et* Roberts sp. nov.

In medio liquido post dies duos cellulae globosae aut ovoidae (4–7 × 5–8 μm), *cellulae singulae et aggregatae, per gemmationem multipolarem reproducentes. Post unum mensem sedimentum formatur. Pseudomycelium nec mycelium non formatur. Post dies octo in agaro glucose et extracti levidinis asci formatur. Asci stabiles. Glucosum fermentatur at non galactosum, maltosum, lactosum, raffinosum nec sucrosum. Glucosum, trehalosum, ethanolum, ribitolum, mannitolum, glucitolum, acidum lacticum et acidum succinicum assimilantur at non galactosum,* L-*sorbosum, sucrosum, maltosum, cellobiosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile,* D-*xylosum,* L-*arabinosum,* D-*arabinosum,* D-*ribosum,* L-*rhamnosum,* D-*glucosaminum, methanolum, glycerolum, erythritolum, methyl* α-D-*glucosidum, salicinum, acidum citricum nec inositolum. Ethylaminum et cadaverinum assimilantur at non kalium nitricum. Crescit in* 30 °C, *at non crescit in* 37 °C. *Non crescit in* 10 % NaCl/5 % *glucosum nec* 50 %

glucosum. Materia amyloidea non formantur. Non crescit in 0.01 % *cycloheximido. Typus stirps* CLQCA-10-114^T (=CBS 12184^T=NCYC 3744^T).

Description of *Saturnispora quitensis* sp. nov.

Saturnispora quitensis (qui.ten′sis. N.L. fem. adj. *quitensis* of or belonging to Quito, the capital of Ecuador, near where this strain was isolated).

Cells are spheroidal to ovoid (4–7 × 5–8 μm) and occur singly or in groups after growth in YM broth for 2 days at 25 °C. Budding is multilateral. Sediment is formed after 1 month, but no pellicle is observed. Pseudomycelia or true mycelia are not formed. After 8 days on agar media with a low nitrogen/carbon ratio (i.e. yeast carbon base with 0.01 % ammonium sulphate), conjugated cells give rise to asci containing one to two spheroidal ascospores ornamented with an equatorial ledge (i.e. saturn-shaped) (Fig. 2a and b). Ascospores are not liberated. Conjugation takes place between individual cells and, more commonly, between cells and their buds (Fig. 2a).

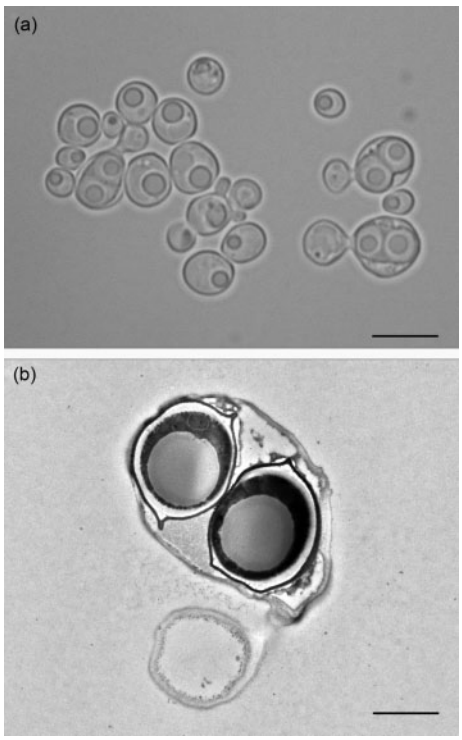

Fig. 2. *Saturnispora quitensis* sp. nov. CLQCA-10-114^T. (a) Photomicrograph of cells grown on yeast carbon base supplemented with 0.01 % ammonium sulphate after 8 days incubation at 20 °C. Note the vegetative cells, conjugation between three separate cells with their respective buds, and asci with two ascospores. Bar, 5 μm. (b) Transmission electron micrograph of a single ascus containing two ascospores, one of which is ornamented with an equatorial ledge. Bar, 1 μm.

Glucose is fermented. Galactose, maltose, lactose, raffinose and sucrose are not fermented. Glucose, trehalose, ethanol, ribitol, D-mannitol, D-glucitol, lactic acid and succinic acid are assimilated. No growth occurs on galactose, L-sorbose, sucrose, maltose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, glycerol, erythritol, methyl α-D-glucoside, salicin, citrate or inositol. Positive for ethylamine hydrochloride and cadaverine, but negative for nitrate. Growth is observed at 30 °C, but not at 37 °C. No growth is observed on YM agar with 10 % NaCl or on 50 % glucose/0.5 % yeast extract. No growth is observed in the presence of 50 % (w/v) glucose. Starch-like compounds are not produced. No growth with 100 μg cycloheximide ml^{-1}.

The type strain is CLQCA-10-114^T (=CBS 12184^T=NCYC 3744^T), isolated from the fruit of an unidentified species of bramble (*Rubus* sp.), Maquipucuna, Ecuador.

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