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ORIGINAL PAPER

Hyphopichia lachancei, f.a., sp. nov., a yeast species from diverse origins

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Abstract Three strains originating from insect frass in South Africa, yellow foxglove in Hungary and soil in France, were characterised phenotypically and by sequencing of the D1/D2 domain of the large subunit and the ITS1-5.8S-ITS2 (ITS)-region of the rRNA gene. The strains have identical D1/D2 domain sequences and only one strain shows a 1 bp indel in a 9 bp homopolymer A/T repeat within the ITS-region. Based on sequence analysis *Hyphopichia burtonii* is the closest related species. The investigated strains differ from the type strain of *H. burtonii* by 1.9% (9 substitutions and an indel) in the D1/D2 domain and by 23 substitutions and 21–22 indels in the ITS-region. Since the sequence variability is very low among the three strains and the sequence divergence with the closely related *H. burtonii* exceeds the level generally encountered between species we propose the new species *Hyphopichia lachancei* f.a., sp. nov. to accommodate the three novel strains. From *H. burtonii* the new species can be distinguished phenotypically by its inability to ferment cellobiose and by the formation of endospores (Holotype: CBS 5999^{T}; Isotype: NCAIM Y.02228^{T}; MycoBank no.: MB833616).

Keywords *Hyphopichia lachancei* · Endospores · Conidia · One new taxon

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Introduction

The genus *Hyphopichia* was erected for a single species, *Hyphopichia burtonii* by von Arx and van der Walt (1976). The authors delimited the genus on the basis of phenotypic characteristics such as heterothallism, formation of septate hyphae and denticulate conidiogenous cells. Kurtzman (1998) did not accept the genus *Hyphopichia* because the phenotypic characteristics being the basis of the genus description can be found in other genera as well. However, rRNA gene sequence analysis revealed that the genus *Hyphopichia* is well separated from other genera (Kurtzman and Robnett 1998; Yamada et al. 1999). Consequently, the genus *Hyphopichia* was reinstated by Kurtzman (2005) and a total of 7 species were assigned to the *Hyphopichia* clade. Later additional related species were described (Limtong et al. 2012; Ren et al. 2015). On the basis of sequence analysis of the D1/D2

domain three distinct phylogenetic clusters were recognised within the genus and a new species, *H. pseudoburtonii*, was described by Groenewald and Smith (2010). Further, ascosporulation was induced in *Candida homilentoma* by crossing haploid strains and its teleomorph was described as *H. homilentoma*. In addition, a new anamorphic species, *H. buzzinii*, was added to the genus and six phylogenetically related *Candida* species were transferred to *Hyphopichia* (Ribeiro et al. 2017).

In the current study three conspecific strains of the genus *Hyphopichia* originating from Hungary, South Africa and France were characterised and the new species *Hyphopichia lachancei* is proposed to accommodate them.

Materials and methods

Three strains were investigated in this study; CBS 5999^T was isolated from insect frass from a tree in South Africa, NCAIM Y.01946 (CBS 15875) from yellow foxglove (*Digitalis grandiflora*) flowers in Hungary and NCAIM Y.02227 from soil under an oak (*Quercus* sp.) in southern France. The strains were phenotypically characterised according to standard methods (Kurtzman et al. 2011). Attempts were made to induce sporulation by inoculating them alone or in mixture on 2% malt agar, McClary's acetate agar, Fowell's acetate agar, corn meal agar, Gorodkowa agar and restricted growth agar, ''Spezieller Nährstoffarmer Agar''(SNA), PDA, GPYA, YM agar, V8 agar, diluted (1:9) V8 agar, yeast-carbon base (YCB) agar and yeast-carbon base agar supplemented with 0.01% ammonium sulphate (YCBAS) (Kurtzman et al. 2011). The cultures were incubated at 15 and 25 °C and examined microscopically regularly for a period of 3 weeks.

Sequencing of the D1/D2 domain of the large subunit of the rRNA gene and of the ITS1-5.8S-ITS2 (ITS) region was done as described before (Brysch-Herzberg and Seidel 2015) with the primer pairs NL1-NL4 (Kurtzman and Robnett 1998) and ITS1-ITS4 (White et al. 1990). Sequence alignments were made with CLC Main Workbench 8.0 (QIAGEN Aarhus A/S). For the D1/D2-domain phylogenetic relationships were calculated with the maximum likelihood method and the Jukes Cantor nucleotide substitution model included in Seaview (Gouy et al. 2010). Bootstrap values were calculated from 1000 iterations. DNA sequences of the type strains of species in the *Hyphopichia* clade as well as of related strains representing undescribed species retrieved from GenBank were included in the analysis.

Results and discussion

Phenotypic characterization

Morphologically all three strains characterised in this study fit well into the genus *Hyphopichia*. Asexual cells are globose, ovoid, elongated or irregular in shape (Fig. 1a–e). Extreme differences can be observed in the cell sizes, 2–5 × 2–30 µm. Budding is multilateral (Fig. 1a, d) and may take place on tube-like outgrowth of yeast cells (Fig. 1e). Denticulate conidiogenous cells are also present (Fig. f–m). Pseudomycelium may be produced (Fig. 1d) and true mycelium is formed (Fig. 1f–h) which grows on and under the agar surface. Endospores may be produced within hyphal cells (Fig. 1n–p) on Gorodkowa agar after 1 week at 25 °C. Although no endospore formation by budding was observed, their variable size and shape clearly distinguish them from ascospores.

As the sequence analysis of the D1/D2 domain of the LSU rRNA gene (see below) revealed that *H. lachancei* sp. nov. is more closely related to *H. burtonii* than to other species in the *Hyphopichia* clade, the physiological characteristics of *H. lachancei* sp. nov. are compared to that of *H. burtonii* as given by Barnett et al. (2000) and Kurtzman et al. (2011). Barnett et al. (2000) found a lot of variation in the reactions to most of the tests among the strains they have examined, making it almost impossible to reliably differentiate *H. burtonii* and *H. lachancei* on the basis of these phenotypic data. Fermentation of cellobiose is the only test for which all strains listed by Barnett et al. (2000) gave positive (delayed) results and the strains characterised in the current study showed negative results. A complete set of growth responses of *H. lachancei* is given below.

Sequence comparison

The three investigated strains of the novel species share an identical 525 bp D1/D2 domain sequence (MN645470), which differs from that of *H. burtonii* by

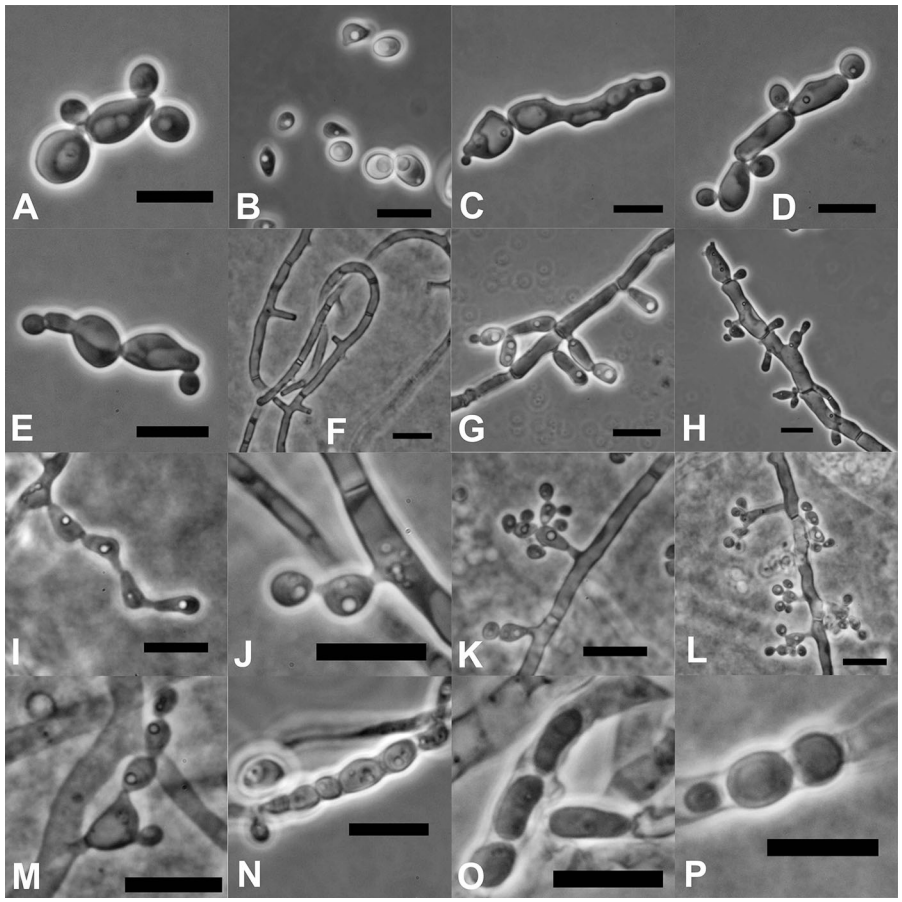

Fig. 1 Micromorphology of *Hyphopichia lachancei* sp. nov.; **a**–**e**: multilateral budding of different cell types after 24 h at 25 °C on GPY Agar; **f**–**h**: true mycelium; **i**–**m**: conidia; **n**–**p**: endospores in true mycelium; (**f**–**m**: after 1 week at 25 °C on GPY Agar; **n**–**p**: after 1 week at 25 °C on Gorodkowa agar). The bar indicates 5 µm. (**a**, **c**, **d**, **e**, **g**, **i**, **j**, **m**, **n**, **o**, **p**: CBS 5999^{T}; **b**, **f**: NCAIM Y.02227; **h**, **k**, **l**: NCAIM Y.01946)

1.9% (9 substitutions and an indel). Kurtzman and Robnett (1998) concluded from their sequence analyses of a broad spectrum of ascomycetous species that strains exhibiting more than 1% divergence in the D1/D2 domain are likely to belong to different species. Later this finding was confirmed by (Vu et al. 2016) who analysed the D1/D2 domain sequences of the type strains and additionally those of several strains per species deposited in the CBS culture collection. Nevertheless, it should be emphasised that species are not solely defined by D1/D2 divergence values and that substantial deviations from the '1% rule' have been reported (Lachance 2018). In *Galactomyces candidus* a high level of interstrain and intrastrain (intragenomic) variation was observed (Alper et al. 2011). Similar results were described for other species like *Metschnikowia pulcherrima* (Sipiczki et al. 2018) or *Clavispora lusitaniae* (Lachance et al. 2003). Because in the current study no sites with ambiguous sequencing results were detected the mechanisms responsible for concerted evolution seem to work efficiently in *H. lachancei* sp. nov. Therefore, it seems unlikely that the new species possesses considerable intragenomic variation in the rDNA copies. Despite the distant geographical origins of the three strains of *H. lachancei* sp. nov., their D1/D2 sequences are identical. This can be interpreted to indicate that the intraspecies heterogeneity is very low and that the species is well separated from the other species in the clade.Thus the D1/D2 domain can be used to reliably identify strains of *H. lachancei* sp. nov. which is not the case for all yeast species: e.g. *Kazachstania*

aerobia and *K. solicola* (Wu and Bai 2005) have identical D1/D2 domain sequences and in other groups like the *Rhodosporidium babjevae* clade or the *Filobasidium floriforme* clade it was shown that a reliable identification of strains based on both the D1/D2 domain and the ITS region is not always possible (Brysch-Herzberg and Seidel 2015). The phylogenetic placement of the new species within the genus on the basis of D1/D2 sequences is illustrated in Fig. 2. The case of a novel species is supported by the phylogenetic species concept as the three strains investigated in this study and involved in the analysis form an early emerging position with respect to their closest relatives *H. burtonii* and *H. khmerensis*.

The ITS region of *H. lachancei* sp. nov. is 425 bp long. Strain NCAIM Y.02227 from France differs from the Hungarian and the South African strain merely by a single indel (1 bp) in a 9 bp homopolymer A/T repeat (MN749307; MN749065). The strain CBS 5999^{T} differs from the type strain (CBS 2352) of *H. burtonii* by 23 substitutions and 22 indels. Again, as in case of the D1/D2 domain, the intraspecific variability is very low whereas the interspecific divergence is high. Taking into account the results of both the D1/D2 domain and the ITS region sequence comparisons the description of the new species, *H. lachancei,* is well supported.

Given that only three strains were considered, it is premature to make any reliable conclusion on their

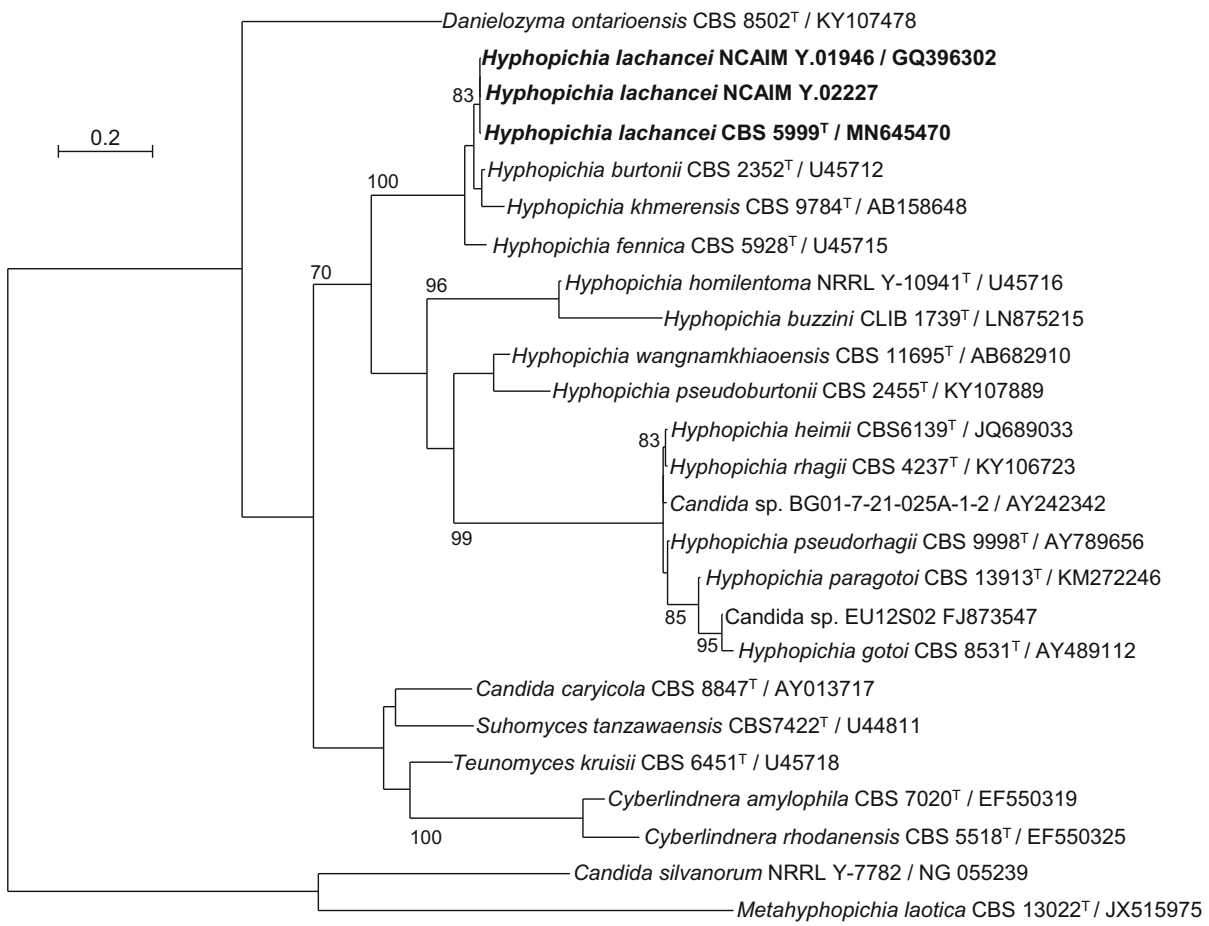

Fig. 2 Phylogenetic placement of *Hyphopichia lachancei* within the *Hyphopichia* clade. The tree is based on DNA sequence analysis of the D1/D2 domain of the nuclear large subunit (LSU) rRNA gene and obtained by maximum likelihood analysis. Percentage bootstrap values of 1000 replicates above 70% are given at each node. GenBank accession numbers are indicated after strain designation. Bar, 20% nucleotide sequence divergence. *Candida silvanorum* and *Metahyphopichia laotica* were used as outgroup

ecology. Their different geographical origins suggest that the novel species is widely distributed but rarely isolated. The connecting link among their isolation substrates (insect frass, flower and soil) may be insects.

Description of *Hyphopichia lachancei,* f.a., sp.nov. Michael Brysch-Herzberg[1], Marizeth Groenewald, Denes Dlauchy, Martin Seidel, Gábor Peter

MycoBank no.: MB833616

Etymology: The specific epithet lachancei (N.L. gen. n. lachancei, pertaining to Lachance) refers to Marc-André Lachance, in recognition of his outstanding contributions to the study of the ecology and taxonomy of yeasts.

After 3 days on GPY Agar at 25 °C the colonies are raised with an irregular filamentous margin. The colony surface is filamentous and the colour is white to off-white.

After 7 days on GPY-Agar at 25 °C budding cells pseudomycelium and true mycelium are formed. True mycelium grows on and under the agar surface. Asexual reproduction proceeds by multilateral budding. Budding may occur on elongated outgrowth of yeast cells. Denticulate conidiogenous cells are present. Yeast cells are irregular in shape, globose, ovoid or elongated (2–5 × 2–30 µm). Endospores are formed on Gorodkowa agar after 1 week at 25 °C.

D-glucose, D-galactose (positive or slow), maltose (positive or slow), Me-α-D-Glucoside (weak and variable), sucrose (weak or slow and variable), trehalose (positive or slow) and starch (weak, delayed and variable) are fermented. Melibiose, lactose, cellobiose, melizitose, raffinose, inulin and xylose are not fermented.

D-glucose, D-galactose, L-sorbose (positive or slow), D-glucosamine (weak), *N*-acetyl-D-glucosamine, D-ribose, D-xylose, L-arabinose (positive or slow), D-arabinose (weak, slow and variable), sucrose, maltose, α-α-trehalose, Me-α-D-Glucoside (positive or weak or slow), cellobiose (slow and variable), salicin (variable), arbutin (positive or slow), melizitose (positive or slow), starch, glycerol, erythritol, ribitol, xylitol, L-arabitol, D-glucitol, D-manitol, D-glucono-1,5-lactone (positive or slow), 2-keto-D-gluconate, D-Gluconate, succinate, citrate, ethanol, propane-1,2-diol (slow and variable), butane-2,3-diol (positive or variable), hexadecane (slow or latent), palatinose. L-rhamnose, melibiose, lactose, raffinose, inulin, galactitol, myo-inositol, D-glucuronate, D-galacturonate, DL-lactate, saccharate, methanol are not assimilated. Ethylamine, L-lysine and cadaverine are assimilated nitrate, nitrite, creatine, creatinine, glucosamine (as nitrogen source) and imidazole are not assimilated. Growth occurs at 25 °C and 30 °C. No growth occurs at 4 °C and at 37 °C. Growth is observed in the presence of 0.001% cycloheximide whereas no growth occurs in the presence of 0.01% cycloheximide. Growth is observed in the presence of 50 and 60% D-glucose and 10% NaCl but not with 1% acetic acid. Starch and acetic acid are not produced. Urea is not hydrolysed. The colour reaction with DBB is negative. Growth occurs in vitamin-free medium.

Holotype: CBS 5999^T; Isotype: NCAIM Y.02228^T, both are permanently preserved in a metabolically inactive state. The type culture was isolated from insect frass from a tree in South Africa.

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Authors contributions MBH wrote parts of the paper, produced the figures, analysed data and performed the light microscopic analysis. MG wrote parts of the paper, made physiological tests and supported the data analysis. DD and MS performed physiological tests and did molecular genetic analysis. GP wrote parts of the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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