Spencermartinsiella europaea gen. nov., sp. nov., a new member of the family Trichomonascaceae

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Ten strains of a novel heterothallic yeast species were isolated from rotten wood collected at different locations in Hungary. Analysis of gene sequences for the D1/D2 domain of the large subunit rRNA, as well as analysis of concatenated gene sequences for the nearly complete nuclear large subunit rRNA, nuclear small subunit rRNA and translation elongation factor 1-α, placed the novel species in the family Trichomonascaceae, but showed that it was distinct from all currently recognized genera. The name Spencermartinsiella europaea gen. nov., sp. nov. is proposed to accommodate the new genus and novel species. The novel species could be distinguished from recognized species of neighbouring genera on the basis of standard phenotypic characteristics. The type and isotype strains of Spencermartinsiella europaea are NCAIM Y.01817T (=NRRL Y-48265T = CBS 11730T) and NCAIM Y.01819I (=NRRL Y-48266I = CBS 11731I), respectively.

The family Trichomonascaceae was erected to accommodate the genera Sugiyamaella, Trichomonascus, Wickerhamiella, Zygosascus and related anamorphs (Kurtzman & Robnett, 2007). The proposal was based on multigene phylogenetic analyses that included gene sequences for the nearly complete large subunit (LSU) rRNA, mitochondrial small subunit (SSU) rRNA and cytochrome oxidase II (COXII). The majority of taxa included in the family Trichomonascaceae form septate hyphae, but species of the genus Wickerhamiella do not (Kurtzman, 1998; Lachance et al., 1998, 2000). Several species form blastoconidia on small denticles. With the exception of Trichomonascus farinosus, all teleomorphic species that form septate hyphae are heterothallic (Smith & de Hoog, 1998; Kurtzman, 2004, 2007; Kurtzman & Robnett, 2007).

During the course of a study of the yeast community of decaying wood in Hungary, ten strains of a filamentous, heterothallic, haploid yeast species were isolated that produced blastoconidia on small denticles and formed ascii with an apical cell. Each ascus contained a single hemispheroid or helmet-shaped ascospore. Analysis of D1/D2 LSU rRNA gene sequences, as well as a multigene phylogenetic analysis of concatenated gene sequences for the nearly entire nuclear large subunit (LSU) rRNA, nuclear small subunit (SSU) rRNA and translation elongation factor 1-α (EF-1α), revealed that the new strains represented a novel yeast species of the family Trichomonascaceae, which was phylogenetically well separated from recognized teleomorphic genera. Based on these findings, a new genus and novel species, Spencermartinsiella europaea gen. nov., sp. nov., are proposed to accommodate these strains.

The strains investigated in this study originated from different rotten wood samples collected at several locations in Mid-North Hungary between 1991 and 2005 (Table 1). Nine of the ten strains were recovered after a two-step enrichment procedure (Dlauchy et al., 2003) in broth containing methanol. These yeast strains were isolated at 25 °C following serial dilution and plating on rose-Bengal chloramphenicol (RBC) agar from those samples where the

Abbreviations: EF-1α, translation elongation factor 1-α; ITS, intergenic transcribed spacer; LSU, large subunit; RBC, rose-Bengal chloramphenicol; SSU, nuclear small subunit; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain nuclear large subunit rRNA gene sequence and ITS sequence of strain NCAIM Y.01817T (=NRRL Y-48265T = CBS 11730T) is GU265922. The accession number for the ITS1, 5.8S rRNA and ITS 2 region of strain NCAIM Y.01817T is GU733454.

A supplementary table is available with the online version of this paper.
second enrichment broth resulted in growth. Since these strains were unable to grow on this carbon source, they represented the minor components of the yeasts in the enrichment liquid, which was otherwise dominated by methanol-assimilating yeast strains. One strain was isolated following serial dilution conducted directly from the suspended sample without enrichment, at 25°C on RBC agar. Phenotypic characterization of the strains was performed by standard methods as described by Yarrow (1998). Sexual reactivity was studied by mixing actively growing cultures on 2% malt extract agar. The mixtures were incubated at 15 and 25°C and examined weekly by light microscopy for up to 21 days. First, five strains (NCAIM Y.01815, NCAIM Y.01816, NCAIM Y.01817T, NCAIM Y.01818 and NCAIM Y.01819I in Table 1) were mixed pairwise, in all possible combinations, and the members of a highly fertile cross (NCAIM Y.01817T × NCAIM Y.01819I) were selected as the type and isotype strains, respectively, of the novel species. The remaining strains were paired with the designated type and isotype strains under the same conditions. Strain NCAIM Y.01817T was designated hT and the isotype strain NCAIM Y.01819I was designated as hI while the mating types of the other strains investigated were defined in reference to them. Observations by transmission electron microscopy (TEM) were made from a 7-day-old culture grown on cornmeal agar. The culture was fixed in 1.5% potassium permanganate for 20 min and stained with uranyl acetate and Reynolds’ lead citrate.

The D1/D2 domain of the large subunit rRNA gene from all strains was sequenced as described by Kurtzman & Robnett (2003, 2007). Regions of uncertain alignment were removed from the analysis for the two rRNA gene sequences and the third position of each codon was removed from the EF-1a sequences for the nearly entire LSU rRNA, SSU rRNA and EF-1a genes. The methods used for DNA isolation and sequencing of these genes were as given by Kurtzman & Robnett (2003, 2007). Sequences for the D1/D2 LSU rRNA gene sequences. Analysis was carried out using the neighbour-joining program with the Kimura two-parameter distance measure included in PAUP* 4.063a (Swofford, 1998).

**Characterization of strains**

As a result of the application of a selective isolation method, numerous methylotrophic yeast strains were isolated from the rotten wood samples. Some of them represented undescribed taxa and were proposed as novel yeast species (e.g. Péter et al., 2003, 2009a). However, from time to time colonies representing minor components of the yeasts in the enrichment medium appeared on the isolation plates, which were unable to grow with methanol as a sole source of carbon. Ten of these strains (Table 1) shared identical D1/D2 LSU rRNA gene sequences and very similar phenotypic characters. Following 2–3 weeks of incubation on 2% malt extract agar at either 15 or 25°C, nine of the strains were able to form one-spored ascii with an apical cell if mixed with a strain of the opposite mating type. The ascospores were hemispheroid or helmet-shaped.

**Table 1.** List of *Spencermartinsiella europaea* sp. nov. strains considered in this study

<table>
<thead>
<tr>
<th>Strain accession number</th>
<th>Mating type</th>
<th>Source of isolation/year of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCAIM Y.01815</td>
<td>hI</td>
<td>Oak (Quercus sp.), Pilis Mountains, Hungary, 1997</td>
</tr>
<tr>
<td>NCAIM Y.01816</td>
<td>hI</td>
<td>Ash (Fraxinus sp.), Pilis Mountains, Hungary, 2001</td>
</tr>
<tr>
<td>NCAIM Y.01817T (= NRRY 48265T = CBS 11730T)</td>
<td>hI</td>
<td>Willow (Salix alba), Dorog, Hungary, 2001</td>
</tr>
<tr>
<td>NCAIM Y.01818</td>
<td>hI</td>
<td>Willow (Salix sp.), Dorog, Hungary, 2001</td>
</tr>
<tr>
<td>NCAIM Y.01819I (= NRRY 48266T = CBS 11731I)</td>
<td>hI</td>
<td>Wild rose (Rosa sp.), Budapest, Hungary, 2001</td>
</tr>
<tr>
<td>NCAIM Y.01897</td>
<td>hI</td>
<td>Willow (Salix sp.), Budapest, Hungary, 2001</td>
</tr>
<tr>
<td>NCAIM Y.01961</td>
<td>hI</td>
<td>Oak (Quercus sp.), near Hollókő, Hungary, 2002</td>
</tr>
<tr>
<td>NCAIM Y.01962</td>
<td>?</td>
<td>Oak (Quercus cerasifera), near Hollókő, Hungary, 2002</td>
</tr>
<tr>
<td>NCAIM Y.01963</td>
<td>hI</td>
<td>Cherry (Prunus avium), near Esztergom, Hungary, 2002</td>
</tr>
<tr>
<td>NCAIM Y.01964</td>
<td>hI</td>
<td>Unidentified tree, Pilis Mountains, Hungary, 2005</td>
</tr>
</tbody>
</table>
The intensity of sporulation was variable depending on the pairings and was usually similar at the two incubation temperatures used. However, for some crosses, incubation at 15 °C proved to be slightly more favorable than at 25 °C. The mix of the designated type and isotype strains (NCAIM Y.01817T × NCAIM Y.01819T) resulted in fair sporulation, while one strain, NCAIM Y.01962, failed to sporulate either with the type or with the isotype strain even after an extended 7-week incubation period. The mating types were distributed roughly equally among the strains investigated (Table 1). The conspecificity of the type strain, NCAIM Y.01817T, of the novel species and the nonsporulating strain, NCAIM Y.01962, was further substantiated by their identical nucleotide sequences in the ITS regions (ITS1, 5.8S rRNA gene and ITS2). Taken together, the identical D1/D2 sequences shared by all of the new strains considered, and their interfertility (or, in the absence of it, their identical ITS sequences), indicated that the ten new strains represented a single species.

### Phylogenetic placement

The D1/D2 sequences placed the novel species in the family Trichomonascaceae. The closest match among recognized species in terms of pairwise sequence similarity in the GenBank database was the type strain of *Candida santjacobensis* with almost 7% sequence divergence (20 substitutions and 20 indels).

The phylogenetic placement of the novel species based on D1/D2 sequences is shown in Fig. 1. Strain NCAIM Y.01817T formed a strongly supported (bootstrap value 99%) small clade along with some undescribed yeast strains. This clade was related to the *Sugiyamaella* and *Trichomonascus* clades and from the D1/D2 tree it appeared to form a well separated lineage, raising the possibility that it represented a new genus. To further investigate the phylogenetic position of the novel species, representative neighbouring taxa were selected from the multigene analysis presented by Kurtzman & Robnett (2007) for comparison. In the present analysis, representative taxa that were either type species of neighbouring teleomorphic genera or phylogenetically isolated neighbouring *Candida* lineages were analysed from concatenated sequences for the nearly entire LSU rRNA, SSU rRNA and EF-1α genes. The species included in the analysis and the GenBank accession numbers are listed in Supplementary Table S1 (available in IJSEM Online). In the multigene phylogenetic tree depicted in Fig. 2, *Spencermartinsiella europaea* sp. nov., *Sugiyamaella smithiae* and *Trichomonascus petasosporus* clustered about equidistantly from each other, supporting the proposal that *Spencermartinsiella europaea* sp. nov. represented a new genus.

### Delineation of the new taxa and occurrence of the novel species

The new genus could only be recognized from DNA sequence analyses since the morphology of the ascus was also shared by some species of the genera *Trichomonascus* and *Sugiyamaella* (Kurtzman & Robnett, 2007; Kurtzman, 2007). The phenotypic distinction between the genera *Spencermartinsiella* gen. nov., *Trichomonascus* and *Sugiyamaella* was difficult when using the standard phenotypic tests applied in yeast systematics because of numerous shared characteristics. Kurtzman & Robnett (2007) prepared a key utilizing nine physiological characters which, with some possible exceptions, allowed the recognition of members of the *Sugiyamaella*, *Trichomonascus* and *Wickerhamiella* clades, including the anamorphic species. They also predicted that the discovery of novel species with atypical growth reactions could render that key inaccurate. The subsequent description of *Trichomonascus apis* (Péter et al., 2009b) supported this supposition, because this species cannot be reliably assigned to the genus *Trichomonascus* with the above mentioned key. However, *Spencermartinsiella europaea* sp. nov. could be distinguished from all recognized species included in the *Sugiyamaella* and *Trichomonascus* clades (as defined by Kurtzman & Robnett, 2007), as well as from other neighbouring taxa, such as *C. santjacobensis*, using standard phenotypic characteristics. As only a few phenotypic differences separate some species, DNA sequence-based identification is desirable.

Strains of *Spencermartinsiella europaea* sp. nov. were recovered infrequently from numerous rotten wood samples collected in this study in Hungary. However, most of the samples were processed only by enrichment in methanol-containing broth, providing adverse conditions for *Spencermartinsiella europaea* sp. nov., which was unable to utilize methanol. It seems likely that, under favourable isolation conditions, *Spencermartinsiella europaea* sp. nov. may be found more frequently in woody habitats. Interestingly, the holotype strain of *Sugiyamaella (Stephanoascus) smithiae*, from a soil sample, was also isolated following incubation of the sample in methanol-containing liquid mineral medium (Gimenez-Jurado et al., 1994). It is noteworthy that, until now, no strains of *Spencermartinsiella europaea* sp. nov. have been isolated from the hundreds of slime flux samples that have also been processed by enrichment in methanol-containing medium. Recently, a D1/D2 rRNA gene sequence (GenBank accession no. FN659776) identical to that of *Spencermartinsiella europaea* sp. nov. has been deposited in GenBank by a Russian group (O. Kamzolkina, E. Bilanenko, O. Shtaer, G. Dolnikova, A. Kachalkin & V. Mukhin, unpublished data). Their strain was recovered from dead birch (*Betula*) tissue.

Several undescribed yeast species cluster near the type strain of *Spencermartinsiella europaea* sp. nov. (Fig. 1), which suggests that the new genus proposed here will not remain monotypic for long. Among the sequences of these undescribed species, three were deposited by the authors of this study. Two of these deposited sequences belong to phylotypes represented only by single isolates, whereas strains of the third undescribed species have not yet been
satisfactorily characterized. Therefore, in this paper, only *Spencermartinsiella europaea* gen. nov., sp. nov. is proposed for the ten strains isolated from rotten wood in Hungary.

**Latin diagnosis of Spencermartinsiella Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki et Kurtzman gen. nov.**


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**Fig. 1.** Phylogenetic tree showing the placement of *Spencermartinsiella europaea* sp. nov. and some related species based on analysis of the D1/D2 domain of the large subunit rRNA gene. Sequences not generated during this study were obtained from GenBank. The tree was reconstructed by neighbour-joining analysis of aligned sequences. *Schizosaccharomyces pombe* was used as a designated outgroup. The numbers indicate bootstrap values of ≥50% based on 1000 replications. Bar, 0.05 proportional sequence divergence. Strain accession numbers starting with ‘Y’ or ‘YB’ designate NRRL strains. A, authentic strain; *, type strain of *Candida bertae*; Bla., Blastobotrys; Bot., Botryozyma; C., Candida; D., Dipodascus; Hyph., Hyphopichia; K., Kodamaea; L., Lipomyces; Schiz., Schizosaccharomyces; Sp., Sporopachydermia; Su., Sugiyamaella; Trich., Trichomonascus; Trig., Trigonopsis; W., Wickerhamiella; Z., Zygoascus.

**Fig. 2.** Phylogenetic tree of *Spencermartinsiella europaea* sp. nov. and representative neighbouring taxa derived from neighbour-joining analysis of gene sequences from the nearly entire genes for LSU rRNA, SSU rRNA and EF-1α. Regions of uncertain alignment were removed from analysis for the two rRNA gene sequences and the third position of each codon was removed from the EF-1α gene sequence. Each gene sequence contributed the following number of characters: LSU = 2840, SSU = 1662, EF-1α = 608, for a total of 5110. Bootstrap values of ≥50% are given at nodes based on 1000 replications. Bar, 1 base substitution per 100 nucleotides.
**Description of Spencermartinsiella Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki and Kurtzman gen. nov.**

*Spencermartinsiella* (Spen.cer.mar.tin.si.el’la. N.L. fem. n. *Spencermartinsiella* in honour of Professor Isabel Spencer-Martins, in recognition of her major contributions to the taxonomy and physiology of yeasts).

The genus belongs to the family Trichomonascaceae Kurtzman & Robnett. Asci are formed following conjugation of complementary mating types. Asci are persistent, spheroid or ellipsoid with a small attached apical cell. Each ascus contains one hemispheroid or helmet-shaped ascospore. Yeast cells are ovoid, drop-shaped or elongated. Cell division proceeds by multilateral budding or by blastocnidium formation. Conidiogenous cells are often denticulate. Pseudohyphae and true hyphae are present. The single known species does not ferment sugars and does not assimilate nitrate. The new genus can be distinguished from related genera by comparison of gene sequences for nuclear LSU rRNA, nuclear SSU rRNA and EF1-α. Type species: *Spencermartinsiella europaea* Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki & Kurtzman.

**Latin diagnosis of Spencermartinsiella europaea**

In agaro malti post dies tres in 25 °C cultura est butyrosa et compressa vel pilosa, alba et leviter nitida vel opaca. Centrum coloniae sublatum est, margine integro vel leviter lobata et fimbriata cum mycelio. Cellulæ sunt ovoidae, ciliiformae vel elongatae (1.5–3.0 x 3.0–8.0 μm), undiqua gemmantes, singulares, binae vel parvis conexae. Hyphae septatae et blastoconidia formantur. In extracto malti post dies tres in 25 °C repens pellicula formatur. In agaro Zea maydis confecto post dies 7 in 25 °C pseudohyphae et hyphae septatae formantur. Species heterothallica. Asci (3.5–6 x 5–6 μm) spheroidæ aut ellipsoideae, cellula apicali coronata, 1 ascospora hemispheroidæ vel galeiformæ non liberæ habent. Sacchara non fermentantur. D-Glucosum, succosum, raffinosum, melibiosum, D-galactosum, lactosum (aliquando lente), trehalosum, maltosum, melezitosum, α-methyl D-glucosidum, cellobiosum, salicinum (variabile), arbutinum (aliquando lente), L-sorbosum, L-rhamnosum (aliquando lente), D-xilosum, L-arabinosum, D-arabinosum (aliquando lente), D-ribosum (aliquando lente), ethanolum (lente, variabile), glycerolum (aliquando lente), meso-erythritolium, ribitolum (aliquando lente), xylitolum, galactitolum (lente, variabile), D-mannitolum, D-glucitolum, myo-inositolum, L-arabinitolium, D-lactatulum (lente, variabile), succinatulum, citratulum, glucono-δ-lactonum (lente, variabile), D-glucuronicium (ali quando lente), D-galacturonicium, D-glucosaminum, N-acetyl-D-glucosaminum et hexadeceanum assimilantur, at non inulimum, amyllum solubile, methanolum, D-glucoum, 2-ketogluconicum, saccharatum, propane-1,2-diolum et butane-2,3-diolum. Natrium nitrosum (exigui, variabile), ethylaminum hydrochloricum, lysisin, cadaverinum.

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**Fig. 3.** Light micrograph showing budding cells and blastoconidia of *Spencermartinsiella europaea* sp. nov. NCAIM Y.01817^T^, 5 % malt extract agar, 3 days, 25 °C. Bar, 10 μm.

**Fig. 4.** Pseudohyphae (a) and septate hyphae (b) of *Spencermartinsiella europaea* sp. nov. NCAIM Y.01817^T^, cornmeal agar, 7 days, 25 °C. Bar, 10 μm.
dihydrochloricum et glucosaminum assimilantur, at non kalium nitricum, creatinum, creatininum, imidazolum. Materia amyloidea iodophila non formatur. Vitamina externa crescentiae sunt necessaria. Crescere potest in 35 °C, at non in 37 °C. Parte una cycloheximidi per mille crescit, at non in agarō extracto fermenti confecto 50 partes glucosi per centum. Ureum non finditur.

**Description of Spencermartinsiella europaea**

Péter, Dlauchy, Tornai-Lehoczki, M. Suzuki & Kurtzman sp. nov.

*Spencermartinsiella europaea* (eu.ro.pae’a. N.L. fem. adj. europaea of or belonging to Europe, European, refers to the geographical origin of the strains investigated).

On 5% malt extract agar after 3 days at 25 °C, the streak culture is butyrous and folded or mycelial, moderately raised, tannish white, semi-glossy or dull. The edge is entire or finely lobed and fringed with filaments. Cells are ovoid, drop-shaped or elongated, and occur singly, in pairs or in short chains (Fig. 3). They measure 1.5–3.0 × 3.0–8.0 μm and are formed by multilateral budding with some cells arising on denticles, on small projections, or on true hyphae. Cells are solitary, in short chains or most often on small denticles. In 5% malt extract after 3 days at 25 °C, a climbing pellicle is present. On slide culture with cornmeal agar after 7 days at 25 °C, pseudohyphae and true hyphae are present (Fig. 4). Examination of ultrathin sections reveals that the hyphal septum has a single narrow central pore (Fig. 5). True hyphae give rise to single or short-catenated blastoconidia, mostly on small denticles. Blastoconidia may form secondary conidia. The species is heterothallic. Individual strains at 15 °C or 25 °C form no ascospores after 3 weeks of incubation. When compatible mating types are mixed, ascosporulation is observed on 2% malt extract agar either at 15 °C or 25 °C within 2–3 weeks. Asci (3.5–6 × 5–6 μm) are persistent, spheroid to ellipsoid and are usually formed on lateral hyphae. Each ascus bears a more or less ellipsoid apical cell and contains one hemispheroid or helmet-shaped ascospore, which from an upper view seems spheroid or ellipsoid (Fig. 6). Fermentation is absent. The carbon compounds assimilated are D-glucose, sucrose, raffinose, melibiose, D-galactose, lactose (positive or slow), α,α-trehalose, maltose, melezitose, methyl α-D-glucoside, cellobiose, salcin (variable), arbutin (positive or slow), L-sorbitose, L-rhamnose (positive or slow), D-xyllose, L-arabinose, D-arabinose (positive or slow), D-ribose (positive or slow), ethanol (slow and variable), glycerol (positive or slow), meso-erythritol, ribitol (positive or slow), xylitol, galactitol (slow and variable), D-mannitol, D-glucitol, myo-inositol, L-arabinitol, D-lactate (slow and variable), succinate, citrate, glucono-δ-lactone (slow and variable), D-glucononate (positive or slow), D-galacturonate, D-glucuronate, N-acetyl-D-glucosamine and hexadecane; no growth occurs on inulin, starch, methanol, D-gluconate, 2-keto-D-gluconate,
saccharate, propane-1,2-diol or butane-2,3-diol. Sodium nitrite (weak and variable), ethylamine hydrochloride, l-lysine, cadaverine dihydrochloride and glucosamine (as nitrogen source) are assimilated; potassium nitrate, creatine, creatinine and imidazole are not assimilated. Amyloid material is not formed. Growth in vitamin-free medium is absent. Growth occurs at 35 °C but is absent at 37 °C. Growth is absent on 50 % (w/w) glucose yeast extract agar, with 10 % NaCl (w/v) and with 1 % acetic acid. Growth with 0.1 % cycloheximide is positive. Urea hydrolysis and colour reaction with Diazonium Blue B are negative.

The type strain, NCAIM Y.01817T, and isotype strain, NCAIM Y.01819T, were recovered, from rotten wood of willow (Salix alba) and wild rose (Rosa sp.), respectively, in Hungary. The strains are deposited as NCAIM Y.01817T (=NRRL Y-48265T=CBS 11730T) and NCAIM Y.01819T (=NRRL Y-48266T=CBS 11731T) in the National Collection of Agricultural and Industrial Microorganisms in Budapest (Hungary). NCAIM Y.01817T and NCAIM Y.01819T represent opposite mating types.

References


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