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Metahyphopichia laotica gen. nov., sp. nov., a novel polymorphic yeast related to Hyphopichia

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Metahyphopichia laotica gen. nov., sp. nov., a novel polymorphic yeast related to *Hyphopichia*

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(Footnote)

The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain of the LSU rRNA gene, the ITS1-5.8S-ITS2 region and the 18S SSU rRNA gene of 11-1006^T are JX515975, JX515976 and JX515977, respectively.

(Abstract)

Four strains alternating between yeast and filamentous growth morphologies were isolated from flowers in two regions of Laos. In liquid environment the isolates propagated by budding and developed irregularly shaped pseudohyphae. On solid media their yeast cells switched to hyphal growth which could return to the yeast phase by developing lateral blastoconidia. The sequences of the D1/D2 domains of the large subunit (LSU) 26S rRNA genes, the internal transcribed spacer (ITS) regions and the small subunit (SSU) 18S rRNA genes were identical in the four strains and differed from the corresponding sequences of other yeast species available in databases by at least 11% (D1/D2), 13% (ITS) and 7% (SSU). In an independent project, two strains with D1/D2 and ITS sequences very similar to those of the Laotian strains were found in bark samples collected in Brazil. The six strains also differed from the closest yeast species in physiological properties, indicating that they represented a hitherto undescribed species. The phylogenetic analysis of the D1/D2 sequences, and the concatenated sequences of the SSU rRNA genes, D1/D2 domains of LSU rRNA genes as well as the protein-encoding genes *ACT1* and *TEF1* placed them close to *Hyphopichia*. To reflect this position, the novel genus name *Metahyphopichia* and the novel species name *Metahyphopichia laotica* are proposed for them. The type strain is 11-1006^T (=CBS 13022^T = CCY 092-001-001^T = NCAIM Y.02126^T) isolated in Luang Prabang (Laos). Mycobank registration numbers are MB 808253 (*Metahyphopichia*) and MB 808254 (*M. laotica*).

Alternation between yeast and filamentous growth phases is a widespread phenomenon in all larger taxonomic groups of Basidiomycota and Ascomycota. The ability to switch between growth phases helps the di- and polymorphic fungi adapt to changes in the environment. For example species are known that propagate by producing yeast cells in liquid substrates and by forming hyphae or pseudohyphae (or both) on/in solid substrates (e.g. Sipiczki *et al*., 1998). In pathogenic species, the morphological transitions are usually associated with changes in pathogenicity (for a review, see Nemecek *et al.*, 2006). The signals that induce phase transitions and the mechanisms by which the organisms reprogramme themselves are poorly understood in most species. Detailed molecular analyses have been performed in a limited number of species (for reviews see, Han *et al*., 2011; Gancedo, 2001) and revealed considerable diversity. In a recent bioinformatics analysis (Nagy *et al.*, 2014), we found that the diversification of Zn-cluster transcription factors may play an important role in the yeast-filamentous transitions. Identification and characterisation of novel species with di- or polymorphic growth cycles could contribute to a better understanding of the phenomenon. Motivated by these perspectives, we isolated yeasts capable of switching to filamentous growth from plant material collected in various geographical localities. Certain isolates turned out to represent novel species of various ascomycetous or basidiomycetous genera (e.g. Sipiczki & Kajdacsi, 2009; Sipiczki, 2011, 2012, 2013). Here we report on another group of strains (Table 1) capable of alternation between yeast and filamentous morphologies. The strains represent a novel species related to *Hyphopichia* and *Danielozyma*.

To isolate yeasts capable of morphological transitions, plant material was collected in various localities in Laos in 2008. The samples were macerated in sterile water and aliquots were streaked on YEA (1% yeast extract, 2% glucose, 2 % agar, w/v). After incubation at 25 °C for 10 days, yeast colonies fringed with mycelia were isolated. Three samples (fallen small flowers of uncertain origin) collected in the outskirts of the town Luang Prabang and one

sample from Vientiane (fallen Dok Champa [*Plumeria alba*] flower) produced colonies with wrinkled surface and mycelium. Representatives of colonies were isolated from each sample and restreaked on fresh YEA plates to select pure clones. The colonies of the Luang Prabang strains were more wrinkled and occasionally segregated into sectors with smoother surface (Fig. 1a,b). Both the more wrinkled and the smoother parts consisted of mixtures of budding yeast cells and pseudohyphae (Fig. 1c) but the proportion of pseudohyphae was lower in the sectors with smoother surfaces. On nutrient-poor media such as corn-meal agar (van der Walt & Yarrow, 1984), the yeast colonies of all isolates were thinner and released rapidly growing mycelium into the medium. Consistent with this colony morphology, all strains developed a mesh of branched hyphae in thin YEA films sandwiched between a glass slide and a cover slip (a modified Dalmau-plate method; Sipiczki, 2011) (Fig. 1e,f). On older parts of the hyphae, blastoconidia were formed which then divided by budding and established satellite yeast colonies along the hyphae (Fig. 1g). In the liquid medium YEL (YEA without agar), budding yeasts and pseudohyphae of irregular shape and size were observed. Blastoconidia were also formed on the pseudohyphae (Fig. 1d). Similar morphological transitions have been observed in many other dimorphic species (Kurtzman *et al.*, 2011).

For molecular analysis, genomic DNA was extracted from overnight cultures of three Luang Prabang isolates and one Vientiane isolate grown in YEL broth as described previously (Sipiczki, 2003). The purified DNA was used for the amplification of the D1/D2 domains of the large subunit (LSU) rRNA genes of the isolates with the primers NL-1 and NL-4 (O'Donnell, 1993). The amplified DNA was purified and sequenced using the amplification primers. The D1/D2 sequences of the isolates were identical. The sequences of the ITS1-5.8S-ITS2 regions and the small subunit (SSU) rRNA genes of one isolate from Luang Prabang (11-1006^T) and one isolate from Vientiane (11-516) were also determined and found identical. The primers used for amplification and sequencing were ITS1 and ITS4 for the ITS regions

(White *et al.*, 1990) and Fungi-18S-up and ITS4 for the 18S rRNA gene (Sipiczki & Kajdacsi, 2009). The results of the sequence comparisons indicated that the isolates were conspecific. The GenBank accession number of the ITS1-5.8S-ITS2 sequence of 11-1006^T is JX515976, the other accession numbers are listed in Table 1S.

The MEGABLAST similarity search with these sequences in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) found no identical sequences. The most similar D1/D2 sequence was from *Candida silvanorum* NRRL Y-7782 (U71068): 11% nucleotide difference (22 substitutions and 16 indels). Many D1/D2 sequences of taxonomically uncharacterized yeasts and strains of the *Danielozyma* (Kurtzman & Robnett, 2014), *Hyphopichia* (Groenewald & Smith, 2010; Limtong *et al.*, 2012), *Metschnikowia* (Lachance, 2011) and *Pichia* (Kurtzman, 2011b) clades as well as the *C. haemulonii* species complex (Cendejas-Bueno *et al.*, 2012) showed 82-88 % identity. The most similar ITS sequences (85-87 % identity) and 18S sequences (92-93 % identity) were also from species belonging to these clades or from taxonomically uncharacterized strains. The significant sequence differences indicated that the Laotian polymorphic isolates represented a hitherto undescribed novel yeast species.

Recently, a D1/D2 sequence (KC206086) was deposited in the database which showed 99% identity with those of the Laotian strains. The very strong similarity suggested that it was from a yeast most probably conspecific with the Laotian strains. This yeast (UFMG-CM-Y6070) was isolated from bark of the tree *Tapirira guianensis* (Anacardiaceae) collected in the Protected Ecological Reserve of Serra do Lajeado, in the city of Taquaruçu, state of Tocantins, Brazil in October 2011, together with the strain UFMG-CM-Y6069, which had an identical D1/D2 sequence. For yeast isolation, the bark samples were inoculated in tubes containing 15 ml of Yeast Nitrogen Base (YNB, Difco, USA) supplemented with 1% raffinose, 8% ethanol and 0.02% chloramphenicol, as described by Sampaio & Gonçalves

(2008). DNA extraction, PCR reactions and sequencing of the D1/D2 domains were done as described by Safar *et al.* (2013). Both Brazilian strains produced both pseudohyphae and mycelium. The ITS1-5.8S-ITS2 sequence (KP262069) of UFMG-CM-Y6070 differed from that of 11-1006^T at 10 positions which is close to the average intraspecific variability (2.51% with a standard deviation of 4.57) determined by Nilsson et al. (2008) for fungi but higher than the usual variability within ascomycetous yeast species (e.g. Chen *et al.*, 2001; Kurtzman, 2012). To further examine the relationship of 11-1006^T and UFMG-CM-Y6070, we amplified and sequenced regions of their genes coding for actin (*ACT1*), the RNA polymerase II (*RPB2*) and the translation elongation factor 1-alpha (*TEF1*) using the primer pairs CA1 and CA5R (for *ACT1*), RPB2-6F and fRPB2-7cR (for *RPB2*) and YTEF-1 and YTEF-6A (for *TEF1*) with the enzyme DreamTaq (Thermo) (Kann, 1993; Kurtzman & Robnett, 2003). The amplification parameters were: initial denaturation step at 95°C for 5 min, 30 cycles at 95°C for 50 s, 55°C for 50 s, 72°C for 70 s and a final elongation step at 72°C for 5 min. The same primers were used for sequencing the amplified fragments (see Table 1S for GenBank accession numbers). The differences found in their blast2 alignments (1 substitution and 1 indel for *ACT1*, 2 substitutions for *RPB2*, and 10 substitutions and 1 indel for *TEF1*) confirmed the close relationship detected between their D1/D2 domains.

Both the Laotian and the Brazilian groups of isolates were tested for physiological properties and sporulation using standard taxonomic methods (van der Walt & Yarrow, 1984) and found to differ in numerous traits from the type strain of *C. silvanorum*, the most closely related species in terms of rDNA sequence similarity (Table 2). No variability was detected among the isolates. Mating and sporulation was tested both in pure cultures and in mixed cultures with other strains by cultivation on acetate agar, malt-extract agar and corn-meal agar at 17 °C and 25 °C for 4 weeks. Neither mating nor sexual sporulation was observed in the cultures.

To determine the phylogenetic position of the strains of the new species, phylogenetic analyses were carried out with the D1/D2 domain sequences of strains 11-1006^T (JX515975), UFMG-CM-Y6070 (KC206086) and the type strains of species of related genera. Sequences which did not overlap the entire variable regions of the domain (Sipiczki et al., 2013) were not involved in the analysis. For multiple alignment of sequences, the CLUSTAL W 1.7 (Thompson *et al.,* 1994) and the MAFT version 6 (Katoh & Toh, 2008) algorithms were used. After the first alignment, the overhangs of the sequences that did not overlap with all other sequences were removed and a new alignment was produced for the phylogenetic analysis. The alignments were then analysed with Bayesian (Mr Bayes 3.2: Ronquist *et al.,* 2012), maximum-likelihood (PHYML 3.0: Guindon *et al.,* 2010), neighbour-joining, and maximum parsimony (PHYLIP version 3.67 software package: Felsenstein, 2007) methods. The Bayesian tree was generated with the General-Time-Reversible (GTR) substitution model for nucleotide sequences (Saccone *et al.,* 1990) and gamma-shaped rate variation with a proportion of invariable sites. The MCMC processes were set so that four chains were run simultaneously for 3,000,000 generations. The average standard deviation of split frequencies was: 0.004469, indicating a convergence. Bayesian posterior probability of the branches was estimated from 1937 trees. In the maximum-likelihood analysis, settings were made according to the best model suggested by the Akaike Information Criterion (AIC) in jModelTest version 2.0.2 (Posada, 2008). In the neighbour-joining analysis, the F84 model of nucleotide substitutions (Felsenstein & Churchill, 1996) was used for computing distance matrices. Confidence limits for this and the parsimony analysis were estimated by bootstrapping based on 1000 replications using the SEQBOOT and CONSENCE (majority-rule) programmes of the PHYLIP package. Trees were visualized with the TreeView (Page, 1996) and FigTree (http://tree.bio.ed.ac.uk/) programmes.

In all analyses, the Laotian and Brazilian strains shared a branch clearly separated from the type strains of all species whose strains were identified in the database search as having similar D1/D2 sequences, confirming that they constitute a distinct species. All methods identified *C. silvanorum* as their closest relative and placed them close to the genera *Hyphopichia, Danielozyma* and *Metschnikowia* (the PhyML tree is shown in Fig. 1S).

In all trees the joint branch of the new strains and the *C. silvanorum* type strain separated from the *Hyphopichia* lineage, but the statistical support of this node was always very weak. Hence, we conducted an analysis with more chromosomal regions of representatives of a broader spectrum of genera. For multilocus tree inference we concatenated D1/D2, 18S SSU (small subunit rRNA), *ACT1* (coding for actin) and *TEF1* (coding for translation elongation factor 1-alpha) gene sequences. As such sequences were not available for all related type strains, we first sequenced their missing genes (Table 1S) using the primers and methods described above and in Kurtzman and Robnett (2003). Sequence alignment and tree inference were performed as described above. The analysis of the concatenated sequences placed 11-1006^T and UFMG-CM-Y6070 near *Hyphopichia* (Fig. 2) on a well-separated branch with strong statistical support. The 11-1006^T sequences differed in Blast alignments from the corresponding sequences of the type strain (CBS 2352) of *H. burtonii*, the type species of *Hyphopichia* at 35 (D1/D2), 84 (SSU rRNS), 78 (*ACT1*), 107 (*TEF1*), and 121 (*RPB2*) positions. Within the ITS1-5.8S-ITS2 segment, similarity (95%) was detected only in the 5.8S gene. These results indicate that the Laotian and Brazilian strains represent a novel species of a novel genus. To accommodate them in the taxonomic system of yeasts, we propose the new genus name *Metahyphopichia* gen. nov. and the species name *Metahyphopichia laotica* sp. nov. which refers to the geographical location of the site, from where the type strain (11-1006^T) was isolated.

194 *M. laotica* is a morphologically variable yeast like its closest relative, the dimorphic
195 *C. silvanorum* originally identified in beetle infestations (van der Walt *et al.*, 1971) and
196 numerous *Hyphopichia* species (Kurtzman, 2011a; Limtong et al., 2012). It can switch from
197 yeast morphology to filamentous morphology, and its hyphae penetrate into solid substrates
198 where they establish satellite yeast colonies during their extension. A similar colonizing
199 strategy was recently observed in a *Pichia* species (Sipiczki, 2013). It is likely that other
200 dimorphic *Pichia* and *Hyphopichia* species also make use of morphological transitions for
201 more effective colonization of solid and semisolid substrates.
202 The occurrence of *M. laotica* associated with flowers and tree barks suggests that these
203 substrates could be its ecological niche. Probably, insects that visit these substrates are the
204 vectors of this new yeast species. Several recently described yeast species, such as *C.*
205 *golubevii*, *Moniliella fonsecae*, *Saccharomycopsis fodiens* and *Kodamaea transpacifica*, are
206 reported to occur in South America and Asia (Rosa et al., 2009, 2010; Lachance et al., 2012;
207 Freitas et al., 2013). Freitas et al. (2013) suggesting that the dispersion of some of these
208 species may be linked to the activity of ancient human populations. The occurrence of *M.*
209 *laotica* in Asia and South America could also be linked to the dispersion of plants with their
210 indigenous microbiota by these ancient populations, however, this hypothesis needs further
211 studies to be proven.
212
213 **Description of *Metahyphopichia* Sipiczki & Pfliegler gen. nov.**
214
215 *Metahyphopichia* (Me.ta.hy.pho.pi'chi.a. Gr. prep. meta, close by; N.L. fem. n. *Hyphopichia* a
216 fungal genus; N.L. fem. n. *Metahyphopichia*, indicating that this genus occurs on the
217 phylogenetic trees adjacent to the *Hyphopichia* clade).

The genus is phylogenetically related to the genera *Hyphopichia* and *Danielozyma*. Colonies are polymorphic with initial yeast growth. Yeast cells divide by multilateral budding. Among the yeast cells, pseudohyphae of irregular shape are frequently formed which produce lateral and terminal blastoconidia. Invasive, branching septate hyphae are developed below the yeast colonies. The hyphae can produce lateral blastoconidia that establish satellite yeast colonies.

The type species is *Metahyphopichia laotica* Sipiczki, Pfliegler, Safar, Morais & Rosa

Description of *Metahyphopichia laotica* Sipiczki, Pfliegler, Safar, Morais & Rosa sp. nov.

Metahyphopichia laotica (la.o'ti.ca N.L. nom.fem. adj. *laotica* pertaining to Laos from where the type strain was isolated).

In the liquid medium YEL, after 2 days of incubation at 25°C, cells are round to long oval, 1-3 x 1.5-4.5 μm, occur singly or in pairs and propagate by budding (Fig. 1c). Surface ring and sparse sediment are present. On YEA, after 1 month at 25 °C, the colonies are white to cream coloured, with venose to wrinkled surface and eroded margin but also with smoother sectors (Fig. 1a,b). Pseudohyphae consisting of irregularly elongated and curved cells are produced both in liquid and on solid media (Fig. 1c,d). Invasive mycelium is formed in the solid medium under and around the yeast colonies. In thin films of YEA sandwiched between glass slides (modified Dalmou plates), elaborate branching mycelium (Fig. 1e,f) of septate hyphae and pseudohyphae is formed. Ovoid to elongate (2-3 x 3-6 μm) blastoconidia develop on the hyphae. The blastoconidia propagate by budding and establish yeast colonies along the extending hypha (Fig. 1g). No ascospores are produced on YEA, acetate agar, malt-extract

agar or corn-meal agar (for the description of media, see van der Walt & Yarrow, 1984) after 4 weeks of incubation at 17 °C and 25 °C. For description of the biochemical and physiological characteristics, see Table 2. *M. laotica* differs from the most closely related species in numerous properties which allow their differentiation by conventional taxonomic tests.

The type strain is 11-1006^T, isolated from fallen flower in Luang Prabang, Laos. It has been deposited in the culture collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 13022^T (= CCY 092-001-001^T = NCAIM Y.02126^T).

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References

Cendejas-Bueno, E., Kolecka, A., Alastruey-Izquierdo, A., Theelen, B., Groenewald, M., Kostrzewa, M., Cuenca-Estrella, M., Gómez-López, A. & Boekhout T. (2012)

Reclassification of the *Candida haemulonii* complex as *Candida haemulonii* (*C. haemulonii*

Group I), *C. duobushaemulonii* sp. nov. (*C. haemulonii* Group II), and *C. haemulonii* var. *vulnera* var. nov.: three multiresistant human pathogenic yeasts. *J Clin Microbiol* **50**, 3641–3651.

Chen, Y-C, Eisner, J. D., Kattar, M. M., Rassoulian-Barrett, S. L., Lafe, K., Bui, U., Limaye, A. P. & Cookson, B. (2001) Polymorphic internal transcribed spacer region 1 DNA sequences identify medically important yeasts. *J Clin Microbiol* **39**, 4042–4051.

Felsenstein, J. (2007) PHYLIP (phylogeny inference package), version 3.67. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA

Felsenstein, J. & Churchill, G. A. (1996) A Hidden Markov Model approach to variation among sites in rate of evolution. *Mol Biol Evol* **13**, 93-104.

Freitas, L. F., Carvajal Barriga, E. J., Barahona, P. P., Lachance, M.-A. & Rosa, C. A. (2013). *Kodamaea transpacifica* f.a., sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galapagos Islands and Malaysia: futher evidence for ancient human transpacific contacts. *Int J Syst Evol Microbiol* **63**, 4324-4329.

Gancedo, J. M. (2001) Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* **25**, 107-123.

Groenewald, M. & Smith, M. T. (2010) Re-examination of strains formerly assigned to *Hyphopichia burtonii*, the phylogeny of the genus *Hyphopichia*, and the description of *Hyphopichia pseudoburtonii* sp. nov. *Int J Syst Evol Microbiol* **60**, 2675–2680.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *System Biol* **59**, 307-321.

Han, T.-L., Cannon, R. D & Villas-Bôas, S. G. (2011) The metabolic basis of *Candida albicans* morphogenesis and quorum sensing. *Fungal Genet Biol* **48,** 747–763.

Kann, V. L. (1993) Polymerase chain reaction for the diagnosis of candidemia. *J Infect Dis* **168**, 779–783.

Katoh, K. & Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* **9,** 286-298.

Kurtzman, C. P. (2011a) *Hyphopichia* von Arx & van der Walt (1976). In The Yeasts: a Taxonomic Study, 5th edn, vol. 1, pp. 435–438. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

Kurtzman, C. P. (2011b) Phylogeny of the ascomycetous yeasts and the renaming of *Pichia anomala* to *Wickerhamomyces anomalus*. *Antonie van Leeuwenhoek* **99**, 13-23.

Kurtzman, C. P. (2012) *Citeromyces hawaiiensis* sp. nov., an ascosporic yeast associated with *Myoporum sandwicense*. *Int J Syst Evol Microbiol* **62,** 1215–1219.

Kurtzman, C. P., Fell, J. W. & Boekhout, T. (2011) The yeasts: a taxonomic study. Elsevier, Amsterdam.

Kurtzman, C. P. & Robnett, C. J. (2003) Phylogenetic relationships among yeasts of the *'Saccharomyces* complex' determined from multigene sequence analyses. *FEMS Yeast Res* **3**, 417-432.

Kurtzman, C. P. & Robnett, C. J. (2014) Three new anascosporic genera of the Saccharomycotina: *Danielozyma* gen. nov., *Deakozyma* gen. nov. and *Middelhovenomyces* gen. nov. *Antonie van Leeuwenhoek* **105**, 933–942.

Lachance, M.-A. (2011). *Metschnikowia* Kamienski. In The Yeasts: a Taxonomic Study, 5th edn, vol. 1, pp. 575–620. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier.

Lachance, M.-A., Rosa, C. A., Carvajal, E. J., Freitas, L. F. & Bowles, J. M. (2012). *Saccharomycopsis fodiens* sp. nov.,a rare predacious yeast from three distant localities. *Int J Syst Evol Microbiol* **62**, 2793-2798.

Limtong, S., Kaewwichian, R., Jindamorakot, S., Yongmanitchai, W. & Nakase, T. (2012) *Candida wangnamkhiaoensis* sp. nov., an anamorphic yeast species in the *Hyphopichia* clade isolated in Thailand. *Antonie van Leeuwenhoek,* **102**, 23-28.

Nagy, L. G., Ohm, R. A., Kovacs, G. M., Floudas, D., Riley, R., Gacser, A., Sipiczki, M., Davis, J. M., Doty, S. L., de Hoog, G. S., Lang, B. F., Spatafora, J. W., Martin, F. M., Grigoriev, I. V. & Hibbett, D. S. (2014) Latent homology and convergent regulatory evolution underlies the repeated emergence of yeasts. *Nat Commun* **5**, 4471.

Nemecek, J. C., Wüthrich, M. & Klein, B. S. (2006) Global control of dimorphism and virulence in fungi. *Science* **312**, 583–588.

Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K.-H. (2008) Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evolutionary Bioinformatics **4**, 193-201

O'Donnell, K. (1993) *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*, pp. 225-233. Edited by D. R. Reynolds & J. W. Taylor. Wallingford, UK: CAB International.

Page, R. D. M. (1996) TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357-358.

Posada, D. (2008) jModelTest: Phylogenetic model averaging. *Mol Biol Evol* **25**, 1253–1256.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A. & Huelsenbeck, J. P. (2012) MrBayes 3.2: Efficient Bayesian

phylogenetic inference and model choice across a large model space. *System Biol* **61**, 539–542.

Rosa, C. A., Jindamorakot, S., Limtong, S., Nakase, T., Lachance, M.-A., Fidalgo-Jiménez, A., Daniel, H. M., Pagnocca, F. C., Inácio, J. & Morais, P. B. (2009). Synonymy of the yeast genera *Moniliella* and *Trichosporonoides* and proposal of *Moniliella fonsecae* sp. nov. and five new species combinations. *Int J Syst Evol Microbiol* **59**, 425-429.

Rosa, C. A., Jindamorakot, S., Limtong, S., Nakase, T., Pagnocca, F. C. & Lachance, M.-A. (2010). *Candida golubevii* sp. nov., na asexual yeast related to *Metschnikowia* clade. *Int J Syst Evol Microbiol* **60**, 704-706.

Saccone, C., Lanave, C., Pesole, G. & Preparata, G. (1990) Influence of base composition on quantitative estimates of gene evolution. *Methods Enzymol* **183**, 570–583.

Safar, S. V. B., Gomes, F. C. O., Marques, A. R., Lachance, M.-A. & Rosa, C. A. (2013) *Kazachstania rupicola* sp. nov., a yeast species isolated from water tanks of a bromeliad in Brazil. *Int J Syst Evol Microbiol* **63**, 1165-1168.

Sampaio, J. P. & Gonçalves, P. (2008) Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus. Appl Environ Microbiol* **74**, 2144–2152.

Sipiczki, M. (2003) *Candida zemplinina* sp. nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines. *Int J Syst Evol Microbiol* **53**, 2079-2083.

Sipiczki, M. (2011) Dimorphic cycle in *Candida citri* sp. nov., a novel yeast species isolated from rotting fruit in Borneo. *FEMS Yeast Res* **11,** 202-208.

Sipiczki, M. (2012) *Pichia bruneiensis* sp. nov., a biofilm-producing dimorphic yeast species isolated from flowers in Borneo. *Int J Syst Evol Microbiol* **62**, 3099-3104.

Sipiczki, M. (2013) Detection of yeast species also occurring in substrates associated with animals and identification of a novel dimorphic species in *Verbascum* flowers from Georgia. *Antonie van Leeuwenhoek* **103**, 567-576.

Sipiczki, M. & Kajdacsi, E. (2009) *Jaminaea angkoriensis* gen. nov., sp. nov., a novel anamorphic fungus containing an S943 nuclear small subunit rRNA group IB intron represents a basal branch of Microstromatales. *Int J Syst Evol Microbiol* **59**, 914-920.

Sipiczki, M., Takeo, K., Yamaguchi, M., Yoshida, S. & Miklos, I. (1998) Environmentally controlled dimorphic cycle in a fission yeast. Microbiol-UK **144**, 1319-1330.

Sipiczki, M., Pfliegler, W.P. & Holb, I. J. (2013) *Metschnikowia* species share a pool of diverse rRNA genes differing in regions that determine hairpin-loop structures and evolve by reticulation. PLoS One, **8,** e67384.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22,** 4673-4680.

van der Walt, J. P., Scott, D. B. & van der Klift, W. C. (1971) Four new, related *Candida* species from South African insect sources. *Antonie van Leeuwenhoek* **37**, 449-460.

van der Walt, J. P. & Yarrow, D. (1984) Methods for the isolation, maintenance, classification and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 3rd edn, pp 45-104. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.

van Uden, N. & Kolipinski, M. C. (1962) *Torulopsis haemulonii* nov. spec. a yeast from the Atlantic ocean. *Antonie van Leeuwenhoek* **28**, 78-80.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols. A Guide to Methods and*

384 *Applications*, pp. 315-322. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White.

385 San Diego, CA: Acadamic Press.

386

387 **Table 1.** List of strains

Strain	Substrate from which the strain was isolated	Location of sample collection	Date of sample collection
11-516	Fallen Dok Champa [*Plumeria alba*] flower	Ventiane, Laos	2008
11-1006^T	Fallen flowers of uncertain origin	Luang Prabang, Laos	2008
12-511	Fallen flowers of uncertain origin	Luang Prabang, Laos	2008
12-777	Fallen flowers of uncertain origin	Luang Prabang, Laos	2008
UFMG-CM-Y6070	Bark of the tree *Tapirira guianensis*	Protected Ecological Reserve of Serra do Lajeado, Taquaruçu, Tocantins state, Brazil	2011
UFMG-CM-Y6070	Bark of the tree *Tapirira guianensis*	Protected Ecological Reserve of Serra do Lajeado, Taquaruçu, Tocantins state, Brazil	2011

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389
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Table 2. Physiological properties of *Metahyphopichia laotica* strains 11-1006^T and UFMG-CM-Y6070. Comparison with *Candida silvanorum* CBS 6274^T. All strains are positive for fermentation of D-glucose; assimilation of D-glucose, D-galactose, D-ribose, D-xylose, L-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl-α-D-glucoside, cellobiose, salicin, arbutin, raffinose, melezitose, glycerol, meso-erythritol, ribitol, xylitol, D-glucitol, D-mannitol, succinate, L-lysine and growth at 50% glucose, 25 °C and 30 °C. All strains are negative for fermentation of lactose and melezitose; assimilation of D-glucosamine (as carbon source), lactose, galactitol, myo-inositol, D-glucuronate, methanol, propane-1,2-diol, butane-2,3-diol, nitrite, imidazole and growth at 0.01% cycloheximide, 1% acetic acid and 60% glucose. No physiological differences were detected among the Laotian strains and between the Brazilian strains. +, growth; -, no growth; v, variable; w, weak growth; d, delayed growth; ^{T}, type strain.

Property	*Metahyphopichia laotica* 11-1006^T	*Metahyphopichia laotica* UFMG-CM-6070	*Candida silvanorum* CBS 6274^{T,*}
Fermentation of carbon sources			
D-Galactose	+	+	w
Maltose	-	-	w
Sucrose	-	-	w
Trehalose	+	+	w
Melibiose	-	-	w
Cellobiose	-	-	-
Raffinose	w	w	w
Assimilation of carbon compounds			
L-Sorbose	-	-	d
D-Arabinose	w	-	d
Melibiose	-	-	+
Inulin	-	-	-
Starch	w	w	+
L-Arabinitol	w	w	+
Citrate	+	+	+
Ethanol	-	-	+
Quinic acid	+	+	-
Assimilation of nitrogen compounds			
Nitrate	w	-	-

Ethylamine	-	-	+
Cadaverine	-	-	+
Creatine	+	+	-
Creatinine	+	+	-
Glucosamine	+	+	-
D-Tryptophan	+	+	-
Other tests			
Growth in vitamin-free medium	v	w	-
Acid production	+	+	-
37 °C	-	w	+

* CBS (Centraalbureau voor Schimmelcultures):

http://www.cbs.knaw.nl/collections/BioloMICS.aspx and Barnett *et al.* (1990)

410 (legends)

411

412 **Fig. 1.** Morphology of *Metahyphopichia laotica*. Colony morphology of strains (a) 11-1006^T
413 and (b) 11-516 on YEA after incubation for 1 month at 25 °C. (c) Yeast cells and
414 pseudohyphae in an overnight culture of 11-1006^T growing in YEL at 25 °C. (d) A
415 pseudohypha of 11-1006^T with a blastoconidium (star) and scars (arrows), where conidia were
416 developed. (e) Formation of mycelium in YEA film sandwiched between a glass slide and a
417 cover slip. (f) Branching hyphae in the mycelium. (g) Blastoconidia (arrow) and satellite yeast
418 colonies formed along a hypha. Bar, 5 μm for (c) and (d), 20 μm for (f) and 25 μm for (g).

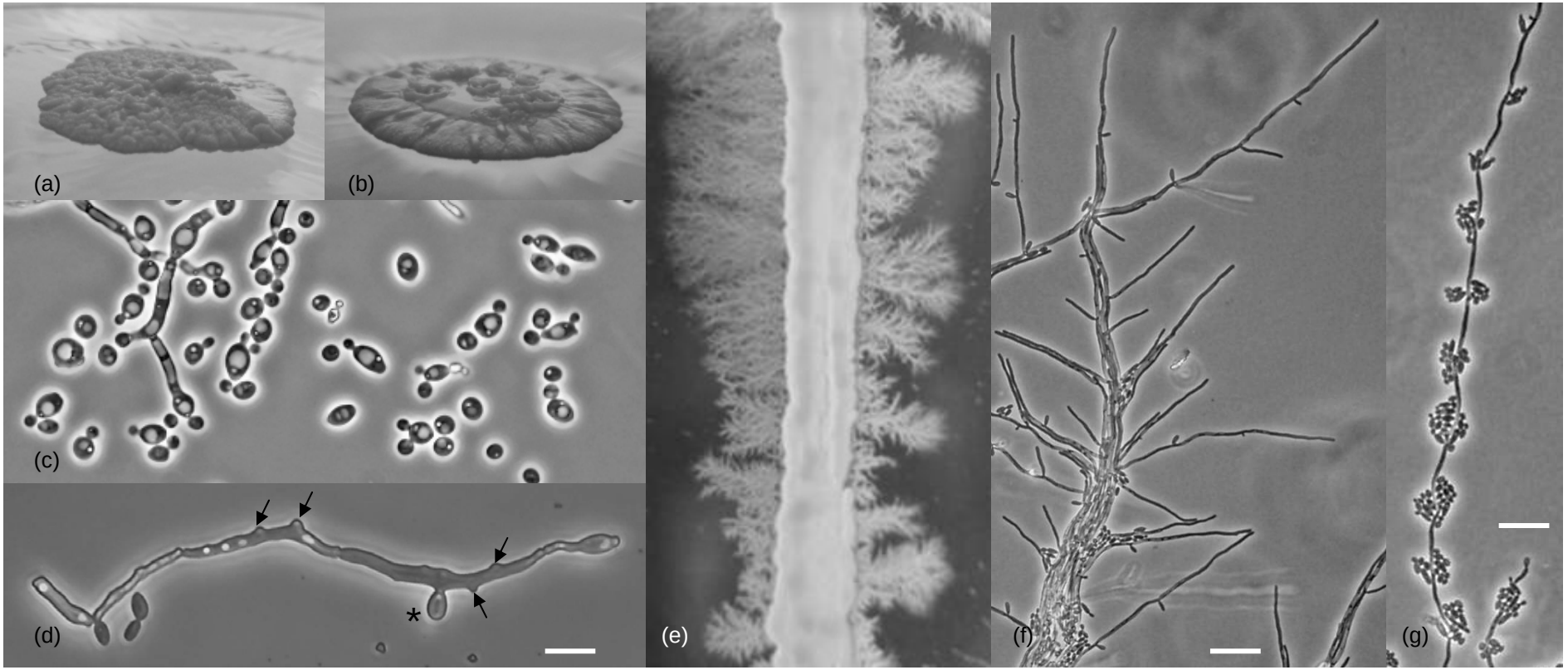
419

420 **Fig. 2.** Phylogenetic relationships of *Metahyphopichia laotica* 11-1006^T and UFMG CM-
421 Y6070 with related species and genera determined from Bayesian analysis of concatenated
422 chromosomal sequences of SSU rRNA, D1/D2 domains of LSU rRNA, *ACT1* and *TEF1*. The
423 type strain of *Schizosaccharomyces pombe* was the outgroup in the analysis. Posterior
424 probability values are given at branch nodes. See Table 1S for sequence accession numbers.
425 For concatenation, Clustal X alignments were prepared for each gene separately and the
426 terminal regions not overlapping with the shortest sequence were removed from all
427 sequences. The shortened sequences were then concatenated and aligned with Clustal X. This
428 alignment was used in the Bayesian analysis.

429

430

(Fig. 1)



(Fig. 2)

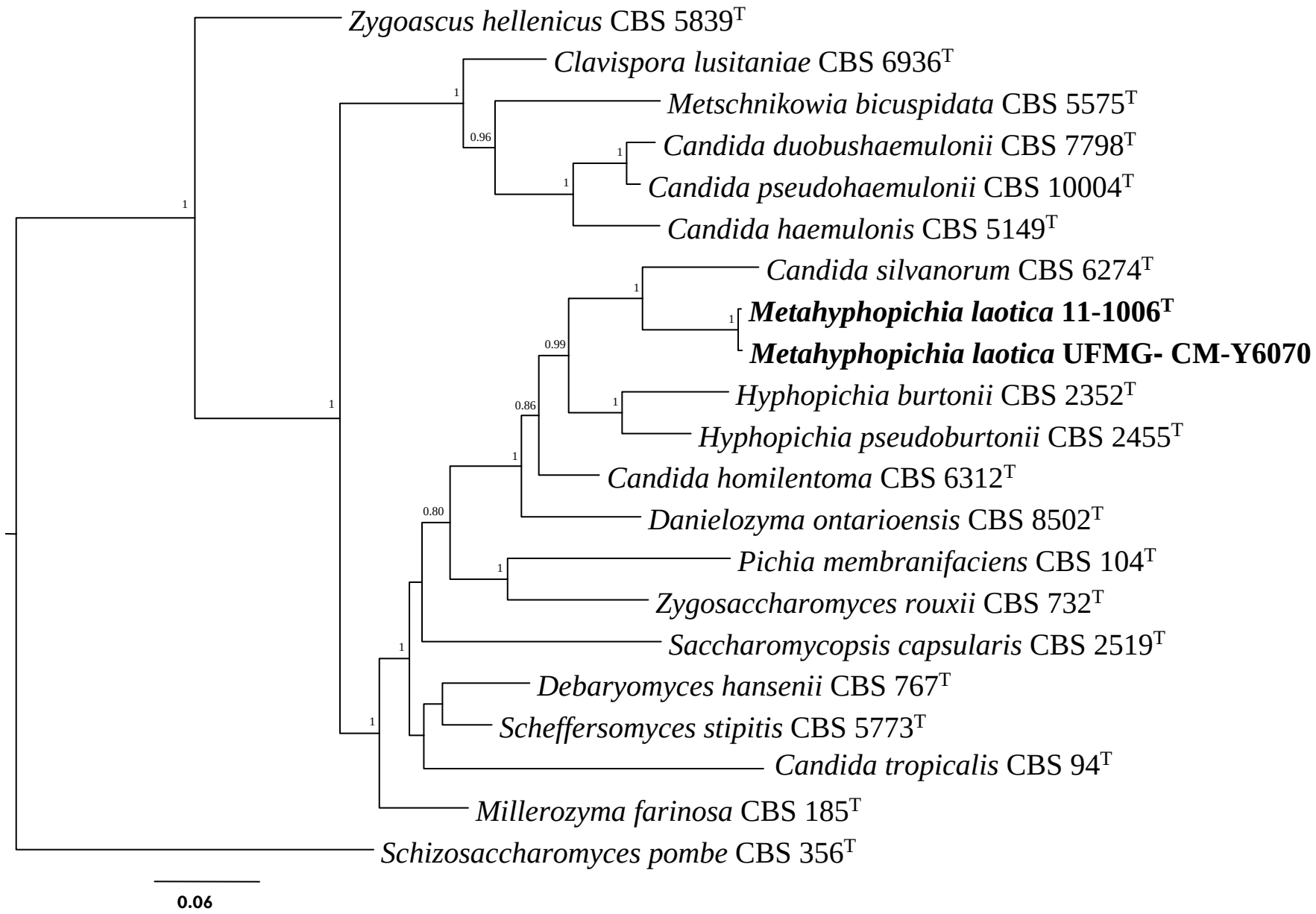


Table 1S. Accession numbers of sequences

Species	CBS*	SSU†	LSU†	*ACT1*†	*TEF1*†
Candidi duobushaemulonii	7798^T	KU557488	JX459765	AJ508472	KU746855
Candida haemulonis	5149^T	AB013572	U44812	KU705473	KU705474
Candida homilentoma	6312^T	AB018166	U45716	KU728670	KU841443
Candida pseudohaemulonii	10004^T	KU570385	AB118792	KU841444	KU841445
Candida silvanorum	6274^T	AB018174	U71068	KU728669	KU728668
Candida tropicalis	94^T	EU348785	U45749	AJ508499	AY497660
Clavispora lusitaniae	6936^T	JQ689030	JQ698900	AJ389065	JQ699057
Danielozyma ontarioensis	8502^T	AY500849	AF017244	KU746856	KF964132
Debaromyces hansenii	767^T	JQ698910	JQ689041	AJ508505	JQ699068
Hyphopichia burtonii	2352^T	AB018177	U45712	AJ508512	KU609071
Hyphopichia pseudoburtonii	2455^T	KU557487	GQ389650	KU609072	KU705472
Metahyphopichia laotica 11-1006^T	13022^T	JX515977	JX515975	KM986114	KM986115
Metahyphopichia laotica UFMG-CM-Y6070		KU609070	KC206086	KP316405	KP316406
Metschnikowia bicuspidata	5575^T	JQ698902	U44822	AJ745130	FJ238407
Millerozyma farinosa	185^T	AB054281	JQ689046	AJ508514	JQ699073
Pichia membranefaciens	107^T	JQ698896	EF550227	AJ389088	JQ699053
Saccharomycopsis capsularis	2519^T	JQ698884	JQ689010	AJ389092	JQ699034
Scheffersomyces stipitis	5773^T	Q698912	JQ689044	AJ508520	JQ699071
Schizosaccharomyces pombe	356^T	JQ698936	AY048171	Y00447	EF552572
Zygoascus hellenicus	5839^T	GU597328	JQ689060	AJ508498	GU597340
Zygosaccharomyces rouxii	732^T	X90758	JQ689016	AJ878414	JQ699040

*Strain number in the culture collection Centralbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands

†Gene sequences. SSU, nuclear small subunit rRNA gene; LSU, D1/D2 domain of the nuclear large subunit rRNA gene; *ACT1*, actin gene; *TEF1*, translation elongation factor 1-α gene.

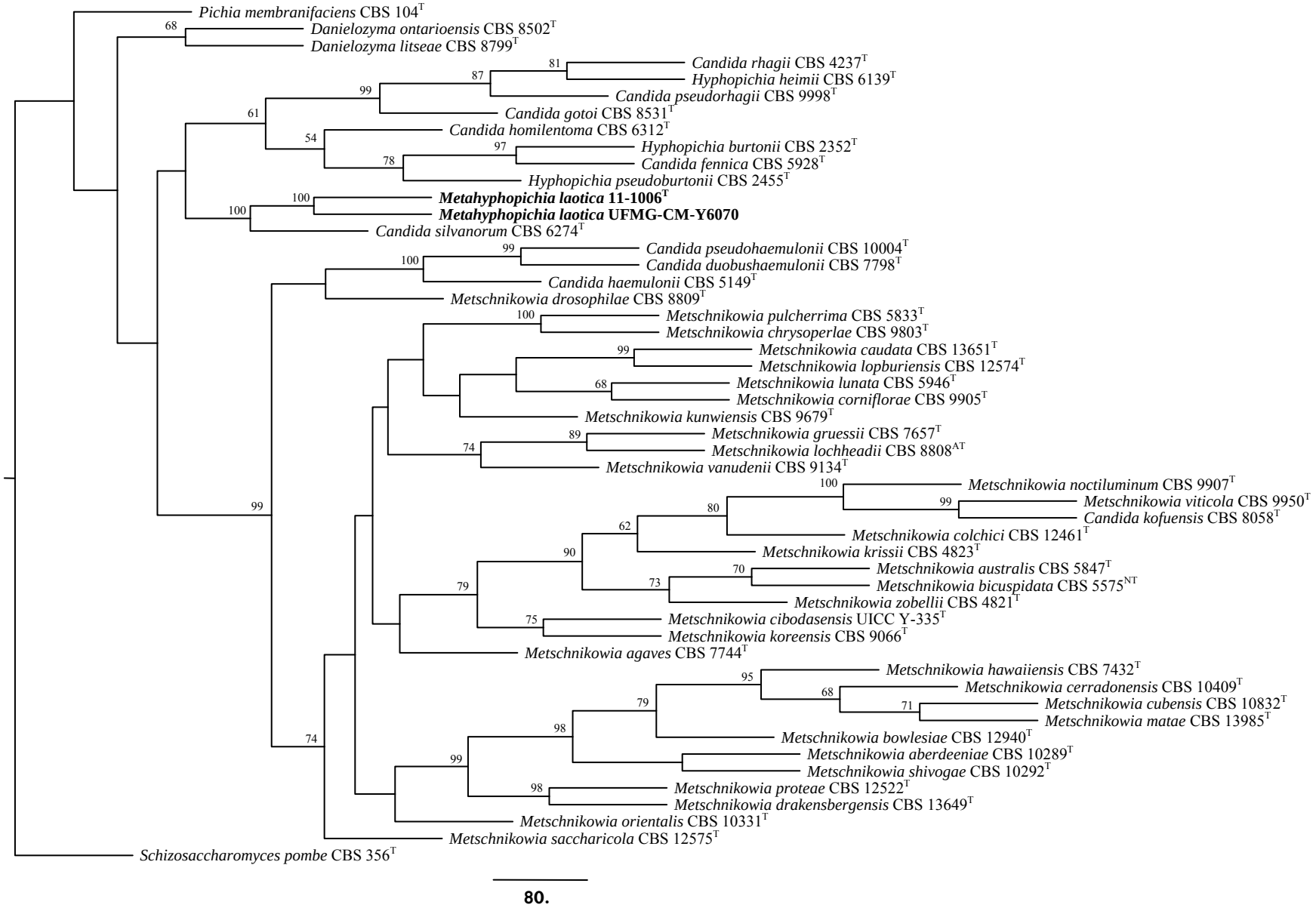


Fig. 1S. A phylogenetic tree based on the PhyML analysis of the sequences of the D1/D2 domains of the large subunit rRNA genes. Bootstrap values >50% based on 1000 resamplings are shown at branch nodes. Outgroup: *Schizosaccharomyces pombe*. GenBank accession numbers of the sequences are shown in brackets.