Species distinction of the ascomycetous heterothallic yeast-like fungus *Stephanoascus ciferrii* complex: description of *Candida allociferrii* sp. nov. and reinstatement of *Candida mucifera* Kocková-Kratochvílová et Sláviková

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The nucleotide sequences of the 18S rRNA gene (rDNA) from nine strains of the heterothallic ascomycetous *Stephanoascus ciferrii* complex were determined and the strains were separated into three groups according to their sequences. 18S rDNA sequences were identical within the same group. In group A the 18S rDNA sequences had no introns; in group B there was one group I intron, Sc1506-1 at position 1506; and in group C there were two group I introns, Sc943 at position 943 and Sc1506-2 at position 1506. Sc1506-1 and Sc1506-2 at position 1506 exhibited 19 base differences but were very similar. Therefore, it is suggested that these introns existed in the common ancestor of groups B and C, and that they were vertically inherited. DNA similarity values showed that the strains within the same group were of identical species. Group B included the isotype strains of *Stephanoascus ciferrii* and the type strains of *Candida ciferrii* and *Sporothrix catenata*; this confirmed that group B strains correspond to *Stephanoascus ciferrii* and that *Candida ciferrii* and *Sporothrix catenata* are synonyms of *Stephanoascus ciferrii*. The single member of group C, strain IFO 10918^T, corresponds to the type strain of *Candida mucifera* and was independent of the other tested strains. Thus, *Candida mucifera* should be regarded as an independent species from *Stephanoascus ciferrii*. It is suggested that group A strains might comprise a new *Stephanoascus* species, but since group A strains could not form asci by themselves in this study they are described as a new species for which the name *Candida allociferrii* sp. nov. (type strain IFO 10194^T) is proposed.

Keywords: *Stephanoascus ciferrii*, *Candida allociferrii* sp. nov., rDNA sequences, group I intron, DNA–DNA hybridization

INTRODUCTION

The definition of a species, whether of higher plants, animals or micro-organisms, is based on the principle of genetic isolation. Members of a species are considered interfertile, whereas genetically separate species are not (Dobzhansky, 1976). However, this definition

is unsuitable for homothallic or anamorphic (asexual) yeasts. Therefore, until the late 1960s, the primary means for defining species of yeasts lay in the subjective appraisal and weighting of phenotypic properties. Nevertheless, the use of growth reactions could result in the erroneous definition of a species, since the assimilation and fermentation of many compounds are controlled by one or only a few genes (Lindegren & Lindgren, 1949; Winge & Roberts, 1949). At present, measurements of DNA reassociation are used to define yeast species on the basis of overall genetic

The DDBJ accession numbers for the sequences reported in this paper are AB000658–AB000662, AB019362 and AB041003–AB041006.

similarity (Kurtzman, 1987; Kurtzman & Phaff, 1987). Furthermore, rRNA gene (rDNA) sequences are used to measure evolutionary relationships and are studied with the aim of rapid yeast identification (Kurtzman & Robnett, 1998).

In this study we found that some strains of *Stephanoascus ciferrii* had 18S rDNA containing group I introns. Group I introns are a class of mobile genetic elements defined in part by the possession of a set of conserved P, Q, R and S sequences (Davies *et al.*, 1982), and they exist in many eukaryotes (Cech, 1988; Sogin & Edman, 1989), prokaryotes (Kuhnel *et al.*, 1990; Reinhold-Hurek & Shub, 1992; Xu *et al.*, 1990) and viruses (Shub *et al.*, 1988; Yamada *et al.*, 1994). They have frequently been found in fungal 18S rDNA (De Wachter *et al.*, 1992; Hibbett, 1996; Gargas *et al.*, 1995; Nishida & Sugiyama, 1995; Takashima *et al.*, 1996; Ueda & Mikata, 1999). It has been reported that introns can be transmitted vertically among lineages as well as horizontally (Hibbett, 1996; Bhattacharya *et al.*, 1996).

Smith & de Hoog (1998) reported that the heterothallic ascomycetous yeast-like fungus *Stephanoascus ciferrii* Smith *et al.* (1976) included three synonyms, *Candida ciferrii* Kreger-van Rij (1965), *Sporothrix catenata* de Hoog & Constantinescu (1981) and *Candida mucifera* Kocková-Kratochvílová & Sláviková (1988). In this paper we report on the DNA similarity, mating, 18S rDNA and 26S rDNA domain D1/D2 sequences of nine strains of the *Stephanoascus ciferrii* complex and discuss their species distinctions.

METHODS

DNA preparation and DNA–DNA hybridization. Yeast strains are listed in Table 1. The strains were cultured at 28 °C with shaking for 10–24 h in 200 ml Erlenmeyer flasks containing 50 ml YPD broth. For protoplast preparation and DNA

extraction the protocol of Holm *et al.* (1986) was followed, as modified by Kaneko & Banno (1991). DNA–DNA homology between strains was studied by the photobiotin microplate hybridization method of Ezaki *et al.* (1988, 1989), as modified by Kaneko & Banno (1991). Calculations were based on the means of five measurements.

18S rDNA sequencing. 18S rRNA gene (rDNA) sequences were determined using a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham Pharmacia Biotech), following the supplier's protocol. PCR for amplification of 18S rDNA and cycle sequencing using PCR products were performed as described by Ueda-Nishimura & Mikata (1999). In addition to some primers described in Ueda-Nishimura & Mikata (1999), the following 5' FITC-labelled primers were also used for sequencing: 1600F (5'-TTGTACACACCGCCCGTCG-3') for the sense strand and 1627R (5'-TAGCGACGGGCG-GTGTGTAC-3') for the reverse strand.

Similarity searches and secondary structure modelling of group I introns. A BLAST search (Altschul *et al.*, 1990) was conducted using DDBJ, GenBank and EMBL databases. Intron secondary structure was drawn following the model of Cech *et al.* (1994). GENETYX-MAC version 8 (Software Development) was used to help predict stem structures in the less conserved portions of the model.

26S rDNA domain D1/D2 sequencing. The 600 nt 26S rDNA domain D1/D2 sequences were determined by using a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (PE Biosystems), CentriSep spin column (PE Biosystems) and an ABI PRISM 310 Genetic Analyzer (PE Biosystems), following the manufacturers' protocols. For amplification of 26S rDNA domain D1/D2 sequences, PCR (Saiki *et al.*, 1988) was performed for 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 45 s with TaKaRa *Taq* DNA polymerase (Takara), using the primer pair NL-1 (5'-GCATATCAAT-AAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTG-TTTCAGACGG-3') (Kurtzman & Robnett, 1997). The PCR products were purified with GFX PCR DNA and the Gel Band Purification Kit (Amersham Pharmacia Biotech), following the supplier's protocol. For sequencing, purified

Table 1. *Stephanoascus ciferrii* complex strains

	Strain			Mating type*	Other information
	IFO	CBS	JCM		
Group A	10193	5165		α	
	10194 ^T	5166 ^T		α	
	10913		2984	—	
Group B	1854 ^{IT}	5295 ^{IT}	7621 ^{IT}	α	Isotype strain of <i>Stephanoascus ciferrii</i>
	1855 ^{IT}	6699 ^{IT}	7622 ^{IT}	a	Isotype strain of <i>Stephanoascus ciferrii</i>
	10192 ^T	4856 ^T	9551 ^T	α	Type strain of <i>Candida ciferrii</i>
	10195	5646		a	
	32952 ^T	215-79 ^T	2921 ^T	—	Type strain of <i>Sporothrix catenata</i>
Group C	10918 ^T	7409 ^T		a	Type strain of <i>Candida mucifera</i>

* Data from Smith & de Hoog (1998).

PCR products were used as a template, and NL-1 and NL-4 were used as primers.

Phylogenetic analysis. Sequence data were manually aligned with rDNA sequences of representative related species obtained from DDBJ and GenBank. Positions that could not be compared among all sequences, corresponding to indels and regions difficult to align between strains, were omitted from the phylogenetic analysis. A phylogenetic tree was constructed by Kimura's two-parameter method (Kimura, 1980) and the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL W. Bootstrap values (Felsenstein, 1985) were calculated from 1000 replicates.

Assimilation and mating test. Physiological, morphological and cultural characteristics and mating types were investigated according to Yarrow (1998). Corn meal agar Dalmau plates were used as sporulation medium.

Ascospore germination. Single spores isolated by a manipulator were cultured on YM agar at 24 °C for 1 week.

RESULTS AND DISCUSSION

Sequencing of 18S rDNA and prediction of group I intron secondary structures

Strains of the *Stephanoascus ciferrii* complex studied and their mating types reported by Smith & de Hoog (1998) are listed in Table 1. 18S rDNA sequences of the nine strains were determined by the direct method. Insertion sequences were found in the 18S rDNA sequences of six strains. The nine strains were separated by 18S rDNA sequences into three groups: group A strains have no insertion in their 18S rDNA, group B strains have an insertion sequence of 404 bases (Sc1506-1) at position 1506 and the group C strain has insertion sequences of 355 and 403 bases (Sc943 and Sc1506-2) at positions 943 and 1506, respectively (nucleotide positions according to Gutell, 1993) (Fig. 1). Sc1506-1 and Sc1506-2 at position 1506 were very similar, but not identical. Group A consists of three strains with identical sequences. Group B consists of five strains, including the isotype strains of *Stephanoascus ciferrii* (IFO 1854^{IT} and IFO 1855^{IT}) and the type strains of *Candida ciferrii* (IFO 10192^T) and *Sporothrix catenata* (IFO 32952^T), and again all have identical sequences. Group C consists of a single

member, the type strain of *Candida mucifera* (IFO 10918^T) (Table 1).

The BLAST search revealed that the three insertion sequences, Sc1506-1, Sc1506-2 and Sc943, are similar to the sequences of many group I introns, and they are therefore thought also to be group I introns. The conserved P, Q, R and S regions of group I introns were found by the BLAST search. The catalytic core sequences corresponding to P, Q, R and S interact to form stems P4 and P7, which are parts of the conserved secondary structure core of group I introns (Cech, 1988). The catalytic core sequences of Sc1506-1 and Sc1506-2 were identical to that of one of the *Tilletiopsis flava* group I introns (GenBank accession numbers D82819 and X13687), while that of Sc943 differed from those of all known introns. The internal guide sequences (IGSs) located just downstream of the 5' splice site and in the upstream part of the 5' splice site interact to form stem P1. Using these sequences and structures (Sogin & Edman, 1989; Wilcox *et al.*, 1992; De Wachter *et al.*, 1992) as guides, the remaining parts of the sequence could be folded to fit the secondary structure model of Cech *et al.* (1994) for group I introns. The complexes P1, P5 and P9 were predicted by use of the RNA secondary structure prediction program in the GENETYX software package. The predicted secondary structures of group I introns Sc1506-1, Sc1506-2 and Sc943 (data not shown) corresponded to subgroup IC1, listed by Michel & Westhof (1990).

In comparison with Sc1506-1, Sc1506-2 has 4 base substitutions, 7 base insertions and 8 base deletions. This closely homologous relation between Sc1506-1 and Sc1506-2 suggests that these introns existed in the common ancestor of groups B and C, and that they were vertically inherited. The substitution rate between the two introns was 1.0% (= 4 substitutions per 396 bases compared). On the other hand, that between 18S rDNA exons of groups B and C was 0.46% (= 8 substitutions per 1754 bases compared). Our observations suggest that group I intron sequences evolve approximately 2.2 times more rapidly than 18S rDNA exon sequences.

DNA–DNA hybridization

DNA–DNA hybridization was tested with DNAs from the nine strains and photobiotin-labelled DNA from IFO 10193 (group A), IFO 1854^{IT} and IFO 32952^T (group B) and IFO 10918^T (group C). The results are shown in Table 2. DNA similarity values among group A strains were 86–100%, and those among group B strains were 79–100%. These data showed that group A strains and group B strains should each be considered to be conspecific. Groups A and B showed intermediate similarity values (41–66%), suggesting that they represent different, but closely related species. IFO 10918^T (group C) showed low similarity (10–41%) to all of the tested strains. Thus, it is suggested that IFO 10918^T (group C) is independent of groups A and B.

Fig. 1. 18S rDNA primary structures of the *Stephanoascus ciferrii* complex. Shaded boxes indicate 18S rDNA coding regions; white boxes indicate insertion sequences.

Table 2. DNA similarity

Hybridization was performed at 45 °C. Boldface type indicates intra-group values; ND, not determined.

Strain	DNA similarity (%)			
	Group A	Group B		Group C
	IFO 10193	IFO 1854 ^{IT}	IFO 32952 ^T	IFO 10918 ^T
Group A				
IFO 10193	100	63	47	29
IFO 10194 ^T	86	41	48	12
IFO 10913	96	66	65	33
Group B				
IFO 1854 ^{IT}	52	100	88	15
IFO 1855 ^{IT}	46	94	79	11
IFO 10192 ^T	50	100	98	14
IFO 10195	42	92	81	10
IFO 32952 ^T	ND	ND	100	ND
Group C				
IFO 10918 ^T	41	33	27	100
<i>Saccharomyces cerevisiae</i>				
IFO 10217	5.5	7.1	ND	1.4

Table 3. Mating test

IFO 10193 and IFO 10913 of group A did not mate with any of the tested strains. i, Ascospores immature; + a, ascus formed, but not ascospores.

Strain	Mating type*	Group A	Group B	
		IFO 10194 ^T α	IFO 1854 ^{IT} α	IFO 10192 ^T α
Group B				
IFO 1855 ^{IT}	a	+ i	+ + +	+
IFO 10195	a	+ i	+	+
Group C				
IFO 10918 ^T	a	+ a	+ i	+ i

* Data from Smith & de Hoog (1998).

Mating tests

Mating abilities between strains were tested and the results are shown in Table 3. The combination of group B strains caused sporulation, with the combination of the isotype strains of *Stephanoascus ciferrii* (IFO 1854^{IT} and IFO 1855^{IT}) causing especially frequent sporulation (Fig. 2a). The combination of groups B and A and of groups B and C also caused sporulation, but ascospores did not mature (Fig. 2b, c). The combination of groups A and C formed asci, but not ascospores. IFO 10193 and IFO 10913 of group A did not mate with any of the tested strains. IFO 10193 (= CBS 5165), which has been shown to be mating type α (Smith & de Hoog, 1998), might have lost mating ability. The fact that all groups could mate with

each other indicated that the three groups are closely related.

The ascospores formed between group B strains could not germinate. The reasons for this finding are not clear, but it is possible that the conditions for maturation or germination of ascospores were inadequate, or that the abilities of maturation or germination were lost. Whatever the reason, the species of tested strains could not be defined by their interfertility in the mating test. Therefore, we attempted to estimate interfertility on the basis of intron composition. In the anamorphic yeast *Candida albicans* and the yeast-like fungus *Pneumocystis carinii*, the group I intron composition of 25S or 16S rDNA has been reported to be variable among strains (McCullough *et al.*, 1999; Liu &

Fig. 2. Photomicrographs of asci on corn meal agar. (a) IFO 1854^{IT} α $\times$ IFO 1855^{IT} a, (b) IFO 10194^T α $\times$ IFO 10195 a, (c) IFO 10192^T α $\times$ IFO 10918^T a. Arrows show mature ascospores. Bars, 5 μ m.

Table 4. Distinguishing characteristics of clusters

+s, Slow growth; +w, weak growth.

Characteristic	Group A	Group B	Group C
Assimilation of:			
Melibiose	+w/+	+w	—
Inulin	+w	—	—
Growth at:			
35 °C	+	+	+s
37 °C	+	+	—

Leibowitz, 1993), while in the *Stephanoascus ciferrii* complex, the intron composition of 18S rDNA was uniform within strains of the same group. This indicates that intron composition spread by sexual reproduction and became fixed in each group. Therefore, although sexual reproduction between group A strains was not observed (Table 3), it is expected that new strains of group A will be isolated and that sexual reproduction between group A strains will be observed.

Physiological and cultural characters

In their physiological characters, all tested strains were similar to each other. Properties usable for discrimination of the groups are presented in Table 4. Group A strains differed from group B and C strains in their ability to assimilate inulin. The group C strain differed from strains in groups A and B by lack of growth at 37 °C. Physiological characters of group B were intermediate between those of groups A and C.

Phylogeny

18S rDNA sequences of the related species (Yamada & Nogawa, 1990) were determined by the direct method and that of *Arxula terrestris* was obtained from DDBJ (Fig. 3a). No group I introns were found in 18S rDNA sequences of related species, except for that at position 1199 in *Arxula terrestris* (Ueda & Mikata, 1999). Fig. 3(a) shows a phylogenetic tree calculated from 18S rDNA exon sequences of groups A, B and C, and related species. It is shown that group C diverged first from the ancestor of the *Stephanoascus ciferrii* complex and that groups A and B are close relatives with 1 base substitution.

In general, the number of base substitutions of 26S rDNA domain D1/D2 sequences is much higher than that of 18S rDNA exon sequences, and the 26S rDNA domain D1/D2 sequences therefore provide a somewhat higher resolution of terminal lineages than do the 18S rDNA exon sequences. Since no differences within the same group were found in the 18S rDNA sequences, the 26S rDNA domain D1/D2 sequences of the *Stephanoascus ciferrii* complex were determined to investigate the diversity within each group. The intra-specific variation in 26S rDNA domain D1/D2 sequences is reported to be up to 3 nt differences, except for contiguous indels (Kurtzman & Robnett, 1998). Sequences were found to be identical within the same group, except that IFO 10192^T of group B has 1 base insertion compared with other strains of group B. Despite this exception, groups A and B were each considered to be a genetically homogeneous population. Fig. 3(b) shows a phylogenetic tree calculated from 26S rDNA domain D1/D2 sequences of *Stephanoascus ciferrii* groups A, B and C, and related species

Fig. 3. Phylogenetic trees calculated by neighbour-joining, depicting relationships among the *Stephanoascus ciferrii* complex and related species analysed by 18S rDNA (a) and 26S rDNA domain D1/D2 (b) sequences. Bootstrap values were calculated from 1000 trees. DDBJ accession numbers are shown in parentheses. Bar, sequence dissimilarity value of 1%.

obtained from GenBank, EMBL and DDBJ. The three groups were found to be close relatives within 5 base substitutions.

Comparison of the 26S rDNA domain D1/D2 sequences revealed only 1 base substitution between groups B and C, indicating that they were the closest relatives (Fig. 3b). This was not consistent with the phylogeny of 18S rDNA exon sequences, which indicated that groups B and C were not the closest relatives (Fig. 3a). For instance, between groups B and C, 8 base substitutions were observed in the 18S rDNA exons, but only 1 base substitution in the 26S rDNA domain D1/D2. The 1 base substitution in the 26S rDNA domain D1/D2 is within the range of conspecificity proposed by Kurtzman & Robnett (1998). Nevertheless, DNA similarity values showed that

group B is clearly a different species from group C, and that it has intermediate similarity to group A (Table 2). Thus, it is suggested that the 18S rDNA exon tree corresponds to the result of DNA similarity (Fig. 3a) and that the 26S rDNA domain D1/D2 does not show the exact phylogenetic distance between groups B and C (Fig. 3b). This in turn suggests that species distinction using only 26S rDNA domain D1/D2 sequences might be mistaken.

Estimation of the group I intron loss and insertion timing

In the course of evolution, it is expected that these strains frequently lose or acquire the group I intron in the 18S rDNA. The fact that no group I introns were

Fig. 4. Photomicrographs of *Candida allociferrii* in YM broth after 2 days at 24 °C. (a) Budding yeast cells, IFO 10194^T; (b) budding yeast cells, IFO 10193; (c) pseudohyphae, IFO 10194^T. Bars, 10 µm.

found in the 18S rDNA sequences of related species, except for that at position 1199 in *Arxula terrestris* (Ueda & Mikata, 1999), suggests that the 18S rDNA of the common ancestor of the *Stephanoascus ciferrii* complex lacked group I introns. Because introns Sc1506-1 and Sc1506-2 must have been vertically transferred, it is suggested that the intronless ancestor of the *Stephanoascus ciferrii* complex first horizontally acquired Sc1506 as the prototype of Sc1506-1 and Sc1506-2. Because the group B strains with Sc1506-1 are more closely related to intronless group A than to group C with Sc1506-2 (Fig. 3a), it can be proposed that the ancestor of group A subsequently lost Sc1506 and that the ancestor of group C acquired the prototype of Sc943.

Classification

DNA similarity values demonstrated that group B strains, including the isotype strains of *Stephanoascus ciferrii* and the type strains of *Candida ciferrii* and *Sporothrix catenata*, are conspecific. Therefore, it was confirmed that group B strains correspond to *Stephanoascus ciferrii* and that *Candida ciferrii* and *Sporothrix catenata* are synonyms of *Stephanoascus ciferrii*. On the other hand, the single member of group C, IFO 10918^T, which corresponds to the type strain of *Candida mucifera*, was shown to be independent of the other tested strains by its low DNA similarity values. Thus, *Candida mucifera* should be regarded as an independent species from *Stephanoascus ciferrii*. The finding of intermediate DNA similarity values between groups A and B and a close relationship between them on the basis of 18S rDNA exon phylogenetic analysis indicates that these groups have speciated recently and that group A might be a new *Stephanoascus* species. However, since group A strains could not form asci by

themselves in this study, they are described as a new species for which the name *Candida allociferrii* sp. nov. is proposed.

Latin diagnosis of *Candida allociferrii* sp. nov.

In medio liquido YM post dies 2 ad 24 °C, cellulae globosae vel elongatae (1.7–4.5 × 2.9–9.4 µm), cellulae singulae aut binae, per gemmationem multilateralem reproducentes. Post unum mensem sedimentum formatur. Pseudohyphae formatur. *In agaro* YM post unum mensem ad 24 °C, coloniae alba vel crenea, farinose aut rugosa. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, maltosum, sucrosus, cellobiosum, trehalosum, melibiosum, raffinose, inulinum (lente), amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, D-gluconatum, arbutinum et inositolum assimilantur, at non lactosum, melezitolum, glucono-1,5-lactonum nec acidum citricum. Ethylaminum assimilantur, at non kalium nitricum. Ad crescentiam vitaminarum externarum necessariae sunt. Augmentum in 37 °C. Typus stirps IFO 10194^T (= CBS 5166^T) isolatus ex *Homo sapiens*, conservatur in collectionibus culturarum quas Institute for Fermentation, Osaka (IFO), Japonia.

Description of *Candida allociferrii* sp. nov.

Candida allociferrii (al.lo.ci.fer'ri.i. Gr. pron. *allo* another; L. gen. masc. n. *ciferrii* of Ciferrii; N.L. gen. masc. n. *allociferrii* another *ciferrii*, different but relating to *Stephanoascus ciferrii*).

After 2 days growth in YM medium at 24 °C, the yeast cells are ovoid or elongate (1.7–4.5 × 2.9–9.4 µm) and

occur singly or in pairs (Fig. 4a, b). A sediment is present. Budding is multilateral. Pseudohyphae are formed (Fig. 4c). After 1 month growth on YM agar medium at 24 °C, colonies are white to slightly cream with a mycelial surface, raised and wrinkled. Pseudohyphae grow from the reverse of the colony into the agar and from the margin of the colony to outside on the surface of agar. Ascospores were not detected in cultures of the three known strains of *Candida allociferii*, nor in mixtures of the three, when grown on YM or corn meal agar at 24 °C *Candida allociferii* is closely related to *Stephanoascus ciferrii* and *Candida mucifera*; for this reason the *Candida allociferii* type strain IFO 10194^T (= CBS 5166^T) mated with some strains of these two species (Table 3). Fermentation is negative. Glucose, galactose, L-sorbose, maltose, sucrose, cellobiose, trehalose, melibiose, raffinose, inulin (weak), soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, methyl- α -D-glucoside, salicin, DL-lactic acid, succinic acid, D-gluconate, arbutin and inositol are assimilated, but not lactose, melezitose, D-glucono-1,5-lactone or citric acid. Ethylamine is assimilated as sole nitrogen source, but not potassium nitrate. Growth in vitamin-free medium is negative. Growth at 37 °C is positive. Source of cultures: IFO 10193 and IFO 10194^T were received from the Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands, as *Stephanoascus ciferrii* (Smith *et al.*, 1976) and had been isolated from a wooden pole in a cow shed and presumed human, respectively; IFO 10913 was received from the Japan Collection of Microorganisms (JCM) as *Stephanoascus ciferrii* isolated from pickled embric myrabolon in Thailand (Suzuki *et al.*, 1987). Cultures of the type strain IFO 10194^T (= CBS 5166^T) have been deposited in the culture collection of the Institute for Fermentation, Osaka (IFO), Japan.

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