Five new additions to the genus *Spathaspora* (Saccharomycetales, Debaryomycetaceae) from southwest China

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Abstract

*Spathaspora* is an important genus of d-xylose-fermenting yeasts that are poorly studied in China. During recent yeast collections in Yunnan Province in China, 13 isolates of *Spathaspora* were obtained from rotting wood and all represent undescribed taxa. Based on morphological and phylogenetic analyses (ITS and nuc 28S), five new species are proposed: *Spathaspora elongata*, *Sp. mengyangensis*, *Sp. jiuxiensis*, *Sp. para-jiuxiensis* and *Sp. rosaee*. Our results indicate a high species diversity of *Spathaspora* waiting to be discovered in rotting wood from tropical and subtropical southwest China. In addition, the two *Candida* species, *C. jeffriesii* and *C. materiae*, which are members of the *Spathaspora* clade based on phylogeny, are transferred to *Spathaspora* as new combinations.

Keywords

Five new species, Debaryomycetaceae, Saccharomycetales, yeast taxonomy, d-xylose-fermenting yeast

Introduction

*Spathaspora* N.H. Nguyen, S.O. Suh & M. Blackw (2006) (Saccharomycetales, Debaryomycetaceae) was introduced, based on a single species, *Spathaspora passalidarum*, which was isolated from a passalid beetle in Louisiana, USA (Nguyen et al. 2006). This species produces asci containing elongate ascospores with curved ends, a unique trait of this
genus (Nguyen et al. 2006; Nguyen et al. 2011). Subsequently, *Spathaspora arborariae*, *S. boniae*, *S. brasiliensis*, *S. girioi*, *S. gorwiae*, *S. hagerdaliae*, *S. piracicabensis*, *S. roraimanensis*, *S. subii* and *S. xylofermentans* were introduced. These were from rotting wood (Cadete et al. 2009, 2013; Lopes et al. 2016; Morais et al. 2017; Varize et al. 2018) and *S. allomyrinae* from insects (Wang et al. 2016). *Spathaspora* has been shown to be a polyphyletic group, containing members placed throughout the larger *Spathaspora/Candida albicans/Lodderomyces* clade of Debaryomycetaceae (Morais et al. 2017; Varize et al. 2018). Several *Candida* species, such as *C. blackwellae*, *C. jeffriesii*, *C. lyxosophila*, *C. materiae*, *C. parablackwellae* and *C. subhashii*, are closely related to *Spathaspora*, based on a phylogenetic analysis of the D1/D2 domain of the nuclear 28S rDNA (nuc 28S) sequences (Cadete et al. 2013; Daniel et al. 2014; Morais et al. 2017; Varize et al. 2018).

Most species of *Spathaspora*, including *S. arborariae*, *S. brasiliensis*, *S. passalidarum*, *S. roraimanensis*, *S. subii* and *S. xylofermentans*, are economically important because of their ability to ferment d-xylose, the second most abundant sugar in lignocellulosic feedstocks (Nguyen et al. 2011; Cadete et al. 2013; Lopes et al. 2016; Morais et al. 2017). These xylose-fermenting species can be used directly for ethanol production or may provide a source of genes, enzymes and/or sugar transporters to engineer industrial strains for the efficient production of bioethanol from renewable biomass (Wohlbach et al. 2011; Cadete et al. 2013).

*Spathaspora* species are associated with rotting-wood substrates and the insects that occupy this ecological niche (Cadete et al. 2009, 2013; Nguyen et al. 2011; Lopes et al. 2016; Wang et al. 2016; Morais et al. 2017; Varize et al. 2018). They can be found in tropical, subtropical and temperate regions on different continents, but most species are presently known from Brazilian regions (Cadete et al. 2009, 2013; Lopes et al. 2016; Morais et al. 2017; Varize et al. 2018). In China, the genus is underexplored with only three published reports of the species *Sp. allomyrinae*, *Sp. gorwiae* and *Sp. passalidarum* (Ren et al. 2013; Wang et al. 2016). Here, we describe five new species of *Spathaspora* discovered in tropical and subtropical areas of southwest China, based on their morphological characters and molecular phylogenetic analyses.

**Materials and methods**

**Sample collection and isolation**

Rotting wood samples were collected in two areas of Yunnan Province, China. The areas are located in the Xishuangbanna Primeval Forest Park of Jinghong (21°98′N, 100°88′E) and Jiuxi Mountain Forest Park of Honghe (24°40′N, 103°68′E). The predominant vegetation is characterised as a tropical and subtropical forest biome. The climate is hot and humid, with annual precipitation between 1,100 to 1,600 mm and an average temperature that ranges from 17.2 to 26.4 °C. Sixty decayed wood samples were collected, thirty from each area, during July to August in 2016–2018. The samples were stored in sterile plastic bags and transported under refrigeration to the
laboratory over a period of no more than 24 h. The yeast strains were isolated from rotting wood samples in accordance with the methods described by Lopes et al. (2016). Each sample (1 g) was added to 20 ml sterile d-xylose medium (yeast nitrogen base 0.67%, d-xylose 0.5%, chloramphenicol 0.02%, pH 5.0 ± 0.2) in a 150 ml Erlenmeyer flask and then cultured for 3–10 days on a rotary shaker. Subsequently, 0.1 ml aliquots of the enrichment culture and appropriate decimal dilutions were spread on d-xylose agar plates and then incubated at 25 °C for 3–4 days. Different yeast colony morphotypes were then isolated by repeated plating on yeast extract-malt extract (YM) agar (1% glucose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract, pH 5.0 ± 0.2) and stored on YM agar slants at 4 °C or in 15% glycerol at –80 °C.

Morphological, physiological and biochemical studies

Morphological and physiological properties were determined according to Kurtzman et al. (2011). Induction of the sexual stage was tested by incubating single or mixed cultures of each of the two strains on cornmeal (CM) agar, 5% malt extract (ME) agar, dilute (1:9 and 1:19) V8 agar or yeast carbon base plus 0.01% ammonium sulphate (YCBAS) agar at 25 °C for 2 months (Cadete et al. 2013; Lopes et al. 2016). Assimilation of carbon and nitrogen compounds and growth requirements were tested at 25 °C. The effects of temperature from 25–40 °C were examined in liquid culture and on agar plates. Ethanol was determined with alcohol oxidase (Sangon Biotech, China) and peroxidase (Sangon Biotech, China), as described previously (Alves et al. 2007).

DNA extraction, PCR amplification and nucleotide sequencing

Genomic DNA was extracted from the yeasts using the Ezup Column Yeast Genomic DNA Purification Kit, according to the manufacturer’s protocol (Sangon Biotech, China). The nuc rDNA ITS1-5.8S-ITS2 (ITS) region was amplified using the primer pair ITS1/ITS4 (White et al. 1990). The D1/D2 domain of the nuc 28S rDNA was amplified using the primer pair NL1/NL4 (Kurtzman and Robnett 1998). The following thermal profile was used to amplify the ITS and nuc 28S regions: an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C and 40 s at 72 °C, with a final extension of 10 min at 72 °C (Liu et al. 2016). PCR products were directly purified and sequenced by Sangon Biotech Inc. (Shanghai, China). We determined the identity and accuracy of the newly-obtained sequences by comparing them to sequences in GenBank and assembled them using BioEdit (Hall 1999). Newly-obtained sequences were then submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/; Table 1).

Phylogenetic analyses

The sequences obtained from this study and the reference sequences downloaded from GenBank (Table 1) were aligned using MAFFT v. 6 (Katoh and Toh 2010) and
Table 1. DNA sequences used in the molecular phylogenetic analysis. Entries in bold are newly generated in this study.

| Species                  | Strain          | ITS          | D1/D2       |
|--------------------------|-----------------|--------------|-------------|
| Candida albicans         | NRRL Y-12983T   | HQ876043     | U45776      |
| Candida argentae         | CBS 12358T      | JF682350/    | JF682353    |
| Candida batostannamensis | CBS 13915T      | KM586743     | KM586733    |
| Candida blackwallei      | CBS 10843T      | EU402940/    | EU402939    |
| Candida bohniensis       | NRRL Y-27737T   | FJ172255     | AY520317    |
| Candida buenavistaensis  | NRRL Y-27734T   | FJ623627     | AY242341    |
| Candida cetoiae          | CBS 12463T      | KC118129     | KC118128    |
| Candida chatuloides      | NRRL Y-27909T   | FJ623621     | DQ655678    |
| Candida coleopterorum    | CBS 14180T      | KU128707     | KU128722    |
| Candida corydalis        | NRRL Y-27910T   | FJ623622     | DQ655679    |
| Candida dubliniensis     | NRRL Y-17841T   | KY102055     | U57685      |
| Candida fujianensis      | NRRL Y-48060T   | EF658666     | EF120596    |
| Candida hyderabadensis   | NRRL Y-27953T   | AM180949     | AM159100    |
| Candida jefferesi        | CBS 9898T       | NR_111398    | NG_042498   |
| Candida jiangfengensis   | CBS 10846T      | EU402936     | EU402935    |
| Candida kantuleensis     | CBS 15219T      | LC317101     | LC317097    |
| Candida labiduridarum    | NRRL Y-27940T   | EF658664     | DQ655687    |
| Candida lyxosophila      | NRRL Y-17539T   | KY102184     | HQ263370    |
| Candida maltosa          | NRRL Y-17677T   | NR_138346    | U45745      |
| Candida margittii        | CBS 14175T      | KU128708     | KU128721    |
| Candida materiae         | CBS 10975T      | FI154790     | FI154790    |
| Candida metapsilosis     | CBS 10907T      | FJ872019     | DQ213057    |
| Candida monoketiae       | NRBC 10509T     | AB696987     | DQ400364    |
| Candida meandricana      | NRRL Y-27057T   | EF658662     | AF245404    |
| Candida orthopolaris     | NRRL Y-2317T    | AJ359374/    | U45793      |
| Candida parablackwelliae| NYNU 17776T     | MG255731     | MG255702    |
| Candida paraparvuloides  | CBS 13928T      | KP054272     | KP054271    |
| Candida parapsilosis     | NRRL Y-12969T   | AJ635316     | U45754      |
| Candida pseudofujengensis| CBS 10847T      | EU402938     | EU402937    |
| Candida pseudoviswanathii| CBS 13916T     | KM586736     | KM586735    |
| Candida sanyaensis       | CBS 12637T      | JQ647915     | JQ647914    |
| Candida sabaoensis       | CBS 12318T      | AB696985     | AB617978    |
| Candida sojaiae          | NRRL Y-17909T   | KJ722419     | U71070      |
| Candida subhassii        | CBS 10753T      | NR_073356    | EU836708    |
| Candida terrigiderum     | NRRL Y-48142T   | FJ623630     | EF120599    |
| Candida theae            | ATCC MYA-4764T  | JQ812707     | JQ812701    |
| Candida tropicalis       | NRRL Y-12968T   | AF287910     | U45749      |
| Candida verbasci         | CBS 12699T      | JX515982     | JX515981    |
| Candida viwanathii       | CBS 4024T       | KY102515     | U45752      |
| Candida xiangnanensis    | CBS 13923T      | KM586732     | KM586731    |
| Candida yunnanensis      | NYNU 17948T     | MG255721     | MG255709    |
| Lodderomyces heijingensis| CBS 14171T     | KU128709     | KU128720    |
| Lodderomyces elongopus   | NRRL YB-4239T   | AY391848     | U45763      |
| Nematodopora anomalaiae  | CBS 13927T      | KP054270     | KP054269    |
| Nematodopora valgi       | CBS 12562T      | KM386993     | HM627112    |
| Scheffersomyces stipitis | NRRL Y-7124T   | JN943257/    | U45741      |
| Spathaspora allomyrinae  | CBS 13924T      | KP054268     | KP054267    |
| Spathaspora arborvariae  | ATCC MYA-4684T  | NR_111592    | NG_042574   |
| Spathaspora boniae       | CBS 13262T      | NR_158910    | KT276332    |
| Spathaspora brasiliensis | CBS 12679T      | JN099271     | JN099271    |
| Spathaspora elongata     | NYNU 18118T     | MK682770     | MK682796    |
| Spathaspora elongata     | NYNU 181110     | MT276033     | MT274662    |
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manually edited using MEGA v. 7 (Kumar et al. 2016). The best-fit nucleotide substitution models for each gene were selected using jModelTest v2.1.7 (Darriba et al. 2012), according to the Akaike Information Criterion.

Phylogenetic analyses of the combined gene regions (ITS and nuc 28S) were performed using the Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Candida argentea CBS 12358 was chosen as the outgroup. ML analysis was performed using MEGA v7 with the GTR+I+G model (Nei and Kumar 2000) and 1,000 rapid bootstrap replicates to estimate branch confidence. BI analysis was performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.0b4 (Ronquist and Huelsenbeck 2003). Two MCMC chains, started from random trees for 1,000,000 generations and trees, were sampled every 100th generation, resulting in a total of 10,000 trees. The first 25% of the trees were discarded as burn-in of each analysis. Branches with significant Bayesian Posterior Probabilities (BPP) were estimated in the remaining 7,500 trees. The phylogenetic trees from the ML and BI analyses were displayed using Mega 7 and FigTree v1.4.3 (Rambaut 2016), respectively.

Results

Phylogenetic analyses

The combined nuclear dataset (ITS and nuc 28S) was analysed to infer the interspecific relationships within the larger Spathaspora/Candida albicans/Lodderomyces clade
of Debaryomycetaceae. The dataset consisted of 72 sequences including the outgroup, *Candida argentea* (culture CBS 12358). A total of 944 characters including gaps (391 for ITS and 553 for nuc 28S) were included in the phylogenetic analysis. The best nucleotide substitution model for ITS and nuc 28S was GTR+I+G. ML and BI analyses of the combined dataset resulted in phylogenetic reconstructions with similar topologies and the average standard deviation of split frequencies was 0.011210 (BI). In the ML phylogenetic tree (Figure 1), thirteen strains formed five single clades with high to full support (100% in ML and 0.99 or 1.00 in BI) and clustered in the clade that comprised most species of *Spathaspora*. Phylogenetically, *S. elongata* and *S. mengyangensis* clustered together with high support (84% in ML and 0.91 in BI), while *S. jiuxiensis* and *S. para-jiuxiensis* clustered together with strong support (100% in ML and 1.00 in BI). Two strains of *S. rosae* formed a unique lineage with *S. allomyrinae*, but with low support.

**Taxonomy**

*Spathaspora elongata* C.Y. Chai & F.L. Hui, sp. nov.

MycoBank: 836444

Figure 2

**Type.** China, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood from a tropical rainforest, August 2018, K.F. Liu & Z.W. Xi (holotype, NYNU 18115T preserved in a metabolically-inactive state), ex-holotype: CICC 33353; CBS 16002.

**Etymology.** *Elongata* refers to the elongate ascospores of this yeast.

**Description.** After 3 days of culture in YM broth at 25 °C, the cells are ovoid (3–4 × 3–7 μm) and occur singly or in pairs (Fig. 2a). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. After 3 days of growth on YM agar at 25 °C, colonies are white to cream-coloured, butyrous and smooth with entire margins. After 14 days at 25 °C, on Dalmau plate culture on CM agar, pseudo-hyphae are present, but true hyphae are not formed (Fig. 2b). Sporulation occurs on dilute (1:19) V8 agar after 14 days at 25 °C. Unconjugated asci are formed from single cells with one elongated ascospore which are tapered and curved at the ends (Fig. 2c). Glucose, galactose, maltose and sucrose are weakly fermented. Xylose fermentation is absent using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Glucose, d-ribose, d-xylitol, d-arabinose, sucrose, maltose, trehalose, methyl α-d-glucoside, cellobiose, salicin, arbutin, inulin, ribitol, d-glucitol, d-mannitol, 2-keto-d-gluconate, succinate, citrate and ethanol are assimilated. No growth occurs with galactose, l-sorbosone, d-glucosamine, l-arabinose, l-rhamnose, melibiose, lactose, raffinose, melezitose, glycerol, erythritol, xylitol, galactitol, *myo*-inositol, d-glucono-1, 5-lactone, 5-keto-d-gluconate, d-gluconate, d-glucuronate, DL-lactate or methanol. For the assimilation of nitrogen compounds, growth on ethylamine, l-lysine, glucosamine or d-tryptophan is present, whereas growth on nitrate, nitrite, cadaverine, creatine, creatinine or imidazole is absent. Growth is observed at 37 °C but not at 40 °C. Growth in the presence of 1% acetic acid is present,
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Figure 1. Phylogenetic tree, based on an ML analysis of a combined DNA dataset of ITS and nuc 28S rDNA sequences for *Spathaspora* species and related taxa in the Debaryomycetaceae. Numbers above the branches indicate ML bootstraps (left, MLBS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.90). The tree is rooted with sequences from *Candida argentea* CBS 12358. Isolates from the current study are shown in bold letters. “-” indicates MLBS < 50% or BPP < 0.90. The scale bar indicates the number of substitutions per site.
but growth in the presence of 10% sodium chloride (NaCl) plus 5% glucose and 0.01% cycloheximide is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

**Additional isolates examined.** CHINA, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood from a tropical rainforest, August 2018, K.F. Liu & Z.W. Xi, NYNU 181112, NYNU 181120 and NYNU 181158.

**Notes.** Four strains, representing *Sp. elongata*, clustered in a well-supported phylogenetic clade that is closely related to *Sp. mengyangensis*, another new species proposed in this paper and *C. subhashii*. The nucleotide differences between *Sp. elongata* and *Sp. mengyangensis* were 2.5% substitutions in the D1/D2 domain and 5.2% substitutions in the ITS region (Groenewald et al. 2016). Similarly, *Sp. elongata* and *C. subhashii* showed differences of 3.9% substitutions in the D1/D2 domain and 5.9% substitutions in the ITS region (Groenewald et al. 2016). Physiologically, *Sp. elongata* can be differentiated from its close relative, *Sp. mengyangensis*, based on its growth in citrate and the presence of 1% acetic acid, which are present for *Sp. elongata* and absent for *Sp. mengyangensis*. Moreover, *Sp. elongata* weakly ferments glucose, galactose, maltose and sucrose and grows at 37 °C, but *Sp. mengyangensis* does not.

*Spathaspora mengyangensis* C.Y. Chai & F.L. Hui, sp. nov.
MycoBank: 836445

**Type.** CHINA, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood from a tropical rainforest, July 2017, K.F. Liu & L. Zhang (holotype, NYNU 17741T preserved in a metabolically-inactive state), ex-holotype: CICC 33267; CBS 15227.

**Etymology.** *Mengyangensis* refers to the geographical origin of the type strain of this species.
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Description. In YM broth after 3 days at 25 °C, cells are ovoid (3–7 × 5–7.5 μm) and occur singly or in pairs (Fig. 3a). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. After 3 days of growth on YM agar at 25 °C, colonies are white to cream-coloured, butyrous and smooth with entire margins. After 14 days at 25 °C on Dalmau plate culture on CM agar, pseudohyphae are present, but true hyphae are not formed (Fig. 3b). Sporulation occurs on CM agar after 14 days at 25 °C. Unconjugated asci are formed from single cells with one elongated ascospore which are tapered and curved at the ends (Fig. 3c). Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Glucose, D-ribose, D-xylene, sucrose, maltose, trehalose, methyl α-D-glucoside, cellubiose, salicin, arbutin, inulin, ribitol, D-glucitol, D-mannitol, 2-keto-D-gluconate, succinate and ethanol are assimilated. No growth occurs with galactose, L-sorbose, D-glucoammine, D-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, raffinose, melezitose, glycerol, erythritol, xylitol, galactitol, myo-inositol, D-glucono-1, 5-lactone, 5-keto-D-gluconate, D-glucurate, D-glucuronate, DL-lactate, citrate or methanol. For the assimilation of nitrogen compounds, growth on ethylamine, L-lysine, glucosamine or D-tryptophan is present, whereas growth on nitrate, nitrite, cadaverine, creatine, creatinine or imidazole is absent. Growth is observed at 30 °C, but not at 35 °C. Growth in the presence of 10% NaCl plus 5% glucose, 0.01% cycloheximide and 1% acetic acid is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

**Figure 3.** *Spathaspora mengyangensis* (NYNU 17741, holotype) **a** budding cells on YM broth after 3 d **b** simple pseudohyphae on CM agar after 14 d **c** ascus and ascospore (arrow) on CM agar after 14 d. Scale bars: 10 μm.
Additional isolate examined. China, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood from a tropical rainforest, July 2017, K.F. Liu & L. Zhang, NYNU 17705.

Notes. Phylogenetic analyses show that Sp. mengyangensis is closely related to Sp. elongata and C. subhashii; however, the independent phylogenetic position and different physiological characters can distinguish Sp. mengyangensis from its sister species Sp. elongata (as mentioned above). Similarly, Sp. mengyangensis differed from C. subhashii by 2.8% substitutions in the D1/D2 domain and 7.8% substitutions in the ITS region (Groenewald et al. 2016). Physiologically, Sp. mengyangensis can be differentiated from C. subhashii by the ability to assimilate d-ribose, trehalose, d-glucitol and d-mannitol and the inability to assimilate galactose, l-arabinose and melezitose. In addition, C. subhashii can grow at 40 °C, but Sp. mengyangensis cannot.

Spathaspora jiuxiensis C.Y. Chai & F.L. Hui, sp. nov.
MycoBank: 836446
Figure 4

Type. China, Yunnan Province, Honghe Prefecture, Luxi County, in rotting wood in Jiuxi Mountain Forest Park, July 2017, K.F. Liu & L. Zhang (holotype, NYNU 17416T preserved in a metabolically-inactive state), ex-holotype: CICC 33264; CBS 15226.

Etymology. Jiuxiensis refers to Jiuxi Mountain, the mountain from which it was collected.

Description. In YM broth after 3 days at 25 °C, cells are ovoid to elongate (3–6 × 3.5–9 μm) and occur singly or in pairs (Fig. 4a); pseudohyphae are present. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. After 3 days of growth on YM agar at 25 °C, colonies are white to cream-coloured, butyrous and smooth with entire margins. After 12 days at 25 °C on Dalmau plate culture on CM agar, pseudohyphae and true hyphae are formed (Fig. 4b). Asci or signs of conjugation were not seen on the sporulation media used. Glucose and maltose are weakly fermented. Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Glucose, d-glucosamine, d-ribose, d-xylose, sucrose, maltose, trehalose, methyl α-d-glucoside, cellobiose, salicin, arbutin, melezitose, inulin, ribitol, d-glucitol, d-mannitol, 2-keto-d-gluconate, DL-lactate, succinate and ethanol are assimilated. No growth occurs with galactose, l-sorbose, l-arabinose, l-ribose, l-rhamnose, melibiose, lactose, raffinose, glycerol, erythritol, xylitol, galactitol, myo-inositol, D-glucono-1, 5-lactone, 5-keto-D-gluconate, d-gluconate, d-glucuronate, citrate, l-arabinitol or methanol. For the assimilation of nitrogen compounds, growth on l-lysine, glucosamine or d-tryptophan is present, whereas growth on nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine or imidazole is absent. Growth is observed at 35 °C, but not at 37 °C. Growth in the presence of 0.01% cycloheximide is present, but growth in the presence of 0.1% cycloheximide, 10% NaCl plus 5% glucose and 1% acetic acid is
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Figure 4. Morphology of *Spathaspora jiuxiensis* (NYNU 17416, holotype) a budding cells and pseudohyphae on YM broth after 3 d b true hyphae with blastoconidia on CM agar after 14 d. Scale bars: 10 μm.

absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

**Additional isolate examined.** China, Yunnan Province, Honghe Prefecture, Luxi County, in rotting wood in Jiuxi Mountain Forest Park, July 2017, K.F. Liu & L. Zhang, NYNU 17417.

**Notes.** The two strains, both representing *Sp. jiuxiensis*, cluster in a well-supported clade in the phylogenetic analysis and are closely related to *Sp. parajiuxiensis*. The nucleotide differences between these two new species were 1.4% substitutions in the D1/D2 domain and 4.6% substitutions in the ITS region (Groenewald et al. 2016). These two sister species can also be differentiated by a few physiological characteristics; *Sp. jiuxiensis* can assimilate dL-lactate and *Sp. parajiuxiensis* can grow at 37 °C.

*Spathaspora parajiuxiensis* C.Y. Chai & F.L. Hui, sp. nov.

MycoBank: 836447

Figure 5

**Type.** China, Yunnan Province, Honghe Prefecture, Luxi County, in rotting wood in Jiuxi Mountain Forest Park, July 2016, R.C. Ren & L. Zhang (holotype, NYNU 16747T preserved in a metabolically-inactive state), ex-holotype: CICC 33162; CBS 14691.

**Etymology.** *Paraluxiensis* refers to its close phylogenetic relationship to *Sp. luxiensis*.

**Description.** In YM broth after 3 days at 25 °C, cells are ovoid to elongate (3.5–4 × 7–15 μm) and occur singly or in pairs (Fig. 5a); pseudohyphae are present. Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. After 3 days of growth on YM agar at 25 °C, colonies are white to cream-coloured, butyrous
and smooth with entire margins. After 12 days at 25 °C on Dalmau plate culture on CM agar, pseudohyphae and true hyphae are formed (Fig. 5b). Sporulation occurs on 5% ME agar after 14 days at 25 °C. Unconjugated asci are formed from single cells with one elongated ascospores which are tapered and curved at the ends (Fig. 5c). Glucose and maltose are weakly fermented. Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Glucose, d-glucosamine, d-ribose, d-xylose, sucrose, maltose, trehalose, methyl a-D-glucoside, cellobiose, salicin, arbutin, melezitose, inulin, ribitol, d-glucitol, d-mannitol, 2-keto-d-gluconate, succinate and ethanol are assimilated. No growth occurs with galactose, L-sorbose, L-arabinose, d-arabinose, L-rhamnose, melibiose, lactose, raffinose, glycerol, erythritol, xylitol, galactitol, myo-inositol, d-glucono-1, 5-lactone, 5-keto-d-gluconate, d-gluconate, d-glucuronate, DL-lactate, citrate, L-arabinitol or methanol. For the assimilation of nitrogen compounds, growth on L-lysine, glucosamine or d-tryptophan is present, whereas growth on nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine or imidazole is absent. Growth is observed at 37 °C, but not at 40 °C. Growth in the presence of 0.01% cycloheximide is present, but growth in the presence of 0.1% cycloheximide, 10% NaCl plus 5% glucose and 1% acetic acid is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

Additional isolate examined. China, Yunnan Province, Honghe Prefecture, Luxi County, in rotting wood in Jiu Xi Mountain Forest Park, July 2016, R.C. Ren & L. Zhang, NYNU 16632.
**Spathaspora rosae** C.Y. Chai & F.L. Hui, sp. nov.
MycoBank: 836448

**Type.** China, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood in a tropical rainforest, July 2017, Z.W. Xi & L. Zhang (holotype, NYNU 17934\textsuperscript{T} preserved in a metabolically-inactive state), ex-holotype: CICC 33271; CBS 15231.

**Etymology.** *Rosae* was named in honour of Carlos A. Rosa for his contributions in yeast taxonomy.

**Description.** In YM broth after 3 days at 25 °C, cells are ovoid to elongate (4–7 × 5–16 μm) and occur singly or in pairs (Fig. 6a). Budding is multilateral. Sediment is formed after a month, but a pellicle is not observed. After 3 days of growth on YM agar at 25 °C, colonies are white to cream-coloured, butyrous and smooth with entire margins. After 7 days at 25 °C, on Dalmau plate culture on CM agar, pseudohyphae and true hyphae are formed (Fig. 6b). Asci or signs of conjugation are not seen on sporulation media used. Xylose fermentation is negative using Durham tubes, but ethanol is produced from xylose when determined with alcohol oxidase and peroxidase tests. Glucose, d-glucosamine, d-xylose, sucrose, maltose, trehalose, methyl α-d-glucoside, cellobiose, salicin, arbutin, inulin, ribitol, d-glucitol, d-mannitol, 2-keto-d-gluconate, dl-lactate, succinate, citrate and ethanol are assimilated. No growth occurs with galactose, l-sorbose, d-ribose, l-arabinose, d-arabinose, l-rhamnose, melibiose, lactose, raffinose, melezitose, glycerol, erythritol, xylitol, galactitol, myo-inositol, d-glucono-1, 5-lactone, 5-keto-d-gluconate, d-gluconate, d-glucuronate, l-arabininitol or methanol. For the assimilation of nitrogen compounds, growth on ethylamine, l-lysine, glucosamine or d-tryptophan is present,
whereas growth on nitrate, nitrite, cadaverine, creatine, creatinine or imidazole is absent. Growth is observed at 35 °C, but not at 37 °C. Growth in the presence of 0.01% cycloheximide is present, but growth in the presence of 0.1% cycloheximide, 10% NaCl plus 5% glucose and 1% acetic acid is absent. Starch-like compounds are not produced. Urease activity and diazonium blue B reactions are negative.

**Additional isolates examined.** China, Yunnan Province, Jinghong City, Mengyang Town, in rotting wood in a tropical rainforest, July 2017, Z.W. Xi & L. Zhang NYNU 17903, NYNU 17909.

**Notes.** Three strains, representing *Sp. rosae*, grouped in a well-supported clade and appear to be most closely related to *Sp. allomyrinae* (Wang et al. 2016). The nucleotide differences between *Sp. rosae* and its close relative, *Sp. allomyrinae*, were 10.2% substitutions in the D1/D2 domain and 11% substitutions in the ITS region (Groenewald et al. 2016). Physiologically, *Sp. rosae* can be differentiated from *Sp. allomyrinae*, based on growth in galactose, melezitose, xylitol and 5-keto-d-gluconate, which are positive for *Sp. allomyrinae* and negative for *Sp. rosae*. Moreover, *Sp. allomyrinae* weakly ferments glucose, galactose, maltose and cellobiose, but *Sp. rosae* does not.

**Two new combinations**

In addition to the previously-described taxa, two new combinations are proposed herein and their descriptions refer to relevant protologues.

*Spathaspora materiae* (Barbosa, Cadete, Gomes, Lachance & Rosa) C.Y. Chai & F.L. Hui, comb. nov.
MycoBank: 836449

**Basionym.** Candida materiae Barbosa, Cadete, Gomes, Lachance & Rosa, International Journal of Systematic and Evolutionary Microbiology 59(8): 2015 (2009).

*Spathaspora jeffriesii* (N.H. Nguyen, S.-O. Suh & M. Blackwell) C.Y. Chai & F.L. Hui, comb. nov.
MycoBank: 836450

**Basionym.** Candida jeffriesii N.H. Nguyen, S.-O. Suh & M. Blackwell, Mycological Research 110(10): 1239 (2006).

**Discussion**

*Spathaspora* is distributed worldwide with 12 species identified from rotting wood and insects. In China, three species of *Spathaspora* have been previously reported (Ren et
Five novel \textit{Spathaspora} species (al. 2013; Wang et al. 2016). In the present study, five additional species, \textit{Sp. elongata}, \textit{Sp. jiuxiensis}, \textit{Sp. mengyangensis}, \textit{Sp. parajiuxiensis} and \textit{Sp. rosae} (Fig. 1), were recorded in addition to previously-known species. Thus, to our knowledge, eight species of \textit{Spathaspora} are currently known from China. Of these eight species, only two species, \textit{Sp. gorwiae} and \textit{Sp. passalidarum}, were reported in China up until 2013 (Ren et al. 2013). The remaining six species, namely \textit{Sp. allomyrinae}, \textit{Sp. elongata}, \textit{Sp. jiuxiensis}, \textit{Sp. mengyangensis}, \textit{Sp. parajiuxiensis} and \textit{Sp. rosae}, were recorded from 2016 to 2018. Given this history, it is most likely that more species will be found. Nonetheless, this number is significant when compared to the total diversity of 11 species of \textit{Spathaspora} reported for South America (Cadete et al. 2009, 2013; Lopes et al. 2016; Morais et al. 2017; Varize et al. 2018). Further studies are needed to document the overall diversity of species of \textit{Spathaspora} in China, especially in the southwest regions.

The phylogenetic relationship of \textit{Spathaspora} has been unclear until now, mainly due to its polyphyletic nature (Daniel et al. 2014; Morais et al. 2017; Varize et al. 2018). In this article, we used more available type strains to revise this genus, based on a phylogenetic analysis of the combined ITS and nuc 28S rDNA sequences. As shown in Figure 1, three main groups were reconstructed and the results showed that \textit{Spathaspora} is not a monophyletic group, but rather is polyphyletic with several \textit{Candida} species included. \textit{Sp. passalidarum}, the type species of the genus, \textit{C. jeffriesii}, \textit{C. materiae}, \textit{Sp. arborariae}, \textit{Sp. brasiliensis}, \textit{Sp. girioi} and \textit{Sp. subii} form a core group that is well supported by phylogeny. This result is similar to the results of previous phylogenetic analyses of nuc 28S rDNA sequences (Morais et al. 2017; Varize et al. 2018). Therefore, two \textit{Candida} species, \textit{C. materiae} and \textit{C. jeffriesii}, are transferred to \textit{Spathaspora} as new combinations because of their phylogenetic placement within that genus.

The second group is composed of ten distinct species, including the four species \textit{Sp. elongata}, \textit{Sp. mengyangensis}, \textit{Sp. jiuxiensis} and \textit{Sp. parajiuxiensis} described in this study. Typical ascospores are formed by \textit{Sp. elongata}, \textit{Sp. mengyangensis}, \textit{Sp. parajiuxiensis} and \textit{Sp. roraimanensis}, but other members of the group are known from their asexual cycle only.

The species \textit{Sp. allomyrinae}, which shares the unique ascospore morphology of the genus, fell outside a larger \textit{Spathaspora} clade, as in the nuc 28S-based phylogeny proposed by Morais et al. (2017). However, this species is joined by \textit{Sp. rosae}, which is described in the current study, in a third cluster consisting of \textit{Spathaspora} in our phylogenetic analysis (Fig. 1). Placement of \textit{Sp. allomyrinae} and \textit{Sp. rosae} is only weakly supported and continued assignment to the genus will require verification from more robust datasets, such as whole genome sequences.

Morais et al. (2017) described the species \textit{Sp. boniae}, based on two strains producing asci containing elongate ascospores with curved ends typical of the genus \textit{Spathaspora}. Our phylogenomic analysis showed that \textit{Sp. boniae} clusters with \textit{C. blackwellae} and \textit{C. parablackwellae} to form a distinct clade outside a larger \textit{Spathaspora} clade. This result was also supported by previous phylogenetic analyses on this clade using nuc 28S rDNA sequences (Morais et al. 2017; Varize et al. 2018; Zhai et al. 2019). These results suggest that the genus \textit{Spathaspora} should be limited to species in the group comprising
the type species *Sp. passalidarum*. This clade, which has been treated previously as members of *Spathaspora*, may represent a separate genus, despite the morphological characteristics of the included species and isolates are similar to *Spathaspora*. Therefore, whole genome sequencing of all *Spathaspora* species and those of related genera, combined with the discovery of new species of the clade, is needed to clarify the possible heterogeneity of this genus.

*Spathaspora* is a cosmopolitan genus, but most known species have relatively-distinct habitats or regional locations. Currently, most *Spathaspora* species are known from East Asia (mainly in China) and South America. Although the taxonomy of *Spathaspora* has received much attention in recent years, many regions in the world are under-sampled and more under-described indigenous *Spathaspora* species will undoubtedly be discovered in the future as with most microfungal genera (Hyde et al. 2020). Our study indicates that there is a high species diversity of *Spathaspora* waiting to be discovered in rotting wood in tropical and subtropical southwest China and nearby areas as with other genera (Hyde et al. 2018).

Acknowledgements

We sincerely thank Dr. Lin Zhang, Dr. Kai-Fang Liu and Dr. Zhi-Wen Xi for their kind help with collecting specimens. This project was supported by Grant No. 31570021 from the National Natural Science Foundation of China (NSFC), P. R. China, and No. 2018001 from the State Key Laboratory of Motor Vehicle Biofuel Technology, Henan Tianguan Enterprise Group Co. Ltd., China.

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