Cryptotrichosporon argae sp. nov., Cryptotrichosporon brontae sp. nov. and Cryptotrichosporon steropae sp. nov., isolated from forest soils

Ana Pontes, Oliver Röhl, Cristina Maldonado, Andrey M. Yurkov and José Paulo Sampaio

Abstract

Yeast strains belonging to the basidiomycetous genus Cryptotrichosporon were isolated from forest soils in Serra da Arrábida Natural Park in Portugal. Similar to the already-known representatives of this genus, the new isolates formed pigmented colonies of a distinctive pale orange colour. Phylogenetic analyses employing concatenated sequences of the D1/D2 domains of the 26S (large subunit) rRNA gene and the internal transcribed spacer (ITS) region supported the recognition of three novel species: Cryptotrichosporon argae sp. nov. (type strain CM 19³=CBS 14376³=PYCC 7010³=DSM 104550³; MycoBank accession number MB 817168), Cryptotrichosporon brontae sp. nov. (type strain CM 1562²=CBS 14303²=PYCC 7011²=DSM 104551²; MycoBank accession number MB 817077) and Cryptotrichosporon steropae sp. nov. (type strain OR 395³=CBS 14302³=PYCC 7012³=DSM 104552³; MycoBank accession number MB 817078).

The genus Cryptotrichosporon was proposed by Okoli et al. [1] to accommodate an asexual species of a trichosporonoid yeast, Cryptotrichosporon anacardii, isolated from cashew tree flowers in Nigeria. This yeast produced mucoid colonies of a pale yellowish-brown colour, and molecular phylogenetic analyses placed it at a basal position within the order Trichosporonales [1]. Wang and Wang [2] described a second species, Cryptotrichosporon tibetense, isolated from plant leaves in Tibet. Contrary to C. anacardii, this species produces ballistoconidia. More recently, the classification of tremellaceous yeasts was revised based on multigene molecular phylogenies [3]. In this study, the delimitation of the genus Cryptotrichosporon was confirmed and the genus Tetragoniomyces was suggested to represent the most closely related known sexual relative of the genus Cryptotrichosporon. During a study aimed at assessing soil yeast diversity in Arrábida Natural Park, a region generally characterized by an Atlantic–Mediterranean climate with 14–16 °C mean temperatures and 600–700 mm annual precipitation [4], and with different microclimatic areas determined by the varying orientation of the landscape and orography. The northern slopes of the Arrábida mountain range are more humid because they have a more pronounced influence of the Atlantic Ocean whereas the southern slopes are mostly sub-humid to semi-arid. Soil samples were collected in October 2013 and May 2014 from two sampling sites, one in humid forest (northern slope) and the other in the maquis scrubland (southern slope, Alto do Jaspe). Details on vegetation, soil properties and soil sampling are provided elsewhere [5]. In brief, 20×20 m plots were defined at the two collecting sites, and multiple soil samples collected within the plot were pooled in four composite samples. Woody debris and stones (>5 mm) were removed aseptically, and soil samples were placed in sterile paper bags and transferred to the

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Keywords: Cryptotrichosporon; basidiomycete yeast; soil microbiology; new yeast species; Mediterranean forests.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers of the LSU and ITS sequences of Cryptotrichosporon argae sp. nov. CBS 14376³ are KU831533 and KUB31541, PYCC 7013 are KUB31542 and KUB31534, and PYCC 7014 are KUB31543 and KUB31537, those of Cryptotrichosporon brontae sp. nov. CBS 14303² are KUB31538 and KUB31544, PYCC 7015 are KUB31545 and KUB31539, and PYCC 7016 are KUB31546 and KUB31540, and those of Cryptotrichosporon steropae sp. nov. CBS 14302² are KT314199 and KUB31547.

Three supplementary figures are available with the online Supplementary Material.
The novel species were found during a comprehensive survey of soil yeasts in Arrábida Natural Park [5]. A total of five isolates of C. argae sp. nov., nine isolates of C. brontae sp. nov. and one isolate of C. steropae sp. nov. were obtained. Whereas C. argae was found in the humid forest (northern slope), C. brontae and C. steropae were found in the dry scrubland (southern slope). C. steropae was collected in autumn, and the other two species were found only in spring. For C. argae and C. brontae, the D1/D2 and ITS sequences did not reveal intraspecific variation. According to the phylogenetic analysis depicted in Fig. 1, which includes sequences from three isolates of C. argae, three isolates of C. brontae and from the only available strain of C. steropae (Table 1), the closest relative of C. argae is C. brontae. These two species differ by 8 substitutions in the D1/D2 domains and 30 substitutions in the ITS region. The third novel species, C. steropae sp. nov., appears to be more distantly related and has as its closest relative C. tibetense. These two species differ by 10 substitutions in the D1/D2 domains and 33 substitutions in the ITS region. An additional possible novel species, represented by the D1/D2 sequence KT314195 (strain OR 1016), was found by us in the same study. However, because the culture recovered after cryopreservation did not correspond to the genus Cryptotrichosporon but to a contaminant yeast, the absence of a living culture made it impossible to advance the study of this possible novel species. Phylogenetic analysis based on D1/D2 sequences (the only molecular sequence data available for OR 1016) suggested that it represents a distinct species closely related to C. steropae sp. nov. (but with 6 substitutions) and to C. tibetense (Fig. S1, available in the online Supplementary Material). In the molecular phylogeny accompanying the description of C. tibetense, Tetragoniomyces uliginosus, the single species in the genus, occupies a basal position in relation to the genus Cryptotrichosporon [2]. This species, formerly known as Tremella uliginosa, exhibits a combination of features that are unique for the order Trichosporonales, the order that includes the genera Trichosporon, Cryptotrichosporon and Tetragoniomyces among other genera according to the most recent studies [3, 11]. Up to now, the genus Tetragoniomyces represented the only sexual stage known in the order Trichosporonales, which is characterized by round, thick-walled, deciduous and four-celled basidia that give rise directly to hyphae, being basidiospores and a yeast stage unknown [12]. However, the association of the genus Tetragoniomyces with the genus Cryptotrichosporon was based on the same sequences from herbarium material [2, 3, 11] that we noticed to
hamper the alignment because they were anomalously short. Here, we used newly obtained sequences from a living culture (PYCC 6958), obtained from F. Oberwinkler and strictly filamentous, which had 513 instead of 393 positions in the D1/D2 region and 465 instead of 440 positions in the ITS region when compared with the older sequences from voucher material. When the newly obtained sequences were used in BLAST searches in the NCBI database, sequences of species of the genus *Papiliotrema* emerged as the closest hits. To confirm this alternative phylogenetic placement, we prepared a phylogenetic tree based on sequences of the D1/D2 domains (Fig. S2) that depicts *Tetragoniomyces uliginosus* PYCC 6958 nested in the genus *Papiliotrema*, thus showing that this species does not belong to the order Trichosporonales.

Because the three novel species are closely related and originate from soils in a Mediterranean ecosystem, they are named after three ciclope brothers in Greek mythology. Ciclopes were imprisoned in the underworld Tartarus and lived on a Mediterranean island after liberation.

**DESCRIPTION OF CRYPTOTRICHOSPORON ARGAE SP. NOV. A. PONTES, O. RÖHL, A. YURKOV & J. P. SAMPAIO**

*Cryptotrichosporon argae* (ar’gae. Gr. masc. gen. n. Argou of Arges, referring to one of the three ciclope brothers in Greek mythology).

Cultures are pale orange, creamy and do not form mycelium or ballistoconidia after 2 weeks on CMA at 17°C. After 3 days of growth on YM agar at 22°C, cells are ellipsoidal to limiform (5–9 × 2.5–4 µm) and do not have capsules (Fig. 2). Proliferation is achieved by polar budding. Crosses of different strains on CMA at 17°C do not result in the development of the sexual stage, and true hyphae are not observed after 2 months. Glucose is not fermented. Carbon compounds assimilated: D-glucose, D-galactose, D-glucosamine (slow), D-ribose (slow), D-xylose, L-arabinose, D-arabinose (variable), sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, melezitose, ribitol (slow), D-glucitol, D-mannitol, *myo*-inositol, glucono-δ-lactone, D-glucunate, D-glucuronate and succinate. No growth on L-sorbose, L-rhamnose, salicin, melibiose, lactose, raffinose, inulin, starch, glycerol, erythritol, galactitol, D,L-lactate, citrate, succinate. No growth on L-sorbose, L-rhamnose, salicin, melibiose, lactose, raffinose, inulin, starch, glycerol, erythritol, galactitol, D,L-lactate, citrate,

### Table 1. New cultures of members of the genus Cryptotrichosporon studied in this work, and relevant information pertaining to them

<table>
<thead>
<tr>
<th>Original designation</th>
<th>CBS</th>
<th>PYCC</th>
<th>Species</th>
<th>GenBank accession number (ITS – D1/D2)</th>
<th>Location</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM 19 T</td>
<td>14376</td>
<td>T 7010</td>
<td><em>C. argae</em></td>
<td>KU831533 – KU831533</td>
<td>Humid forest</td>
<td>Spring</td>
</tr>
<tr>
<td>CM 281</td>
<td>7013</td>
<td>C. argae</td>
<td>KU831542 – KU831534</td>
<td>Humid forest</td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td>CM 348</td>
<td>7014</td>
<td><em>C. argae</em></td>
<td>KU831543 – KU831537</td>
<td>Humid forest</td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td>CM 1562 T</td>
<td>14303</td>
<td>T 7011</td>
<td><em>C. brontae</em></td>
<td>KU831544 – KU831538</td>
<td>Dry scrubland</td>
<td>Spring</td>
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<tr>
<td>CM 1564</td>
<td>7015</td>
<td><em>C. brontae</em></td>
<td>KU831545 – KU831539</td>
<td>Dry scrubland</td>
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<td></td>
</tr>
<tr>
<td>CM 1599</td>
<td>7016</td>
<td><em>C. brontae</em></td>
<td>KU831546 – KU831540</td>
<td>Dry scrubland</td>
<td>Spring</td>
<td></td>
</tr>
<tr>
<td>OR 395 T</td>
<td>14302 T 7012</td>
<td><em>C. steropae</em></td>
<td>KU831547 – KT314199</td>
<td>Dry scrubland</td>
<td>Autumn</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Micrographs of *Cryptotrichosporon argae* sp. nov., *Cryptotrichosporon brontae* sp. nov. and *Cryptotrichosporon steropae* sp. nov. Yeast cells of *C. argae* sp. nov. PYCC 7010 (a), *C. brontae* sp. nov. PYCC 7011 (b) and *C. steropae* sp. nov. PYCC 7012 (c) on YM agar. Bars, 10 µm.
methanol, ethanol, L-tartaric acid, L-malic acid or protocatechue acid. Nitrogen compounds assimilated: cadaverine, creatine or creatinine. No growth on nitrate, nitrite, ethylamine, creatine or creatinine. Growth is positive at 30 °C and negative at 35 °C. Soluble-starch-like extracellular carbohydrates are produced. DBB reaction and urease activity are positive.

The type strain, CM 19 T (=CBS 14376 T =PYCC 7010 T =DSM 104550 T), was isolated from soil from a humid Mediterranean forest of Arrábida Natural Park in Portugal, in 2014. A culture derived from the type (PYCC 7010 T, ex-type strain) is permanently preserved in a metabolically inactive state. The type strain (CBS 14376 T) was deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands. The MycoBank accession number is MB 817168.

### DESCRIPTION OF CRYPTOTRICHOSPORON BRONTAE SP. NOV. A. PONTES, O. RÖHL, A. YURKOV & J. P. Sampaio

Cryptotrichosporon brontae (bron’tae. Gr. masc. gen. n. Brontou of Brontes, referring to one of the three cíclope brothers in Greek mythology).

Cultures are pale orange, creamy and do not form mycelium or ballistoconidia after 2 weeks on CMA at 17 °C. After 3 days of growth on YM agar at 22 °C, cells are ellipsoidal to limiform (6–9×2.5–4 µm) and have capsules (Fig. 2). Proliferation is achieved by budding at the oblong end of the cells. Glucose is not fermented. Carbon compounds assimilated: D-glucose, D-galactose (slow), D-glucosamine (slow), D-ribose (slow), D-xylose, L-arabinose, D-arabinose (slow), D-maltose, trehalose, cellobiose, salicin, melezitose, glyceral (slow), ribitol (slow), D-glucitol (slow), D-mannitol, myo-inositol, glucono-δ-lactone, δ-glucuronate and succinate. No growth on L-sorbose, L-rhamnose, sucrose, methyl α-D-glucoside (variable), cellobiose, salicin, melezitose, glycerol (variable), ribitol (slow), xylitol, D-glucitol, D-mannitol, myo-inositol (slow), glucono-δ-lactone, δ-glucuronate and D-glucuronate and succinate (variable). No growth on L-sorbose, D-glucosamine, melibiose, lactose, raffinose, inulin, starch, erythritol, xylitol, galactitol, D,L-lactate, citrate, methanol, ethanol, L-tartaric acid, L-malic acid or protocatechuic acid. Nitrogen compounds assimilated: L-lysine and cadaverine. No growth on nitrate, nitrite, ethylamine, creatine or creatinine. Growth is positive at 30 °C and negative at 35 °C. Soluble-starch-like extracellular carbohydrates are produced. DBB reaction and urease activity are positive.

The type strain, OR 395 T (=CBS 14302 T =PYCC 7012 T =DSM 104552 T), was isolated from soil from a sub-humid Mediterranean forest of Arrábida Natural Park in Portugal, in 2013. A culture derived from the type (PYCC 7012 T, ex-type strain) is permanently preserved in a metabolically inactive state. The type strain (CBS 14302 T) was deposited in the CBS yeast collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands. The MycoBank accession number is MB 817078.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>Growth on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Methyl α-D-glucoside</td>
<td>s/+</td>
<td>s/+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>s/+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>s/+</td>
<td>s</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>+</td>
<td>s</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin-free medium</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Growth at 30 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 35 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Species: 1. C. argae sp. nov.; 2. C. brontae sp. nov.; 3. C. steropae sp. nov.; 4. C. anacardii; 5. C. tibetense. +, Positive; –, negative; s, positive but slow.
Besides their distinctive D1/D2 and ITS sequences, the novel species exhibit a few particular phenotypic traits as shown in Table 2. The combination of the results of growth tests on \( \alpha \)-rhamnose, sucrose and myo-inositol allows the differentiation of the five species presently known in the genus. Overall, \( C. \) tibetense appears to be the species most sensitive to high temperatures, being unable to grow at 30°C, whereas \( C. \) anacardii is the most tolerant, being the sole species able to grow at 35°C. A peculiar feature of all species of the genus Cryptotrichosporon is the distinctive pale orange colour of their cultures (Fig. S3), an uncommon trait in the order Trichosporonales.

References

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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