**Diddensiella caesifluorescens** gen. nov., sp. nov., a riboflavin-producing yeast species of the family Trichomonascaceae

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Four strains of a novel heterothallic yeast species were isolated from rotten wood collected in or near the Pilis Mountains in Hungary. The strains produced riboflavin in liquid culture. Analysis of gene sequences for the D1/D2 domains of the LSU nuclear rRNA, as well as analysis of concatenated gene sequences for the D1/D2 nuclear LSU rRNA and cytochrome oxidase II placed the novel species in a small clade including only two recognized species, *Candida santjacobensis* and *Candida transvaalensis*, in the family Trichomonascaceae. DNA sequence analyses demonstrated that the novel species was distinct from all currently recognized teleomorphic yeast genera. The name *Diddensiella caesifluorescens* gen nov., sp. nov. is proposed to accommodate the novel genus and species. The new genus proposed here can be recognized only from gene sequence analysis, because the characters of its asexual reproduction and ascospore formation are shared by several members of the genera *Trichomonascus*, *Sugiyamaella* and *Spencermartinsiella*. The type and isotype strains of *D. caesifluorescens* are NCAIM Y.01949T (=NRRL Y-48781T=CBS 12613T) and NCAIM Y.01956I (=NRRL Y-48782I=CBS 12614I), respectively. In view of their close relatedness to *D. caesifluorescens*, *C. santjacobensis* and *C. transvaalensis* are transferred to the genus *Diddensiella* as new combinations in accordance with changes in the International Code of Nomenclature for algae, fungi and plants.

The family Trichomonascaceae, which was erected based on multigene phylogenetic analysis (Kurtzman & Robnett, 2007), currently comprises the teleomorphic genera *Spencermartinsiella*, *Sugiyamaella* and *Spencermartinsiella*. The type and isotype strains of *D. caesifluorescens* are NCAIM Y.01949T (=NRRL Y-48781T=CBS 12613T) and NCAIM Y.01956I (=NRRL Y-48782I=CBS 12614I), respectively. In view of their close relatedness to *D. caesifluorescens*, *C. santjacobensis* and *C. transvaalensis* are transferred to the genus *Diddensiella* as new combinations in accordance with changes in the International Code of Nomenclature for algae, fungi and plants.

Abbreviations: ITS, internal transcribed spacer; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain nuclear LSU rRNA, ITS1–5.8S rRNA gene–ITS2, mitochondrial SSU rRNA and cytochrome oxidase II sequences of strain NCAIM Y.01949T (=NRRL Y-48781T=CBS 12613T) are GU195654, JF899509, JX136867 and JX136865, respectively. The MycoBank accession number for *Diddensiella* gen. nov. is MB564855 and that of strain NCAIM Y.01949T is MB564856. Those of *Diddensiella santjacobensis* (C. Ramírez & A. González) Péter, Dlauchy & Kurtzman comb. nov. and *Diddensiella transvaalensis* (Kurtzman) Péter, Dlauchy & Kurtzman comb. nov. are MB105986 and MB510411, respectively. A supplementary figure is available with the online version of this paper.

Sugiyamaella, Trichomonascus, Wickerhamiella and Zygoascus (Kurtzman, 2011a; Péter et al., 2011). The above-noted genera, except for the genus *Wickerhamiella*, are characterized by the formation of septate hyphae. The assignment of the non-hyphal *Wickerhamiella* to the Trichomonascaceae is tentative due to the weak support of the basal lineages of the phylogenetic trees (Kurtzman, 2011a). Anamorphic species related to members of the genus *Trichomonascus* are assigned to the genus *Blastosporos* (Kurtzman & Robnett, 2007), while the other anamorphic species of the family are currently members of the polyphyletic genus *Candida* (Lachance et al., 2011). A few members of the genus *Candida* that are not closely affiliated to any teleomorphic genus of the family Trichomonascaceae are also members of the family. Based on multigene phylogenetic analysis, some species, such as *Candida santjacobensis* and *Candida transvaalensis* form isolated and very well supported clades (Kurtzman & Robnett, 2007).
In this paper we report the isolation of four strains of a filamentous, heterothallic, haploid yeast species from decaying wood in Hungary. The strains produce budding cells, as well as blastoconidia, on short denticles or projections, and typically form ascii bearing an apical cell, a projection or a protuberance. They also produce riboflavin. Analysis of D1/D2 LSU rRNA gene sequences alone, as well as a multigene phylogenetic analysis of concatenated gene sequences for the D1/D2 LSU rRNA, mitochondrial SSU rRNA and cytochrome oxidase II sequences revealed that the above-noted strains represent an undescribed teleomorphic yeast species in the C. santjacobensis clade. As this clade is phylogenetically well separated from currently recognized teleomorphic genera assigned to the family Trichomonosaceae, we propose the new genus Diddensiella gen. nov. and the novel species D. caesifluorescens gen. nov., sp. nov. to accommodate these strains. We also propose reassignment of C. santjacobensis and C. transvaalensis to this new genus because neither of these two members of the genus Candida are closely related to Candida tropicalis, the type species of the genus Candida, and they therefore do not belong in the genus Candida. Transfer of these two species to the genus Diddensiella gen. nov. will resolve this taxonomic issue because the new International Code of Nomenclature for algae, fungi and plants permits inclusion of teleomorphs and anamorphs in the same genus, so that each phylogenetically placed fungus species shall have only one name (e.g. Knapp et al., 2011). The genus Candida, which will be recircumscribed around C. tropicalis, is likely to be retained as a valid genus in preference to the less recognized ascosporic genus Lodderomyces, which is also a member of the C. tropicalis clade.

Strains of the novel species originated from different rotten wood samples collected at several locations in, or very near to, the Pilis Mountains, Hungary (Table 1). All yeast strains were isolated after a two-step enrichment procedure (Dlauchy et al., 2003) in broth containing 0.5 % (v/v) methanol. The strains were isolated at 25°C, following serial dilution and plating on Rose-Bengal Chloramphenicol agar for those samples that were unable to grow with methanol as a sole carbon source. Some of these strains, which represent minor components of the yeast population in the enrichment medium, belong to the family Trichomonosaceae, based on comparisons of their D1/D2 LSU rRNA gene sequences. Ten of these minor component strains were used for the proposal of Spencermartinsiella europaea gen. nov., sp. nov. (Péter et al., 2011). Four additional strains (Table 1) that differed from S. europaea shared the same ITS sequences and three had identical D1/D2 LSU rRNA gene sequences, while the fourth strain, NCAIM Y.01948, exhibited one substitution in reference to the type (NCAIM Y.01956T) strain (NCAIM Y.01956T) as hT, while the mating types of the other strains were defined in reference to them.

The D1/D2 domains of the LSU rRNA gene from all strains were sequenced as described by Kurtzman & Robnett (1998). Gene sequences for mitochondrial SSU rRNA and cytochrome oxidase II were determined as reported by Kurtzman & Robnett (2007). A sequence similarity search was performed against the GenBank sequence database using the BLAST 2.2.26 database search program (Zhang et al., 2000). The sequences generated during this study, along with sequences of representative related species retrieved from GenBank, were aligned and concatenated, and phylogenetic trees were constructed by using the neighbour-joining (Kimura two-parameter distance measure) and maximum-parsimony programs in PAUP* 4.0 (Swofford, 1998), and the maximum-likelihood (ML) program in MEGA version 5 (Tamura et al., 2011). Positions with gaps were excluded from the analysis. Bootstrap support (Felsenstein, 1985) for the trees was determined from 1000 replications. The internal transcribed spacer (ITS) regions (ITS1, 5.8S rRNA gene and ITS2) from all strains were amplified and sequenced as described by Péter et al. (2009).

Isolation and characterization of strains

Our studies on methylotrophic yeasts in some natural habitats in Hungary, in addition to methylotrophic yeasts, resulted in the regular isolation of strains from rotten wood samples that were unable to grow with methanol as a sole carbon source. Some of these strains, which represent minor components of the yeast population in the enrichment medium, belong to the family Trichomonosaceae, based on comparisons of their D1/D2 LSU rRNA gene sequences. Ten of these minor component strains were used for the proposal of Spencermartinsiella europaea gen. nov., sp. nov. (Péter et al., 2011). Four additional strains (Table 1) that differed from S. europaea shared the same ITS sequences and three had identical D1/D2 LSU rRNA gene sequences, while the fourth strain, NCAIM Y.01948, exhibited one substitution in the D1/D2 region. The high sequence similarities clearly suggest conspecificity of the four strains. This idea is also supported by their similar phenotypic characteristics and the results of the mating experiments. The strains did not sporulate if incubated alone, but all of them formed ascospores if mixed with the opposite mating type. One hemispherical or helmet-shaped ascospore was formed in each ascus, which usually had an apical cell, a projection or a protuberance. The mating type of each strain was determined in reference to the type (NCAIM Y.01949T) and isotype (NCAIM Y.01956T) strains and these are indicated in Table 1. No ascospore formation was observed in mixtures of the type strain of C. santjacobensis with any strain of the novel species.

Liquid cultures in carbon assimilation tubes had a faint yellow hue with most of the carbon sources, including glucose. These cultures exhibited bluish fluorescence when observed under
UV light (365 nm). The fluorescent compound was identified as riboflavin by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS using a Bruker Microflex instrument (Bruker Daltonics) in positive ion reflectron mode. Fluorescent material from cultures was mixed with a saturated matrix (2,5-dihydrobenzoic acid in acetonitrile) and applied to standard 100-place stainless steel targets. The laser excitation wavelength was at 337.1 nm, typically at 60% of 150 mJ maximum output, and 2000 shots were accumulated. Pseudo-molecular $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions for riboflavin were observed at $m/z$ 377.9, $m/z$ 399.9 and $m/z$ 416.0, respectively (C17H20N4O6, calculated 376.36 Da), and were assigned by comparison with a commercial riboflavin standard (Fig. S1, available in IJSEM Online).

Riboflavin (vitamin B2) is produced by yeasts in many clades, most notably by members of the genus *Eremothecium*, and to a lesser extent, *Meyerozyma guilliermondii* and neighbouring taxa (Johnson & Echavarri-Erasun, 2011; Kurtzman & de Hoog, 2011). Aqueous solutions of riboflavin are yellow, showing a green fluorescence with a maximum at 565 nm (Budavari, 1996). The best-known biochemically active coenzymes formed from riboflavin are flavin mononucleotide and flavin adenine dinucleotide (Stahmann et al., 2000).

### Phylogenetic placement

Pairwise comparison of D1/D2 sequence similarity of the novel species with entries in the GenBank database showed the closest match to be the type strain of *C. santjacobensis*, NRRL Y-17667T, with more than 3% sequence divergence (12 substitutions and 8 indels). An undescribed *Candida* species (HA1034) is also a member of the clade but exhibits more than 2% divergence in the D1/D2 sequences compared with the novel species. On the basis of D1/D2 sequence analysis, *D. caesifluorescens* sp. nov. is genetically separate from known species and forms a small isolated clade with *C. santjacobensis* and *C. transvaalensis*. Because D1/D2 sequence analysis seldom provides strong support for deep lineages, the hypothesis that *D. caesifluorescens* sp. nov. represents a separate genus was tested by analysing concatenated gene sequences from D1/D2, mitochondrial SSU rRNA and cytochrome oxidase II. Reference species included nearly all known neighbouring species assigned to the family Trichomonascaceae (Kurtzman, 2007a, b, c; Kurtzman & Robnett, 2007). ML analysis (Fig. 1) showed the proposed genus *Diddensiella* gen. nov. to be well separated from other clades (genera) of the family Trichomonascaceae.

### Table 1. List of strains considered in this study

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Mating type*</th>
<th>Source</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. caesifluorescens</td>
<td>hT</td>
<td>Brown rotten wood of an unidentified tree, Pilis Mountains, Hungary, 2005</td>
<td>GU195656 JX136865</td>
</tr>
<tr>
<td>NCAIM Y.01948T</td>
<td>hT</td>
<td>Brown rotten ash (<em>Fraxinus</em> sp.) wood, Pilis Mountains, Hungary, 2005</td>
<td>JF895509 JX136867</td>
</tr>
<tr>
<td>NCAIM Y.01949T</td>
<td>hT</td>
<td>Brown rotten oak (<em>Quercus cerris</em>) wood, Pilis Mountains, Hungary, 2005</td>
<td>GU195654 JX136867</td>
</tr>
<tr>
<td>NCAIM Y.01950I</td>
<td>hI</td>
<td>Brown rotten wood of willow (<em>Salix</em> sp.), Dorog, Hungary, 2001</td>
<td>GU195652 JX136868</td>
</tr>
<tr>
<td>NCAIM Y.01951I</td>
<td>hI</td>
<td>Rotten willow (<em>Salix alba</em>) wood, Dorog, Hungary, 2001</td>
<td>GU195662 JF895510</td>
</tr>
<tr>
<td>S. europaea</td>
<td>hT</td>
<td>Rotten willow (<em>Salix alba</em>) wood, Dorog, Hungary, 2001</td>
<td>GU195662 JF895510</td>
</tr>
</tbody>
</table>

* Mating type identical to the type strain; hT, mating type identical to the isotype strain.
Dinnensiella caesifluorescens gen. nov., sp. nov.

Fig. 1. Phylogeny of D. caesifluorescens sp. nov., D. santjacobensis comb. nov., D. transvaalensis comb. nov. and related species from ML analysis of concatenated gene sequences from the D1/D2 domains of LSU rRNA, mitochondrial SSU rRNA and cytochrome oxidase II. (Sequences not generated during this study were from Kurtzman, 2007a, b, c; and Kurtzman & Robnett, 2007.) Schizosaccharomyces pombe was designated the out-group. Bootstrap values (1000 replicates) ≥50% are given at branch nodes. Strain numbers are NRRL, unless otherwise indicated. The ML analysis is based on the Hasegawa–Kishino–Yano model and the tree with the highest log-likelihood (−34974.2351) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [four categories (+G, parameter=0.8064)]. All ambiguous positions were removed for each sequence pair and there were 1620 positions in the final dataset. Bar, 1 base substitution per 100 nt.

Delineation of the new taxa and occurrence of the novel species

The new genus can only be recognized from DNA sequence analyses, because ascus morphology is shared by some members of the genera Trichomonascus, Sugiyamaella and Spencermartinsiella (Kurtzman & Robnett, 2007; Kurtzman, 2007a, b, 2011b; Péter et al., 2011; Smith et al., 2011). The distinction of the genus Dinnensiella from neighbouring genera is difficult using the standard phenotypic tests applied in yeast systematics because of numerous shared characters. The formation of buds on denticles is also a widespread character among the filament-forming species of the family Trichomonascaceae. The production of riboflavin is a widespread character among the filament-forming species of yeasts. As for S. europaea, it can be anticipated that the occurrence of the novel species in woody habitats is widespread. Known strains of C. santjacobensis were isolated from a fallen tree that was in the advanced stages of decay in Chile and from sap flux from a tree in Hawai‘i Island, USA (Lachance et al., 2011). The only known strain of C. transvaalensis is from forest litter, Transvaal, South Africa (Kurtzman, 2007b). In addition to their related isolation substrates, known strains of D. caesifluorescens sp. nov., C. santjacobensis and C. transvaalensis collected from three different continents exhibit similar carbon source assimilation spectra. Strains of all three species are able to grow on cellobiose and xylose, the building blocks of cellulose and hemicellulose, two major constituents of wood.

Description of Dinnensiella Péter, Dlauchy & Kurtzman gen. nov.

Dinnensiella (Did.den.si.el’a. N.L. fem. dim. n. Dinnensiella named in honour of Dr Harmanna Antonia Diddens in recognition of her major contributions to the taxonomy of yeasts).

The genus belongs to the family Trichomonascaceae Kurtzman & Robnett. Persistent asci are formed following conjugation of complementary mating types. Asci are spheroid, subspheroid or ellipsoid and commonly form a small attached apical cell, a projection or a protuberance.

Fig. 2. Budding cells and septate hyphae of D. caesifluorescens sp. nov. NCAIM Y.01949T grown on 5% malt extract agar for 3 days at 25 °C. Bar, 10 µm.

Fig. 3. Slide culture of D. caesifluorescens sp. nov. NCAIM Y.01949T cells grown on cornmeal agar for 7 days at 25 °C. Bar, 10 µm.
Each ascus contains one hemispheroid or helmet-shaped ascospore. Yeast cells are ellipsoid or elongated. Cell division proceeds by multilateral budding or by blastocnidium formation. Conidiogenous cells are often dentate. Some cells show tapered outgrowths that form blastoconidia. Pseudohyphae and true hyphae are present. The single known species, along with the two recognized phylogenetically closely related anamorphic species, ferments some sugars and do not assimilate nitrate. The new genus can be distinguished from the related genera by comparison of gene sequences for D1/D2 domains of nuclear LSU rRNA, mitochondrial SSU rRNA and comparison of gene sequences for D1/D2 domains of cytochrome oxidase II. The type species is *Diddensiella caesifluorescens* Péter, Dlauchy & Kurtzman sp. nov.

The MycoBank number is MB 564855.

**Description of Diddensiella caesifluorescens**  
Péter, Dlauchy & Kurtzman sp. nov.


Fig. 4. Ascosporulating culture of *D. caesifluorescens* sp. nov. NCAIM Y.01949’xNCAIM Y.01956’ grown on PDA for 21 days at 25 °C. Bar, 10 μm. The inserted image in the bottom-right corner was taken from a different microscopic field but is of the same culture.

New species combinations

*Diddensiella santjacobensis* (C. Ramírez & A. González) Péter, Dlauchy & Kurtzman comb. nov.

The MycoBank number is MB 105986.

Diddensiella transvaalensis (Kurtzman) Péter, Dlauchy & Kurtzman comb. nov.


The MycoBank number is MB 510411.

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References


