Received: 6 January 2023 | Revised: 26 April 2023 | Accepted: 30 April 2023

DOI: 10.1002/yea.3857

Yeast WILEY

RESEARCH ARTICLE

Fantastic Yeast

Schizosaccharomyces lindneri sp. nov., a fission yeast occurring in honey

Michael Brysch-Herzberg[1] | Guo-Song Jia[2] | Matthias Sipiczki[3] | Martin Seidel[1] | Wen Li[2] | Imen Assali[1] | Li-Lin Du[2,4]

[1]Laboratory for Wine Microbiology, Department International Business, Heilbronn University of Applied Sciences, Heilbronn, Germany

[2]National Institute of Biological Sciences, Beijing, China

[3]Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen, Hungary

[4]Tsinghua Institute of Multidisciplinary Biomedical Research, Tsinghua University, Beijing, China

Correspondence

Michael Brysch-Herzberg, Laboratory for Wine Microbiology, Department International Business, Heilbronn University of Applied Sciences, Max-Planck-Str. 39, 74081 Heilbronn, Germany.
Email: michael.brysch-herzberg@hs-heilbronn.de

Matthias Sipiczki, Department of Genetics and Applied Microbiology, University of Debrecen, 4032 Debrecen, Hungary.
Email: gecela@post.sk

Funding information

Ministry of Science and Technology; Deutsche Bundesstiftung Umwelt

Abstract

Two strains of fission yeast were isolated from honey. They differ from the type strain of *Schizosaccharomyces octosporus* by three substitutions in the D1/D2 domain of the nuclear 26S large subunit ribosomal RNA (rRNA) gene sequence, resulting in a 99.5% identity. In the internal transcribed spacer (ITS) region (consisting of ITS1, 5.8S rDNA, and ITS2), the strains differ from *S. octosporus* by 16 gaps and 91 substitutions, which is equivalent to an identity of 88.1%. Genome sequencing on one of the new strains revealed that the average nucleotide identity (ANI) between its genome and the reference genome of *S. octosporus* is 90.43% and there exist major genome rearrangements between the two genomes. Mating analysis revealed that *S. octosporus* and one of the new strains are completely reproductively separated. A strong prezygotic barrier exists and the few mating products consist of diploid hybrids that do not form recombinant ascospores. In the new strains, asci are either zygotic, arising from conjugation, or they develop without conjugation from asexual cells (azygotic). Compared to the currently recognized *Schizosaccharomyces* species, the spectrum of nutrients that are assimilated by the new strains is restricted. Of the 43 carbohydrates that were included in the physiological standard tests, only 7 were assimilated. According to the results of the genome sequence analysis, the mating trials, and the phenotypic characterization, the new species *Schizosaccharomyces lindneri* is described to accommodate the two strains (holotype: CBS 18203^T and ex-type: MUCL 58363; MycoBank no.: MB 847838).

KEYWORDS

genomic rearrangements, hybridization, *Schizosaccharomyces lindneri*, taxogenomics

---

Michael Brysch-Herzberg, Matthias Sipiczki, and Guo-Song Jia contributed equally to this study.

1 | INTRODUCTION

Species in the genus *Schizosaccharomyces* are characterized by a typical haplontic life cycle in which the diploid phase is restricted to the zygote, which arises from the conjugation of two haploid cells of opposite mating types. As an exception, the asexual reproduction of diploid cells in *Schizosaccharomyces pombe* and *Schizosaccharomyces versatilis*, which is seen as synonymous with *Schizosaccharomyces japonicus*, was shown (Suminoe & Dukmo, 1963). Later, it was confirmed that in the haplophase of *S. pombe*, rare diploid cells arise, which form asci without conjugating with another cell and produce haploid ascospores (Leupold, 1970a).

Further characteristics of the genus are the asexual reproduction by fission, the fermentation of different sugars, the inability to use nitrate as the sole nitrogen source, a positive starch test in Wickerham's medium, the formation of coenzyme Q9 and Q10, a negative Diazonium Blue B reaction, and a positive urease test (Vaughan-Martini & Martini, 2011).

Currently, five species in the genus *Schizosaccharomyces* are recognized: *S. pombe* (Lindner, 1893), *S. octosporus* (Beijerinck, 1894), *S. japonicus* (Yukawa & Maki, 1931), *S. cryophilus* (Helston et al., 2010), and *S. osmophilus* (Brysch-Herzberg et al., 2019). An overview of the long-lasting discourse concerning species delimitation and phylogeny in the genus is given by Brysch-Herzberg et al. (2019). It was proposed that the genus should be split into three genera (Kudrjawzew, 1960; Yamada & Banno, 1987). As these proposals were never broadly accepted, we will name the species as given in "The Yeast, a taxonomic study" (Vaughan-Martini & Martini, 2011).

In an extensive isolation study focusing on the ecology of the different *Schizosaccharomyces* species, hundreds of *Schizosaccharomyces* strains were isolated from various substrates by Brysch-Herzberg et al. (2022). The authors investigated 386 honey samples and isolated 61 strains identified as *S. octosporus*. Among them, two strains showed atypical sporulation behavior and relevant differences in the ribosomal RNA (rRNA) gene repeat. In view of the high number of honey samples that were already examined and the high number of samples from other substrates, including bee hive materials that were investigated, no further attempts to isolate more strains of the new type were made. In the current study, the two atypical strains (SZ1623-FG-A = CBS 18203^T, SZ1865-YA) were further investigated and the new species *Schizosaccharomyces lindneri* is shown to accommodate them.

2 | MATERIALS AND METHODS

2.1 | Isolation procedure

The two strains investigated in this study were isolated from honey by means of an enrichment step. The enrichment medium used was a high osmotic medium containing 5% glucose (w/w) and 55% fructose (Medium FG) (Brysch-Herzberg et al., 2022) and YPD medium (Kurtzman et al., 2011) as described before. The further isolation procedure was as described before (Brysch-Herzberg et al., 2022). Because both strains grow better on a high osmotic medium than on a YPD medium containing 2% glucose only, the osmotic pressure of the medium was increased by the addition of 30% glycerol. On this medium, both strains showed good growth.

Take-away

- A new fission yeast species, *Schizosaccharomyces lindneri* sp. nov., is described.
- The new species is closely related to *Schizosaccharomyces octosporus*. Delimitation of *S. octosporus* and *S. lindneri* sp. nov. is based on whole-genome sequencing data and on hybridization trials.
- An almost complete prezygotic and postzygotic sterility barrier was detected between *S. octosporus* and *S. lindneri* sp. nov.
- Genome rearrangements most likely play an important role in hybrid infertility between *S. octosporus* and *S. lindneri* sp. nov. Most likely, speciation of *S. lindneri* was sympatric.
- *S. lindneri* sp. nov. was isolated from honey bee honey.

2.2 | Molecular genetic characterization

In both strains, the D1/D2 domain of the nuclear large subunit (LSU) 26S rRNA gene and the ITS1-5.8S-ITS2 (internal transcribed spacer [ITS]) region were sequenced as described before (Brysch-Herzberg et al., 2019). PCR products were sequenced by Eurofins Genomics.

Sequence alignments were generated in MAFFT (Katoh et al., 2019) and trimmed in Jalview (Kuraku et al., 2013). Mega X was used for phylogenetic analysis (Kumar et al., 2018). The Tamura–Nei model (Tamura & Nei, 1993) was applied as a substitution model. The neighbor-joining algorithm (Saitou & Nei, 1987) was employed to construct trees. Statistical significance was estimated with the bootstrap approach (Felsenstein, 1985) from 1000 iterations. The tree was edited in TreeGraph 2 (Stöver & Müller, 2010). In TreeGraph 2, the tree was rooted with the outgroup species.

2.3 | Genome sequencing of the *S. lindneri* sp. nov. type strain

To sequence the genome of the *S. lindneri* sp. nov. type strain CBS 18203^T, we collected cells grown on solid YES plates and extracted genomic DNA using the MasterPure Yeast DNA Purification Kit (Epicentre). We prepared an Illumina sequencing library using homemade Tn5 transposase as previously described (Tao et al., 2019). The library was submitted to Novogene Co., Ltd. for paired-end sequencing on the Illumina NovaSeq. 6000 System (2 × 150 bp read pairs). Approximately 1.65 Gb of raw Illumina read data was obtained and deposited at the NCBI SRA database under the accession number SRR23651721 (Supporting Information: Table S1).

Preprocessing and quality assessment of the Illumina sequencing data were carried out using fastp (v0.2.0, https://github.com/OpenGene/fastp) with the parameter "–length_required 70" (Chen et al., 2018). About 1.53 Gb of Illumina read data was retained after this step and deposited in the NCBI SRA database under the accession number SRR23651720.

To further clean up the data, we removed read pairs containing low-frequency k-mers using KAT (v2.4.2, https://github.com/TGAC/KAT) (Mapleson et al., 2017). Specifically, we calculated the 27-mer frequency distribution of the fastp-processed reads using the KAT hist tool and obtained the peak 27-mer frequency (the "global_maxima" value in the JSON format output of the KAT hist tool). We then set a low-frequency k-mer cutoff by multiplying the peak 27-mer frequency by 0.2 and removed read pairs containing low-frequency k-mers using the KAT filter tool. About 842.5 Mb of Illumina read data was retained and deposited at the NCBI SRA database under the accession number SRR23651719.

2.4 | De novo assembly of the *S. lindneri* sp. nov. draft nuclear genome

To obtain a draft nuclear genome for *S. lindneri* sp. nov., we performed de novo assembly on fastp-processed Illumina reads using SPAdes (v3.15.5, https://github.com/ablab/spades) with the "--isolate" option (Prjibelski et al., 2020). A total of 3879 contigs were obtained and their total length is 11,955,064 bp. We mapped the KAT-filtered reads to this genome assembly using BWA (v0.7.17-r1188, https://github.com/lh3/bwa) (Li & Durbin, 2009) and calculated the average read depth in 1 kb windows. We then calculated the median read depth of all windows in all contigs. Contigs with half or more of the windows having read depth lower than 30% of the global median depth were removed. Additionally, we removed mitochondrial genome contigs identified by BLAST and contigs smaller than 1 kb. The thus-obtained draft nuclear genome consists of 113 contigs and has a total length of 10,873,930 bp (Supporting Information: Table S2). We deposited it at the NCBI GenBank database under the accession number JARFMH000000000.

2.5 | Calculation of the average nucleotide identity (ANI) values

To obtain pairwise ANI values for all combinations of the type strains of fission yeast species, we employed the OrthoANIu (OAU) tool (v1.2, http://www.ezbiocloud.net/tools/orthoaniu) (Yoon et al., 2017). The genome assemblies used as input for the OAU analysis are as follows: *S. pombe* type strain (CBS 356^T = JB861) assembly from https://figshare.com/articles/dataset/SGA_de_novo_assemblies_of_all_wild_strains/3978279 (Jeffares et al., 2015), *S. octosporus* type strain (CBS 371^T = ATCC 420^T = yFS286) assembly and *S. cryophilus* type strain (CBS 11777^T = OY26) assembly from http://bifx-core.bio.ed.ac.uk/~ptong/genome_assembly/ (Tong et al., 2019), *S. japonicus* type strain (CBS 354^T = IFO 1609^T = yFS275) assembly from the Ensembl-Fungi database (http://fungi.ensembl.org/Schizosaccharomyces_japonicus/Info/Index) (Rhind et al., 2011), *S. osmophilus* type strain (CBS 15793^T) assembly from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/assembly/GCA_027921745.1/) (Jia et al., 2022), and the *S. lindneri* sp. nov. type strain (CBS 18203^T) draft nuclear genome obtained in this study.

2.6 | Species tree inference and divergence time estimation

To perform genome-based phylogenetic analysis, we used single-copy Benchmarking Universal Single-Copy Orthologs (BUSCO) genes for species tree estimation as previously described (Jia et al., 2023). We firstly submitted the *S. lindneri* sp. nov. draft nuclear genome to BUSCO (v3.0.2, https://busco.ezlab.org/) to obtain the presence/absence status of 1315 predefined single copy orthologs in Ascomycota (the ascomycota_odb9 gene set) (Simão et al., 2015). We then used a total of 1057 "complete and single-copy" BUSCO genes present in *S. lindneri* sp. nov., the five previously recognized fission yeast species, and the outgroup species *Saitoella complicata* for phylogenetic analysis. We performed amino acid sequence alignment for each BUSCO gene separately using MAFFT (v7.475) with parameters "--thread 4 --auto --maxiterate 1,000" (Katoh & Standley, 2013) and then trimmed the multiple sequence alignment using trimAL (v1.4. rev15, http://trimal.cgenomics.org/) with parameters "-gappyout" (Capella-Gutiérrez et al., 2009). Alignments were concatenated using catsequences (https://github.com/ChrisCreevey/catsequences) (https://zenodo.org/record/4409153#.ZAHewnZBybg) to generate a supermatrix for species tree inference.

To infer the concatenation-based species tree, we estimated the best-fitting model for each trimmed alignment using the IQ-TREE built-in ModelFinder (IQ-TREE v2.0.3, https://github.com/Cibiv/IQ-TREE) with options "-m TESTONLY -nt 1" (Kalyaanamoorthy et al., 2017) and found that the"LG+G4" model was the best-fitting model for a majority of BUSCO genes (60.17%, 636 of 1057 BUSCO genes). We thus inferred the species tree using IQ-TREE (v2.0.3, https://github.com/Cibiv/IQ-TREE) with parameters "-m LG+G4 -alrt 1,000 -bb 1,000" (Minh et al., 2020).

To estimate the divergence time between *S. lindneri* sp. nov. and *S. octosporus*, we used the RelTime method in MEGA11 (Tamura et al., 2021). *S. complicata* served as the outgroup in the analysis. We used the following calibration nodes: the *S. japonicus*–*S. pombe* split (207.2 million years ago), the *S. pombe*–*S. octosporus* split (108.2 million years ago), the *S. octosporus*–*S. cryophilus* split (29.4 million years ago) (Shen et al., 2020), and the *S. octosporus*–*S. osmophilus* split (15.7 million years ago) (Jia et al., 2023). The time-calibrated phylogenetic tree was visualized in FigTree (v1.4.4, http://tree.bio.ed.ac.uk/software/figtree/) and the branch of the outgroup species *S. complicata* was removed manually in Adobe Illustrator.

2.7 | Genome alignment and visualization

To investigate chromosomal rearrangements between *S. lindneri* sp. nov. and *S. octosporus*, we performed genome alignment between the draft nuclear genome of *S. lindneri* sp. nov. and the *S. octosporus* genome assembly from http://bifx-core.bio.ed.ac.uk/~ptong/genome_assembly/ (Tong et al., 2019) using the Mauve plugin in Geneious Prime (v2023.0.4, https://www.geneious.com) (Darling et al., 2004). Contigs of the *S. lindneri* sp. nov. draft nuclear genome were ordered against the *S. octosporus* genome assembly using MAUVE Contig Mover (Rissman et al., 2009). Genome alignment was performed using progressive Mauve (Darling et al., 2010) and visualized using the Mauve viewer. Images generated by the Mauve viewer were manually adjusted using Adobe Illustrator.

2.8 | Phenotypic characterization

The physiological characterization of the strains was done according to the standard methods described by Kurtzman et al. (2011). Pretests had shown that none of the strains was able to grow in YNB with 40% xylitol as the sole carbon source and that the strains showed good growth in 40% xylitol supplemented with 2% glucose. Therefore, 40% xylitol was added to all test media to increase the osmotic pressure in the media except those media used to test tolerance to 50% and 60% glucose as well as to 10% and 16% NaCl. The standard media (Kurtzman et al., 2011) were used in these cases.

To provoke the production of true hyphae, the yeasts were inoculated on corn meal agar supplemented with 40% xylitol in Petri dishes and as slide cultures on the same agar at 25°C for up to 10 days. Sporulation was observed after 2 days on Edinburgh minimal medium agar (EMMA) (Mitchison, 1970).

2.9 | Strains and culture media used for hybridization

The ex-type strain of *S. octosporus* (CCY 44-2-1^T) was used as the mating partner for the holotype strain of *S. lindneri* sp. nov. (CBS 18203^T) isolated in this study and is the type strain of the newly proposed species. The mutants used in the current study are listed in Table 1. The culture media used were: YEA (yeast extract agar), YEL (yeast extract liquid), SML (synthetic minimal liquid), and SMA (synthetic minimal agar) (Sipiczki & Ferenczy, 1977). EMM was used without (EMM − N) and with NH_4Cl (EMM). EMM was used in solid (EMMA) and liquid form (EMML).

2.10 | Isolation and characterization of mutants

The isolation and characterization mainly followed the methods described by Sipiczki and Ferenczy (1978). Cells of CCY 44-2-1^T and CBS 18203^T were mutagenized with UV light. Cells of logarithmic-phase cultures were plated on YEA and irradiated with UV light to about 20% survival. After mutagenesis, the plates were incubated at 25°C for at least 7 days. To identify the auxotrophic mutants, the colonies produced were replica-plated onto a minimal medium. The colonies whose replicas did not grow on a minimal medium were isolated and nutrient requirements were investigated on a minimal medium using the procedure described by Sipiczki and Ferenczy (1978).

2.11 | Hybridization

Hybridization was done according to the methods described by Sipiczki and Ferenczy (1978). One milliliter of samples of early log-phase cultures of the partners grown overnight in YEL was mixed. Two milliliters of fresh YEL was added to the mixed culture, which

TABLE 1 List of yeast strains and auxotrophic mutants.

Species	Strain	Reference
Schizosaccharomyces octosporus	CCY 44-2-1Ta wild type	-
S. octosporus	CCY 44-2-1^T met$^-$	This study
S. octosporus	CCY 44-2-1^T met$^-$ ade$^-$	This study
S. octosporus	CCY 44-2-1^T lys$^-$	Sipiczki and Ferenczy (1978)
S. octosporus	CCY 44-2-1^T arg$^-$	Sipiczki and Ferenczy (1978)
Schizosaccharomyces lindneri sp. nov.	CBS 18203^{Tb} wild type	This study
S. lindneri sp. nov	CBS 18203^T lys$^-$	This study
S. lindneri sp. nov	CBS 18203^T ade$^-$	This study
S. lindneri sp. nov	SZ1865-YA wild type	This study

Abbreviations: CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CCY, Culture Collection of Yeasts, Bratislava, Slovakia.

[a] Ex-type strain of *S. octosporus*.

[b] Type strain of *S. lindneri* sp. nov.

was subsequently incubated at room temperature for 2 days. Cells were separated from the medium by centrifugation and washed with sterile water. The pellet was resuspended in 100 µL sterile water. Twenty microliters of the suspension was dropped on EMMA − N and incubated at room temperature for 3 days to induce conjugation, zygote formation, and sporulation. After 3 days of incubation, the presence of ascospores was examined microscopically. The sporulating culture was suspended in 200 µL of sterile water and the resulting suspension was spread on two minimal medium agar plates. Growing prototrophic colonies were isolated as putative hybrids and isolated onto fresh synthetic minimal medium agar. The same procedure was also performed separately with both partners (without mixing with each other) to test them for back mutation. Sporulation of hybrid cultures was investigated microscopically.

2.12 | Characterization of hybrids

To examine the viability of ascospores produced by the hybrids, 40 ascospores were separated by micromanipulation technique and incubated on YEA at 25°C. In a second approach, sporulating hybrid cultures were treated with lysing enzyme (Sigma-Aldrich; product no. L1412) overnight and plated onto a complete medium. The developing colonies were replica-plated onto SMA agar. Auxotrophic colonies, if any, were characterized concerning their nutrient requirements.

2.13 | PCR fingerprinting

PCR fingerprinting was employed to verify the hybrid nature of prototrophic colonies. The oligonucleotide $(GAC)_5$ was used to generate PCR fingerprints. The composition of the 25-µL PCR mixture was as follows: 18.2 µL MilliQ water, 2.5 µL DreamTaq 10× buffer, 0.3 µL DreamTaq polymerase, 1 µL DNTP mix, 2 µL $(GAC)_5$ primer, and 1 µL genomic DNA. The PCR program was as follows: 5 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 52.6°C, and 1 min at 72°C. The fragments of the amplified DNA were separated on a 1.5% agarose gel by electrophoresis at 120 V for 150 min.

3 | RESULTS AND DISCUSSION

3.1 | Phenotypic characterization

The results of the physiological tests are given in Table 2. For comparison and since not all tests were performed with all species in the current study results reported by Barnett et al. (2000), Helston et al. (2010) and Brysch-Herzberg et al. (2019) are also included in Table 2. CBS 18203^T solely fermented glucose. Additionally, SZ1865-YA was able to ferment sucrose and maltose weakly. All three sugars are fermented at least by some strains of each species in the genus *Schizosaccharomyces*. Both strains assimilate inulin, glycerol, and D-glucono-1,5-lactone besides glucose. In addition, SZ1865-YA assimilates sucrose and maltose. Assimilation of D-glucono-1,5-lactone and glycerol is variable in *S. pombe* and *S. octosporus*. The ability to assimilate sucrose and maltose is present in all *Schizosaccharomyces* species except *S. osmophilus*, which does not assimilate sucrose. Glycerol can be used by strains of *S. pombe*, *S. octosporus*, and *S. osmophilus*. The only nitrogen sources both strains used were NH_4 and ethylamine. Cadaverine was assimilated by SZ1865-YA only. Exclusively based on the physiological characteristics, it is not possible to distinguish *S. lindneri* sp. nov. from *S. octosporus* and *S. osmophilus*, which are closest related within the genus *Schizosaccharomyces* (see below).

After 4 days at 25°C on YPD agar supplemented with 30% (w/v) glycerol, asexual cells of *S. lindneri* sp. nov. measure 3–9 µm × 3–9 µm. Their shapes are globose, oval, egg-shaped, and rarely rod-shaped (Figure 1a). Conjugation may take place between two cells (Figure 1b) and give rise to zygotic asci. These are irregular in shape measuring up to 20 µm × 12 µm. Zygotic asci mainly contain four ascospores (Figure 1c–e) and rarely eight ascospores (Figure 1g). Azygotic asci are produced from asexual cells without prior conjugation (Figure 1f). Azygotic asci are globose, measure 7–10 µm, and contain 1–4 ascospores. Usually, ascospores are globose, oval, and egg shaped and measure 3–5 µm, but other irregularly shaped ascospores can be found as well. Mycelium was never observed. *S. octosporus* differs from *S. lindneri* sp. nov. in a way that it does not form azygotic asci. In *S. octosporus*, conjugation always precedes ascus formation (Figure 2). Generally, species within the genus *Schizosaccharomyces* are expected to have a strictly haplontic lifecycle (Vaughan-Martini & Martini, 2011), although the exceptional occurrence of diploid cells along with haploid cells in one and the same clonal culture was described before for *S. pombe* (Leupold, 1955). The formation of diploid cells in a predominantly haploid culture was ascribed to endomitosis (Leupold, 1970b). The formation of azygotic asci with deviating spore numbers in diploid cultures of *S. pombe* was reported before (Hoffman et al., 2015; Molnar & Sipiczki, 1993). Although diploid asexual propagation may occur, it is not the rule, but exceptional in the genus. By far, *S. octosporus* was not as intensively investigated as it is in the case of *S. pombe*. Therefore, it cannot be excluded that some strains of *S. octosporus* similarly produce diploid cells, as it seems to be the case with *S. lindneri* sp. nov. For this reason and because the other phenotypic characteristics of the newly proposed species are very similar to those of *S. octosporus*, a reliable differentiation on basis of the phenotypes seems questionable.

3.2 | Analysis of DNA sequences of the rRNA gene repeat

The D1/D2 domain of the 26S LSU gene of *S. lindneri* sp. nov. is 604 bp long (accession no.: OQ067231; OQ067232). It exhibits three substitutions equivalent to an identity of 99.5% compared to

TABLE 2 Phenotypic characters of *Schizosaccharomces* species.

	S. pombe	*S. japonicus*	*S. octosporus*	*S. cryophilus*	*S. osmophilus*	*S. lindneri* sp. nov.
Fermentations						
D-Glucose	+	+	+	+	+	d
Inulin	v	v	–	w	–	–
Sucrose	+	+	v	+	d	vdw
Raffinose	v	+	–	w	–	–
Melibiose	+	v	–	–	–	–
Galactose	v	–	–	–	–	–
Lactose	–	–	–	–	–	–
Trehalose	–	–	d	–	–	–
Maltose	v	v	v	+	d	vdw
Melezitose	–	–	–	–	–	–
Methyl-α-D-glucoside	v	d	v	+	–	–
Soluble starch	–	–	–	–	–	–
Cellobiose	–	–	v	–	–	–
Assimilations						
Glucose	+	+	v	+	+	+
Inulin	v	v	v	d	v	+
Sucrose	+	v	v	+	–	v
Raffinose	+	v	–	+	–	–
Melibiose	v	v	–	–	–	–
Galactose	v	–	–	–	–	–
Lactose	–	–	–	–	–	–
Trehalose	v	–	v	w	–	d+
Maltose	v	v	v	d	v	v
Melezitose	v	–	–	–	–	–
Methyl-α-D-glucoside	v	–	v	+	v	–
Soluble starch	v	–	v	–	–	–
Cellobiose	v	–	v	w	–	–
Salicin	–	–	–	–	–	–
L-Sorbose	–	–	–	–	–	–
L-Rhamnose	–	–	–	–	–	–
D-Xylose	v	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–
D-Arabinose	–	–	–	–	–	–
D-Ribose	–	–	–	–	–	–
Methanol	–	–	–	–	–	–
Ethanol	v	–	–	–	–	–
Glycerol	v	–	v	–	+	d
Erythritol	–	–	–	–	–	–

TABLE 2 (Continued)

	S. pombe	*S. japonicus*	*S. octosporus*	*S. cryophilus*	*S. osmophilus*	*S. lindneri* sp. nov.
Ribitol	–	–	–	–	–	–
Galactitol	–	–	–	–	–	–
D-Mannitol	–	–	–	–	–	–
D-Glucitol	v	–	–	–	–	–
Myo-inositol	–	–	–	–	–	–
DL-Lactate	–	–	–	–	+	–
Succinate	–	–	–	–	–	–
D-Galacturonate	–	–	–	–	–	–
Citrate	–	–	–	–	–	–
D-Gluconate	v	–	–	–	+	–
D-Glucuronate	–	–	–	–	–	–
D-Glucosamine	–	–	–	–	–	–
N-Acetyl-D-glucosamine	n	–	–	–	–	–
2-Keto-D-gluconate	v	–	–	–	–	–
5-Keto-D-gluconate	–	–	–	–	–	–
Saccharate	–	–	–	–	–	–
Palatinose	v	–	v	d	–	–
Xylitol	–	–	–	–	–	–
Arabinitol	–	–	–	–	–	–
Arbutin	–	–	–	–	–	–
Propane-1,2-diol	–	–	–	–	–	–
Butane-2,3-diol	–	–	–	–	–	–
D-Glucono-1,5-lactone	v	–	v	–	–	d
Quinate	–	–	–	–	–	–
Nitrate	–	–	–	n	–	–
Nitrite	–	–	–	n	–	–
Cadaverin	v	–	v	n	+	v
Creatine	–	–	–	n	–	–
Creatinine	–	–	–	n	+	–
Lysine	v	v	–	n	–	–
Ethylamine	v	–	v	n	–	+
Glucosamine	–	–	–	n	–	–
Tolerances						
Cycloheximide 0.001%	+	–	–	+	–	–
Cycloheximide 0.01%	v	–	–	–	–	–
Cycloheximide 0.1%	–	–	–	–	–	–
Acetic acid 1%	v	–	v	+	–	–
NaCl 10%	v	–	+	–	+	+
NaCl 16%	–	–	v	–	v	+

(Continues)

TABLE 2 (Continued)

	S. pombe	*S. japonicus*	*S. octosporus*	*S. cryophilus*	*S. osmophilus*	*S. lindneri* sp. nov.
Glucose 50%	+	−	v	+	+	+
Glucose 60%	v	−	v	−	+	+
Temperatures						
25°C	+	+	+	+	+	+
30°C	+	+	+	−	+	+
33°C	n	n	n	n	+	+
35°C	+	+	+	n	v	+
37°C	+	+	v	n	−	+
40°C	v	v	−	n	−	−
42°C	v	v	−	n	n	n

Note: Data for species other than *Schizosaccharomyces lindneri* sp. nov. are taken from von Barnett et al. (2000), Brysch-Herzberg et al. (2019), and Helston et al. (2010).

Abbreviations: d, delayed; n, not tested; v, variable response; w, weak; +, positive result; −, negative result.

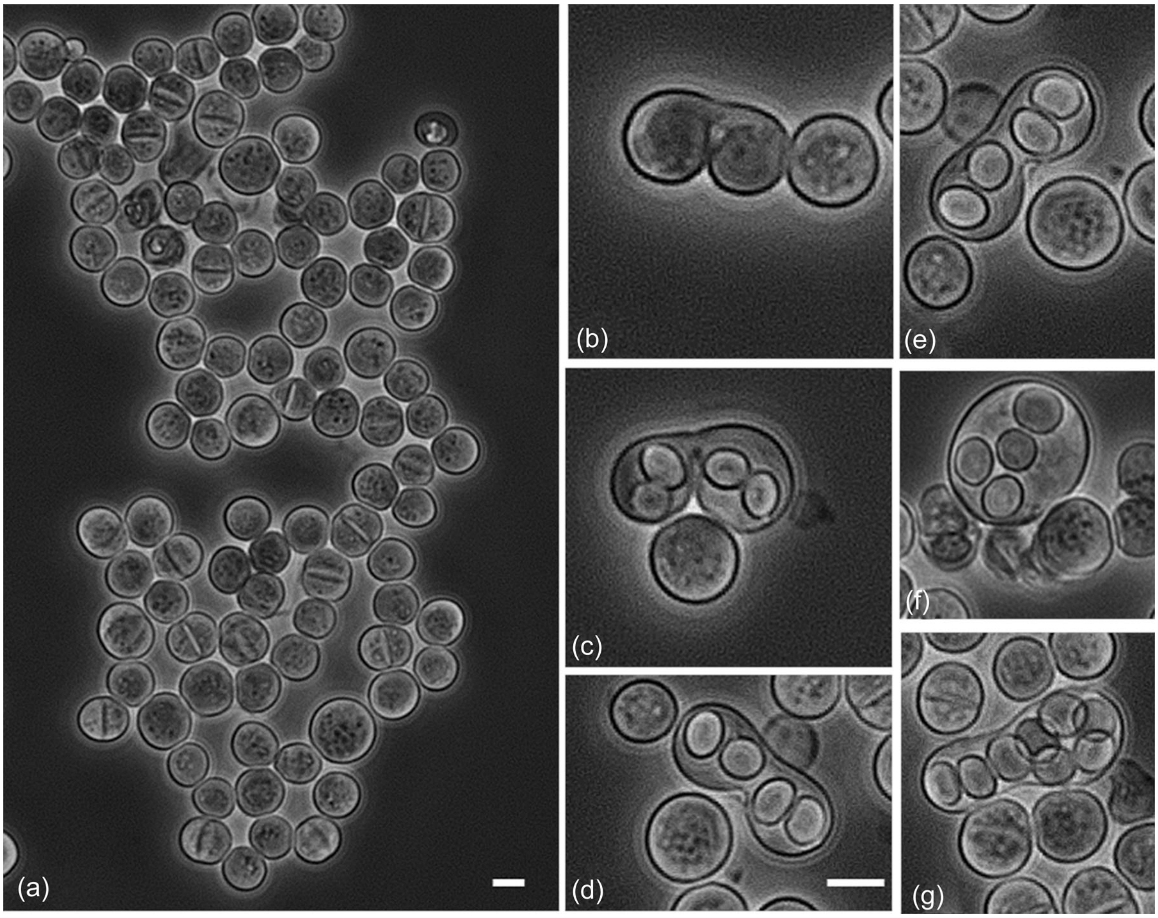

FIGURE 1 Cell and sporulation morphology of *Schizosaccharomyces lindneri* sp. nov. (CBS 18203^T). (a) Cells of an exponential-phase culture in YEL (yeast extract liquid). (b) Conjugated pair of cells. (c–e) Zygotic (conjugation) asci containing four ascospores. (f) Rare four-spored azygotic ascus. (g) Rare eigth-spored zygotic ascus. Culture media: YEL (yeast extract-glucose liquid medium) for (a) and EMMA − N (Edinburgh minimal medium agar without NH_4Cl) for (c–g). Incubation was at 20°C for 3 days. Bars = 5 µm.

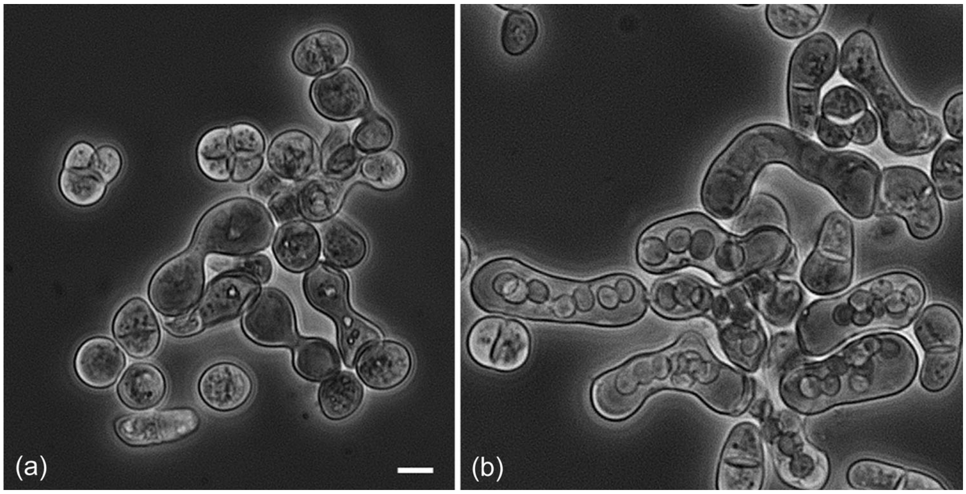

FIGURE 2 Conjugation and sporulation in ex-type cultures of the *Schizosaccharomyces octosporus* (CCY 44-2-1^T). (a) Conjugating cell pairs and (b) zygotic (conjugated) asci on EMMA − N (Edinburgh minimal medium agar without NH_4Cl) after 3 days at 20°C. Bar = 5 µm.

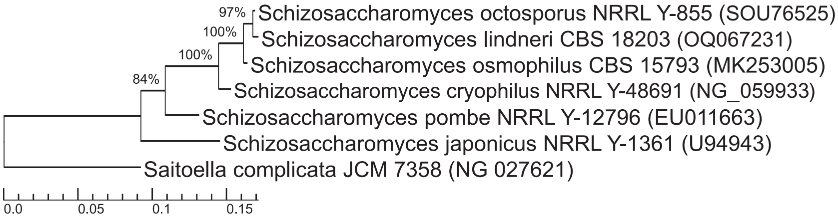

FIGURE 3 Evolutionary tree based on the D1/D2 domain for the large subunit ribosomal RNA (rRNA) gene as inferred by the neighbor-joining method (Saitou & Nei, 1987) and based on the Tamura–Nei substitution model (Tamura & Nei, 1993). Distances are in the units of the number of base substitutions per site. Percentage bootstrap values of 1000 replicates are given at each node. Alignment positions containing gaps and missing data were eliminated before analysis. A total of 548 positions were in the final data set.

S. octosporus, which has the most similar D1/D2 sequence. Figure 3 illustrates the phylogenetic position of *S. lindneri* sp. nov. in the genus *Schizosaccharomyces*. Brysch-Herzberg et al. (2019) showed that nine *S. octosporus* isolates originating from five continents shared identical D1/D2 domain sequences, whereas the two strains of *S. lindneri* sp. nov. deviate considerably. In this context, a divergence of 3 bp in the D1/D2 domain between the proposed species and *S. octosporus* sits within the range of polymorphism encountered in yeast species by Kurtzman and Robnett (1998), who further suggested that a distance of 1% (here 6 substitutions) would serve as a strong hint that two strains represent distinct species. In an extensive study including more than 9000 yeast strains, Vu et al. (2016) established threshold values for species delimitation based on the D1/D2 domain and the ITS region. The authors found that strains belonging to the same species usually show an identity in their D1/D2 domain of more than 99.51% equivalent to three substitutions in the case of *S. lindneri* sp. nov. According to this finding, *S. lindneri* sp. nov. would just be at the limit that frequently defines species boundaries. However, it should be kept in mind that deviations from these threshold values have been observed in different genera (Lachance, 2018).

The ITS region (ITS1-5.8S-ITS2) of *S. lindneri* sp. nov. is 800 bp long (accession no.: OQ621317; OQ621318). It is identical in both strains examined. According to a blastn search against GenBank, the ITS region of *S. octosporus* (accession no.: NR_121469) is most similar to the ITS region of *S. lindneri* sp. nov., which is 810 bp long. The ITS region sequences of the two species differ by 16 indels, which comprise 84 alignment positions and 91 substitutions, corresponding to an 88.1% identity calculated on the basis of the alignment length after subtraction of the positions with gaps. Because of the many indels in the alignment, the precise number of substitutions is hard to determine. All differences are located in ITS1 and ITS2, while none is located in the 5.8S rRNA gene. Vu et al. (2016) calculated that the identity of ITS region sequences between strains of the same species is higher than 98.41% in most of the cases. Accordingly, in its ITS region sequence *S. lindneri* sp. nov. is well separated from *S. octosporus*.

While the analysis of the D1/D2 domain did not lead to an unambiguous justification to describe the examined strains as a separate species, the analysis of the ITS region left little doubt that the strains represent a new undescribed species. Also, the genetic species concept could be applied here to justify the description of a new species; it should be kept in mind that from the beginning species delimitation based on DNA sequences was calibrated by species that were previously defined using the biological species concept. The D1/D2 domain sequences and the ITS region sequences are rather indicators that can be used to identify strains with a high potential to represent a new species than a way of defining species boundaries (Boekhout et al., 2021).

3.3 | Whole-genome sequencing and draft nuclear genome assembly

We sequenced the genome of the *S. lindneri* sp. nov. type strain CBS 18203^T using Illumina sequencing and assembled a draft nuclear genome consisting of 113 contigs (Supporting Information: Table S2). The total length of the *S. lindneri* sp. nov. draft nuclear genome (10.87 Mb) is slightly smaller than the lengths of the reference nuclear genomes of the five previously recognized fission yeast species (12.57, 11.27, 11.52, 11.13, and 11.42 Mb for *S. pombe*, *S. octosporus*, *S. cryophilus*, *S. japonicus*, and *S. osmophilus*, respectively) (Jia et al., 2023; Rhind et al., 2011; Wood et al., 2002), presumably because the repetitive regions are underrepresented in the *S. lindneri* sp. nov. draft nuclear genome owing to the limitation of the short-read sequencing technology.

3.4 | ANI

To assess the relationships between *S. lindneri* sp. nov. and other fission yeast species using genome sequences, we calculated genome-wide pairwise ANI values using the OAU tool (Yoon et al., 2017). The results are shown in Table 3. Consistent with results obtained by the analysis of the D1/D2 region sequence, *S. lindneri* sp. nov. shares the highest ANI value (90.43%) with the reference genome of *S. octosporus*. The reference genome (accession no.: PRJNA264110) was derived from strain yFS286 as it is a one-spore culture of the ex-type strain ATCC 4206 (Rhind et al., 2011). An ANI value alone without further structural genome analysis and investigations of the reproductive compatibility of the strains in question does not allow us to determine species boundaries unless the ANI value is exceedingly high. At least in the genus *Schizosaccharomyces*, too little is known about intraspecies and interspecies variation of ANIs. In the present case, the value of 90.43% is just in the range of ANIs describe before, between closely related species such as the pairs of *Saccharomyces jurei* and *Saccharomyces mikatae* (90.5%) or *Saccharomyces eubayanus* and *Saccharomyces uvarum* (90.0%) (Libkind et al., 2020). The sister species pair *Metschnikowia drakensbergensis* and *Metschnikowia proteae* showed an ANI of 95.2%, and interbreeding strains of *Metschnikowia kamakouana*, an ANI of 94.3% (Lachance et al., 2020). Because of the exceptional phylogenetic position of the genus *Schizosaccharomyces* in the Taphrinomycotina, which are in a basal phylogenetic position to all Ascomycota (Ebersberger et al., 2012), it is not self-evident that the ANIs given above for species pairs in the Saccharomycotina can be applied for species delimitation in the current case. On the other hand, the ANI of 90.43% for the pair of *S. octosporus* and *S. lindneri* sp. nov. for which we present data that show hybrid sterility could be interpreted as a hint that sibling species in the Taphrinomycotina that delimitated based on the biological species concept seem to show ANIs similar to sibling species in the Saccharomycotina.

3.5 | Tree inference and divergence time estimation

To assess the gene content completeness of the *S. lindneri* sp. nov. draft nuclear genome, we analyzed the presence/absence status of a set of 1315 BUSCO (ascomycota_odb9 gene set) (Simão et al., 2015). We found that in the *S. lindneri* sp. nov. draft nuclear genome, 1225 (93.16%) of the BUSCO genes are "complete and single copy" and 37 (2.81%) of the BUSCO genes are "complete and duplicated" (Supporting Information: Table S3). Such a level of BUSCO gene completeness is similar to those of the published reference genomes of other fission yeast species (Jia et al., 2023).

We further estimated the divergence time between *S. lindneri* sp. nov. and *S. octosporus*. A maximum likelihood species tree was inferred using a concatenation supermatrix of 1057 "complete and single-copy" BUSCO genes. Using the RelTime method (Tamura et al., 2018; Tamura & Nei, 1993), we inferred that *S. octosporus* and *S. lindneri* sp. nov. diverged about 5.1 million years ago (Figure 4). This is similar to the divergence time between humans and chimpanzees

TABLE 3 Average nucleotide identities (ANIs) for species pairs in the genus *Schizosaccharomyces*.

	S. pombe	*S. japonicus*	*S. cryophilus*	*S. octosporus*	*S. osmophilus*	*S. lindneri*
S. pombe	100.00	68.94	70.76	70.78	70.76	70.69
S. japonicus		100.00	69.06	69.24	69.18	69.15
S. cryophilus			100.00	79.12	81.29	79.15
S. octosporus				100.00	83.57	90.43
S. osmophilus					100.00	83.88
S. lindneri sp. nov.						100.00

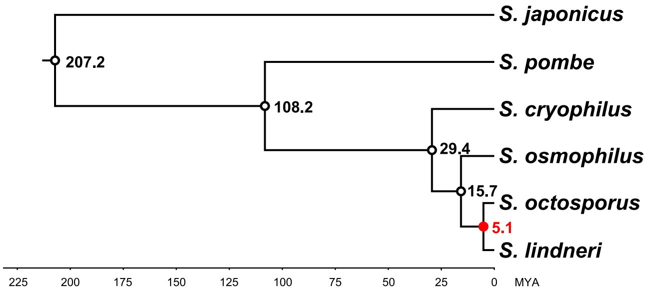

FIGURE 4 Time-calibrated phylogeny of fission yeast species including *Schizosaccharomyces lindneri* sp. nov. A concatenation supermatrix of 1057 “complete and single-copy” BUSCO (Benchmarking Universal Single-Copy Orthologs) genes from six fission yeast species and the outgroup species *Saitoella complicata* was used for maximum likelihood phylogeny inference. The RelTime method was used to estimate the divergence time between *Schizosaccharomyces octosporus* and *S. lindneri* sp. nov. (filled red circle) given four time calibration nodes (empty black circles).

(Kumar et al., 2022). Therefore, the divergence time estimated supports the classification of *S. lindneri* sp. nov. as a separate species.

3.6 | Chromosomal rearrangements between *S. lindneri* sp. nov. and *S. octosporus*

To investigate chromosomal rearrangements between *S. lindneri* and *S. octosporus*, we aligned the *S. lindneri* draft nuclear genome to a chromosome-level *S. octosporus* genome assembly using Mauve (Darling et al., 2004; Tong et al., 2019). Two small intrachromosomal inversion events were revealed by the Mauve alignment (Figure 5a). The size of the inversion on chromosome I of *S. octosporus* is approximately 6 kb and the size of the inversion on chromosome II of *S. octosporus* is approximately 73 kb. In addition, we observed a reciprocal interchromosomal translocation (Figure 5b). As the two translocation breakpoints in the *S. octosporus* genome are located in the middle of chromosome I and chromosome II, respectively, this translocation is expected to cause an up to 50% reduction of spore viability in a cross between *S. octosporus* and *S. lindneri*, due to the loss of essential genes in up to half of the spore progeny. This assessment is made based on the results derived from intraspecies hybridizations in *S. cerevisiae* (Hou et al., 2014). Yet, the transfer of such values from a distantly related species to another is always associated with uncertainty.

We investigated the breakpoints of this translocation by examining genes located at or in the vicinity of the breakpoints. Interestingly, we found that in *S. octosporus*, translocation breakpoints fall within two genes belonging to the *wtf* gene family (De Carvalho et al., 2022; Hu et al., 2017; Nuckolls et al., 2017), *wtf12* and *wtf49*, located on chromosome 1 and chromosome 2, respectively (Figure 5c). In the *S. lindneri* draft nuclear genome, manual gene annotation showed that translocation breakpoints also fall within two *wtf* genes (Figure 5c). One of them is a pseudogene located on the NODE_37 contig, while the other is an intact *wtf* gene located on the NODE_34 contig. Thus, this reciprocal interchromosomal translocation event appears to have happened through recombination between *wtf* genes. We note that the fragmented nature of the draft nuclear genome of *S. lindneri* limited our ability to identify chromosomal rearrangements, and there are likely additional chromosomal rearrangements between *S. lindneri* and *S. octosporus*.

3.7 | Mutant selection and hybridization

The mutants isolated and characterized in the current study are listed in Table 1. Mating of an *S. octosporus* Leu$^-$ mutant and an *S. octosporus* Arg$^-$ mutant yielded numerous prototrophic colonies on EMMA (Figure 6a). Mating of an *S. octosporus* Arg$^-$ mutant with an *S. lindneri* sp. nov. Lys$^-$ mutant produced very few colonies on EMMA (Figure 6b). This low yield of prototrophic colonies indicates a strong but incomplete prezygotic sterility barrier between *S. octosporus* and *S. lindneri* sp. nov.

The asci and ascospores in the prototrophic colonies arising from mating the *S. octosporus* Leu$^-$ mutant and the *S. octosporus* Arg$^-$ mutant are of the usual size and shape. They mainly contain eight ascospores. Azygotic asci were formed, presumably because the prototrophic hybrids are diploids (Figure 7a–c). Mating of an *S. linderni* sp. nov. Ade$^-$ mutant and an *S. lindneri* sp. nov. Lys$^-$ mutant also yielded high numbers of prototrophic colonies. Predominantly, in these colonies, zygotic asci of shape and size typical for *S. lindneri* sp. nov. were formed, indicating that the colonies were formed by recombinant spores produced in sporulating zygotes. Only 2 out of 40 colonies contained azygotic asci that looked exactly like the azygotic asci in *S. octosporus*.

In contrast to the intraspecific mating, the asci and ascospores in prototrophic colonies produced by mating *S. octosporus* × *S. lindneri* sp. nov. are aberrant (Figure 7d–i). The spore number is frequently low and often it is neither 4 nor 8. Frequently not all ascospores are of the usual size or the ascospores are aberrant in shape (Figure 7d–i). The sporulation frequency is low. In some hybrids, sporulation does not occur at all.

To ascertain if the very few prototrophic colonies produced by the mating of *S. octosporus* and *S. lindneri* sp. nov. are diploid hybrids or haploid segregants (recombinants), single ascospores were separated from sporulating prototrophic colonies by micromanipulation technique. None of the 40 ascospores that were separated grew. In a second approach, sporulating hybrid cultures were treated with lysing enzymes to lyse the walls of asexual cells. The cell suspensions treated with lysing enzymes were spread on YEA. Only a few colonies grew, indicating that only a few ascospores were viable. The few colonies were replica-plated on EMMA. About 10% of the colonies were auxotrophic, indicating that the prototrophic cultures produced by crossing auxotrophic strains of *S. octosporus* and *S. lindneri* sp. nov. were diploid hybrids. They contained both the wild-type allele and the mutant allele. None of the

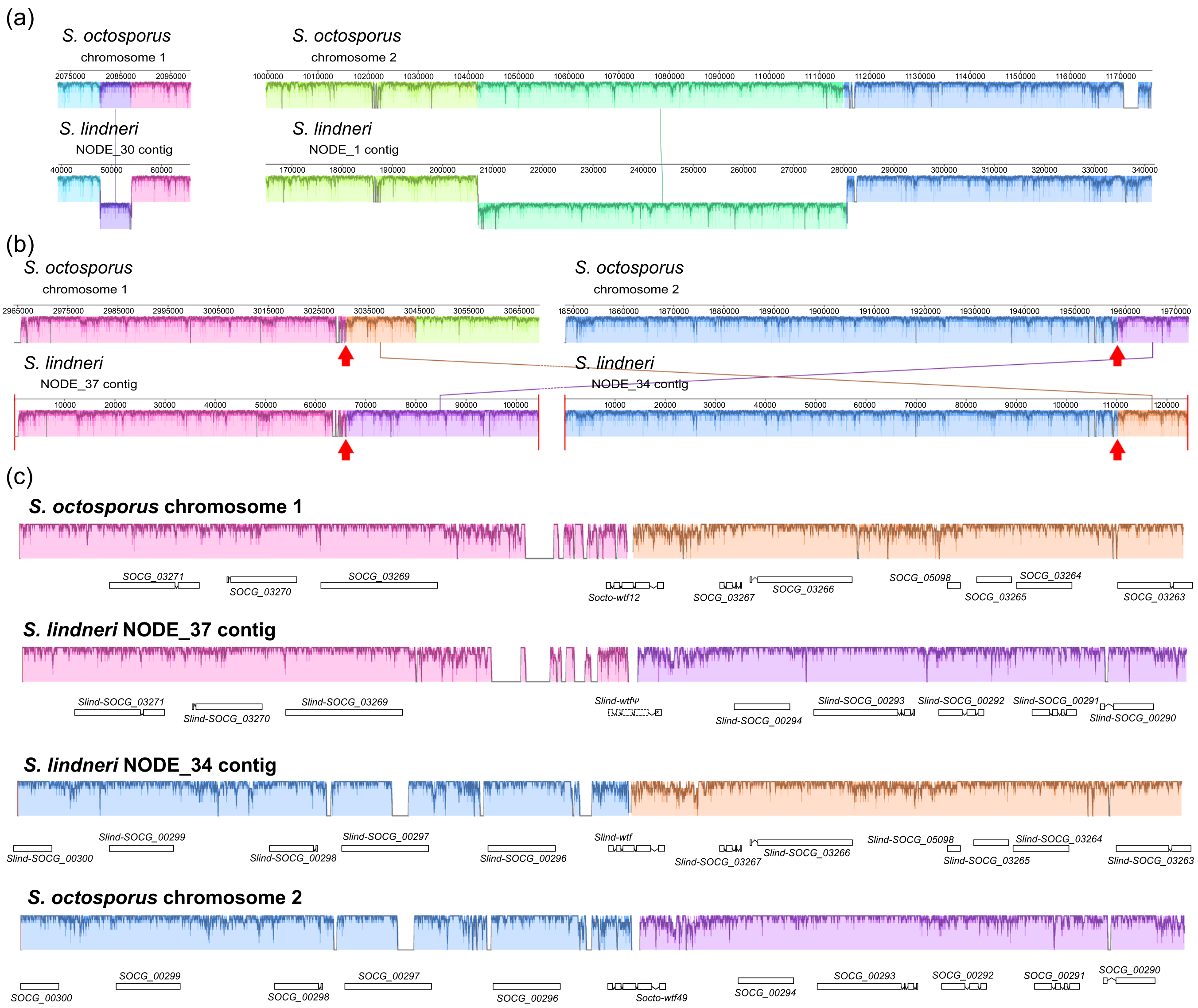

FIGURE 5 Chromosomal rearrangements between *Schizosaccharomyces lindneri* sp. nov. and *Schizosaccharomyces octosporus*. A Mauve-generated genome alignment between a chromosome-level *S. octosporus* genome assembly and the *S. lindneri* sp. nov. draft nuclear genome was visualized using the Mauve viewer. Colored blocks represent locally collinear blocks (LCBs) detected by Mauve. (a) Two intrachromosomal inversions were revealed by the Mauve genome alignment. For the *S. lindneri* sp. nov. draft nuclear genome, LCBs shown in the top track are in the forward orientation relative to the *S. octosporus* genome, and LCBs shown in the bottom track are in the reverse orientation relative to the *S. octosporus* genome. (b) A reciprocal interchromosomal translocation was revealed by the Mauve genome alignment. Red arrows point to the four translocation breakpoints. The boundaries of the *S. lindneri* sp. nov. contigs are indicated by red vertical lines. (c) Close-up views of translocation breakpoint regions in the *S. octosporus* genome (the first and the fourth rows) and in the *S. lindneri* sp. nov. genome (the second and the third rows). Except for *wtf* genes, *S. octosporus* gene annotations were from http://bifx-core.bio.ed.ac.uk/~ptong/genome_assembly/. *S. octosporus wtf* gene annotations were from a recently published study (De Carvalho et al., 2022). *S. lindneri* sp. nov. genes were annotated based on homology to *S. octosporus* genes.

ascospores had received both auxotrophic markers from the parental genomes.

The results of the (GAC)$_5$ PCR fingerprinting show that the banding pattern of most prototrophic colonies contains all major parental bands (Figure 8). Thus, these colonies are diploid hybrids containing both parental genomes. Only one of the prototrophic colonies investigated by (GAC)$_5$ PCR fingerprinting did not show all parental bands. Since the banding pattern of this colony is indistinguishable from that of the *S. octosporus* parent, its prototrophy could be the result of a mutational event that converts the auxotrophy marker gene in the *S. octosporus* parent back to a functional state.

The results of hybridization not only show that between *S. octosporus* and *S. lindneri* sp. nov. an almost complete prezygotic sterility barrier exists but also that the subgenomes of the two parental strains in the hybrids are not compatible, which means that

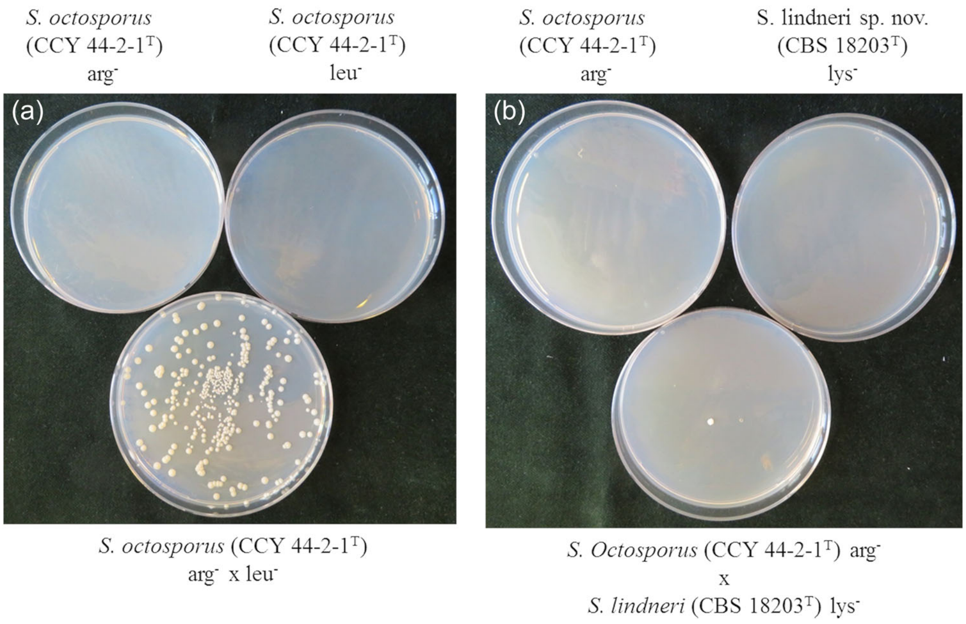

FIGURE 6 Hybridization. (a) Hybridization of the *Schizosaccharomyces octosporus* auxotrophic mutants arg$^-$ and leu$^-$. (b) Hybridization of the *S. octosporus* (CCY 44-2-1^T) auxotrophic mutant arg$^-$ with the auxotrophic lys$^-$ mutant of *Schizosaccharomyces lindneri* sp. nov. (CBS 18203^T). Only prototrophic colonies can grow on the selective medium SMA (synthetic minimal agar).

between *S. octosporus* and *S. lindneri* sp. nov., an almost complete postzygotic sterility barrier exists. For these reasons, it seems reasonable to assume that the two are separate biological species.

Although we did not show direct evidence that genomic rearrangements are causative for the hybrid sterility, the assumption that they mainly establish the species boundary between *S. octosporus* and *S. lindneri* sp. nov. seems reasonable. Both strains of *S. lindneri* sp. nov. were investigated in the current study and many strains of *S. octosporus* (Brysch-Herzberg et al., 2022) were isolated from honey. Brysch-Herzberg et al. (2022) showed that honey is of outstanding importance as a habitat for *S. octosporus*. Additionally, the newly proposed species is almost indistinguishable in its physiological characteristics from *S. octosporus*. Therefore, it seems likely that both species share honey as a common habitat. One strain of *S. lindneri* sp. nov. was isolated from Mexican honey. *S. octosporus* is frequent in Mexican honey. It was detected in about 45% of Mexican honey samples (Brysch-Herzberg et al., 2022). Foraging bees fly long distances and bee hives are often transported from region to region year-round by the beekeepers. For this reason, it seems highly unlikely that the geographic isolation of *S. lindneri* sp. nov. played an important role in its speciation. Everything indicates that speciation was sympatric in the case of *S. lindneri* sp. nov.

3.8 | Conclusions

The molecular genetic analysis revealed that *S. octosporus* and *S. lindneri* sp. nov. are closely related. The distance between their D1/D2 sequences is just at the limit of what generally is expected within a yeast species. Yet, in *S. octosporus*, strains from different continents show no deviation in their D1/D2 sequences. In contrast, *S. lindneri* sp. nov. differs much more in its ITS region sequence from *S. octosporus* than is the case within the great majority of yeast species. The ANI calculated on the basis of the whole genomes for the pair of *S. lindneri* sp. nov. and *S. octosporus* is within the range that deviates from other closely related yeast species. More decisive are the major rearrangements observed in *S. lindneri* sp. nov. in comparison to *S. octosporus*, which alone would be a strong argument to anticipate reproductive incompatibility between the species. The hybridization trials show that between *S. lindneri* sp. nov. and *S. octosporus*, strong prezygotic and postzygotic sterility barriers exist, which means that the two new strains investigated in the current study fully comply with the requirements of the biological species concept. The description of *S. lindneri* sp. nov. is justified.

4 | DESCRIPTION OF *Schizosaccharomyces lindneri* BRYSCH-HERZBERG, SIPICZKI, JIA, SEIDEL, DU, SP. NOV.

MycoBank number 847838: The specific epithet lindneri (N. L. gen. n. lindneri, pertaining to Lindner) refers to Paul Lindner who described *S. pombe* as the first fission yeast species. With the description of *S. pombe*, Paul Lindner laid the basis for fission yeast research.

After 1 week at 25°C on YPD agar supplemented with 30% (w/v) glycerol, colonies with dull, greyish white with a rough surface and an entire margin are raised. After 4 days at 25°C on YPD agar supplemented with 30% (w/v) glycerol, asexual cells are globose, oval, egg-shaped, and rarely rod-shaped measuring 3–9 µm × 3–9 µm. Asexual growth proceeds by fission. Asci are either zygotic and formed

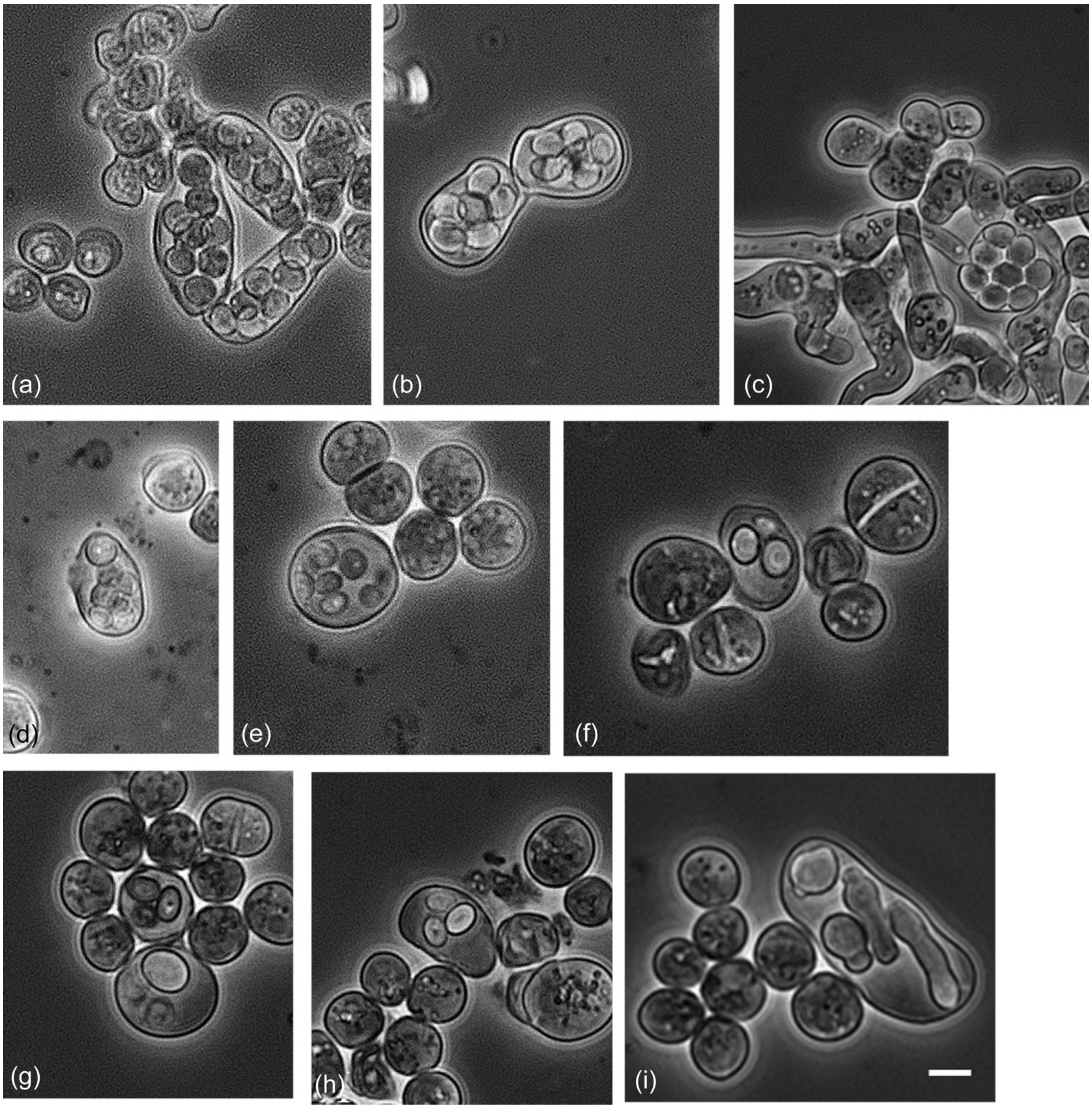

FIGURE 7 Sporulation of hybrids on EMMA – N (Edinburgh minimal medium agar without NH_4Cl) after 3 days at 20°C. (a–c) Azygotic asci in the culture of a *Schizosaccharomyces octosporus* (CCY 44-2-1^T) arg$^-$ × *Schizosaccharomyces octosporus* (CCY 44-2-1^T) leu$^-$ hybrid. (d–h) Azygotic asci in the culture of *S. octosporus* (CCY 44-2-1^T) arg$^-$ × *Schizosaccharomyces lindneri* sp. nov. (CBS 18203^T) lys$^-$ hybrid. (i) Zygotic ascus in the culture of a *S. octosporus* (CCY 44-2-1^T) arg$^-$ × *S. lindneri sp. nov*. (CBS 18203^T) lys$^-$ hybrid. Bar = 5 µm.

after conjugation of two cells or azygotic and formed without conjugation. Azygotic asci are globose, measure (4–9 µm), and contain 1–4 ascospores. Zygotic asci are irregular in shape measuring up to 20 µm × 12 µm and contain 6–8 ascospores. Ascospores are globose, oval, egg shaped, or irregular in shape and measure 3–5 µm. Mycelium was never observed. On a liquid medium, a pellicle is not formed.

Glucose, sucrose (variable, delayed, weak), and maltose (variable, delayed, weak) are fermented. Inulin, raffinose, melibiose, galactose, lactose, trehalose, melezitose, methyl-α-D-glucoside, soluble starch, and cellobiose are not fermented.

Glucose, inulin, sucrose (variable), trehalose (delayed and positive), maltose (variable), glycerol, and D-glucono-1,5-lactone are assimilated. Raffinose, melibiose, galactose, lactose, melezitose, methyl-α-D-glucoside, soluble starch, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, D-ribose, methanol, ethanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, succinate, D-galacturonate, citrate, quinate, D-glucuronate, D-gluconate, D-glucosamine, *N*-acetyl-D-glucosamine, 2-keto-D-gluconate, 5-keto-D-gluconate, saccharate, palatinose, xylitol, L-arabinitol, arbutin, propane-1,2-diol, and butane-2,3-diol are not assimilated.

Ethylamine is assimilated, whereas nitrate, nitrite, cadaverine, creatine, creatinine, L-lysine, and glucosamine are not assimilated.

No growth occurs in the presence of 0.001% cycloheximide and 1% acetic acid. Growth occurs with 16% NaCl and 60% glucose. Growth takes place at 25°C, 30°C, and 37°C, but not at 40°C.

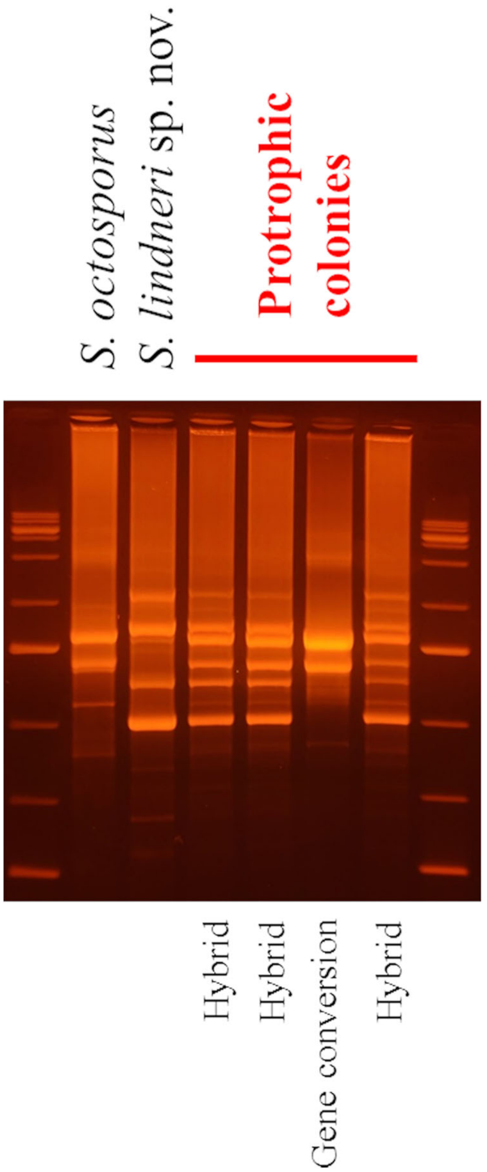

FIGURE 8 (GAC)$_5$ PCR fingerprinting of parental strains and prototrophic colonies growing on the selective medium to distinguish between hybrids and segregants (recombinants).

Holotype: CBS 18203^T, isotype: MUCL 58363; the strains are permanently preserved in a metabolically inactive state. The type culture was isolated from honey bee honey of unknown origin.

AUTHOR CONTRIBUTIONS

Michael Brysch-Herzberg isolated the yeasts, assessed the phenotypic characteristics, and wrote the manuscript. Matthias Sipiczki studied the mating of strains and took microphotographs, and wrote the paper. Guo-Song Jia conducted the genome data analysis and wrote the manuscript. Martin Seidel worked on molecular genetics, assessed the phenotypic characteristics, and wrote the manuscript. Wen Li performed the genome data analysis. Imen Assali isolated the yeasts and assessed the phenotypic characteristics. Li-Lin Du analyzed the data and wrote the manuscript.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Bundesstiftung Umwelt DBU (German Federal Environmental Foundation) [34053/01-32] to Michael Brysch-Herzberg and grants from the Ministry of Science and Technology of China to Li-Lin Du. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov. All data relevant to the study are accessible.

ORCID

Michael Brysch-Herzberg http://orcid.org/0000-0002-3166-2010
Wen Li http://orcid.org/0000-0002-2289-6894

REFERENCES

Barnett, J. A., Payne, R. W., & Yarrow, D. (2000). *Yeasts: Characteristics and identification* (3rd ed.). Cambridge University Press.

Beijerinck, M. (1894). *Schizosaccharomyces octosporus*, eine achtsporige Alkoholhefe. *Zentralblatt Bakteriologie Parasitenkunde*, *16*, 49–58.

Boekhout, T., Aime, M. C., Begerow, D., Gabaldón, T., Heitman, J., Kemler, M., Khayhan, K., Lachance, M. A., Louis, E. J., Sun, S., Vu, D., & Yurkov, A. (2021). The evolving species concepts used for yeasts: From phenotypes and genomes to speciation networks. *Fungal Diversity*, *109*(1), 27–55.

Brysch-Herzberg, M., Jia, G.-S., Seidel, M., Assali, I., & Du, L.-L. (2022). Insights into the ecology of *Schizosaccharomyces* species in natural and artificial habitats. *Antonie Van Leeuwenhoek*, *115*(5), 661–695.

Brysch-Herzberg, M., Tobias, A., Seidel, M., Wittmann, R., Wohlmann, E., Fischer, R., Dlauchy, D., & Peter, G. (2019). *Schizosaccharomyces osmophilus* sp. nov., an osmophilic fission yeast occurring in bee bread of different solitary bee species. *FEMS Yeast Research*, *19*(4), foz038.

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, *25*(15), 1972–1973.

De Carvalho, M., Jia, G.-S., Nidamangala Srinivasa, A., Billmyre, R. B., Xu, Y.-H., Lange, J. J., Sabbarini, I. M., Du, L. L., & Zanders, S. E. (2022). The wtf meiotic driver gene family has unexpectedly persisted for over 100 million years. *eLife*, *11*, e81149.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890.

Darling, A. C. E., Mau, B., Blattner, F. R., & Perna, N. T. (2004). Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Research*, *14*(7), 1394–1403.

Darling, A. E., Mau, B., & Perna, N. T. (2010). progressiveMauve: Multiple genome alignment with gene gain, loss and rearrangement. *PLoS One*, *5*(6), e11147.

Ebersberger, I., de Matos Simoes, R., Kupczok, A., Gube, M., Kothe, E., Voigt, K., & Von Haeseler, A. (2012). A consistent phylogenetic backbone for the fungi. *Molecular Biology and Evolution*, *29*(5), 1319–1334.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, *39*(4), 783–791.

Helston, R. M., Box, J. A., Tang, W., & Baumann, P. (2010). *Schizosaccharomyces cryophilus* sp. nov., a new species of fission yeast. *FEMS Yeast Research*, *10*(6), 779–786.

Hoffman, C. S., Wood, V., & Fantes, P. A. (2015). An ancient yeast for young geneticists: A primer on the *Schizosaccharomyces pombe* model system. *Genetics*, *201*(2), 403–423.

Hou, J., Friedrich, A., de Montigny, J., & Schacherer, J. (2014). hromosomal rearrangements as a major mechanism in the onset of reproductive isolation in *Saccharomyces cerevisiae*. *Current Biology*, *24*(10), 1153–1159.

Hu, W., Jiang, Z.-D., Suo, F., Zheng, J.-X., He, W.-Z., & Du, L.-L. (2017). A large gene family in fission yeast encodes spore killers that subvert Mendel's law. *eLife*, *6*, e26057.

Jeffares, D. C., Rallis, C., Rieux, A., Speed, D., Převorovský, M., Mourier, T., Marsellach, F. X., Iqbal, Z., Lau, W., Cheng, T. M. K., Pracana, R., Mülleder, M., Lawson, J. L. D., Chessel, A., Bala, S., Hellenthal, G., O'Fallon, B., Keane, T., Simpson, J. T., … Bähler, J. (2015). The genomic and phenotypic diversity of *Schizosaccharomyces pombe*. *Nature Genetics*, *47*(3), 235–241. https://doi.org/10.1038/ng.3215

Jia, G.-S., Zhang, W.-C., Liang, Y., Liu, X.-H., Rhind, N., Pidoux, A., & Du, L.-L. (2023). A high-quality reference genome for the fission yeast *Schizosaccharomyces osmophilus*. *G3*, *13*(4), jkad028.

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., Von Haeseler, A., & Jermiin, L. S. (2017). ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nature Methods*, *14*(6), 587–589.

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, *20*(4), 1160–1166.

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780.

Kudrjawzew, V. (1960). *Die Systematik der Hefen*. Akademie-Verlag.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549.

Kumar, S., Suleski, M., Craig, J. M., Kasprowicz, A. E., Sanderford, M., Li, M., Stecher, G., & Hedges, S. B. (2022). TimeTree 5: An expanded resource for species divergence times. *Molecular Biology and Evolution*, *39*(8), msac174.

Kuraku, S., Zmasek, C. M., Nishimura, O., & Katoh, K. (2013). aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. *Nucleic Acids Research*, *41*(W1), W22–W28.

Kurtzman, C. P., Fell, J. W., Boekhout, T., & Robert, V. (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The yeasts: A taxonomic study* (5th ed., pp. 87–110). Elsevier Science Imprint, Elsevier Science & Technology Books.

Kurtzman, C. P., & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie Van Leeuwenhoek*, *73*(4), 331–371.

Lachance, M.-A. (2018). CP Kurtzman's evolving concepts of species, genus and higher categories. *FEMS Yeast Research*, *18*(8), foy103.

Lachance, M.-A., Lee, D. K., & Hsiang, T. (2020). Delineating yeast species with genome average nucleotide identity: A calibration of ANI with haplontic, heterothallic *Metschnikowia* species. *Antonie Van Leeuwenhoek*, *113*, 2097–2106.

Leupold, U. (1955). Methodological Information on the genetics of *Schizosaccharomyces pombe*. *Schweizerische Zeitschrift fur Pathologie und Bakteriologie. Revue suisse de pathologie et de bacteriologie*, *18*, 1141–1146.

Leupold, U. (1970a). Chapter 8. Genetical methods for *Schizosaccharomyces pombe*. In D. M. Prescott, (Ed.), *Methods in cell biology* (4, pp. 169–177). Academic Press.

Leupold, U. (1970b). Genetical methods for *Schizosaccharomyces pombe*. In D. M. Prescott, (Ed.), *Methods in cell biology* (4, pp. 169–177). Elsevier.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

Libkind, D., Čadež, N., Opulente, D. A., Langdon, Q. K., Rosa, C. A., Sampaio, J. P., Gonçalves, P., Hittinger, C. T., & Lachance, M. A. (2020). Towards yeast taxogenomics: Lessons from novel species descriptions based on complete genome sequences. *FEMS Yeast Research*, *20*(6), foaa042.

Lindner, P. (1893). *Schizosaccharomyces pombe* n. sp., ein neuer Gährungserreger. *Wochenschrift für Brauerei*, *10*, 1298–1300.

Mapleson, D., Garcia Accinelli, G., Kettleborough, G., Wright, J., & Clavijo, B. J. (2017). KAT: A K-mer analysis toolkit to quality control NGS datasets and genome assemblies. *Bioinformatics*, *33*(4), 574–576.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*(5), 1530–1534.

Mitchison, J. (1970). Physiological and cytological methods for *Schizosaccharomyces pombe*. In D. M. Prescott (Ed.), *Methods in cell biology* (Vol. 4, pp. 131–165). Elsevier.

Molnar, M., & Sipiczki, M. (1993). Polyploidy in the haplontic yeast *Schizosaccharomyces pombe*: Construction and analysis of strains. *Current Genetics*, *24*, 45–52.

Nuckolls, N. L., Bravo Núñez, M. A., Eickbush, M. T., Young, J. M., Lange, J. J., Yu, J. S., Smith, G. R., Jaspersen, S. L., Malik, H. S., & Zanders, S. E. (2017). wtf genes are prolific dual poison–antidote meiotic drivers. *eLife*, *6*, 20. https://doi.org/10.7554/eLife.26033

Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., & Korobeynikov, A. (2020). Using SPAdes de novo assembler. *Current Protocols in Bioinformatics*, *70*(1), e102.

Rhind, N., Chen, Z., Yassour, M., Thompson, D. A., Haas, B. J., Habib, N., Wapinski, I., Roy, S., Lin, M. F., Heiman, D. I., Young, S. K., Furuya, K., Guo, Y., Pidoux, A., Chen, H. M., Robbertse, B., Goldberg, J. M., Aoki, K., Bayne, E. H., … Nusbaum, C. (2011). Comparative functional genomics of the fission yeasts. *Science*, *332*(6032), 930–936.

Rissman, A. I., Mau, B., Biehl, B. S., Darling, A. E., Glasner, J. D., & Perna, N. T. (2009). Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics*, *25*(16), 2071–2073.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406–425.

Shen, X.-X., Steenwyk, J. L., LaBella, A. L., Opulente, D. A., Zhou, X., Kominek, J., Li, Y., Groenewald, M., Hittinger, C. T., & Rokas, A. (2020). Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota. *Science Advances*, *6*(45), eabd0079.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212.

Sipiczki, M., & Ferenczy, L. (1977). Protoplast fusion of *Schizosaccharomyces pombe* auxotrophic mutants of identical mating-type. *Molecular and General Genetics*, *151*(1), 77–81.

Sipiczki, M., & Ferenczy, L. (1978). Enzymic methods for enrichment of fungal mutants. I. Enrichment of *Schizosaccharomyces pombe* mutants. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *50*(2), 163–173.

Stöver, B. C., & Müller, K. F. (2010). TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics*, *11*(1), 7.

Suminoe, K., & Dukmo, H. (1963). The life cycles of *Schizosaccharomyces* with reference to the mode of conjugation and ascospore formation. *The Journal of General and Applied Microbiology*, *9*(2), 243–247.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Tamura, K., Tao, Q., & Kumar, S. (2018). Theoretical foundation of the RelTime method for estimating divergence times from variable evolutionary rates. *Molecular Biology and Evolution*, *35*(7), 1770–1782.

Tao, Y.-T., Suo, F., Tusso, S., Wang, Y.-K., Huang, S., Wolf, J. B. W., & Du, L.-L. (2019). Intraspecific diversity of fission yeast mitochondrial genomes. *Genome Biology and Evolution*, *11*(8), 2312–2329.

Tong, P., Pidoux, A. L., Toda, N. R. T., Ard, R., Berger, H., Shukla, M., Torres-Garcia, J., Müller, C. A., Nieduszynski, C. A., & Allshire, R. C. (2019). Interspecies conservation of organisation and function between nonhomologous regional centromeres. *Nature Communications*, *10*(1), 2343.

Vaughan-Martini, A., & Martini, A. (2011). *Schizosaccharomyces* Lindner (1893). In C. Kurtzman, J. Fell, & T. Boekhout (Eds.), *The yeasts* (Vol. 2, 5th ed., pp. 779–784). Elsevier.

Vu, D., Groenewald, M., Szöke, S., Cardinali, G., Eberhardt, U., Stielow, B., de Vries, M., Verkleij, G. J. M., Crous, P. W., Boekhout, T., & Robert, V. (2016). DNA barcoding analysis of more than 9000 yeast isolates contributes to quantitative thresholds for yeast species and genera delimitation. *Studies in Mycology*, *85*, 91–105.

Wood, V., Gwilliam, R., Rajandream, M.-A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J., Peat, N., Hayles, J., Baker, S., Basham, D., Bowman, S., Brooks, K., Brown, D., Brown, S., Chillingworth, T., Churcher, C., Collins, M., Connor, R., … Nurse, P. (2002). The genome sequence of *Schizosaccharomyces pombe*. *Nature*, *415*(6874), 871–880.

Yamada, Y., & Banno, I. (1987). *Hasegawaea* gen. nov., an ascosporogenous yeast genus for the organisms whose asexual reproduction is by fission and whose ascospores have smooth surfaces without papillae and which are characterized by the absence of coenzyme Q and by the presence of linoleic acid in cellular fatty acid composition. *The Journal of General and Applied Microbiology*, *33*(3), 295–298.

Yoon, S.-H., Ha, S., Lim, J., Kwon, S., & Chun, J. (2017). A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek*, *110*, 1281–1286.

Yukawa, M., & Maki, T. (1931). *Schizosaccharomyces japonicus nov. spec La Bul. Sci. Falkultato Terkultura* (Vol. 4, pp. 218–226). Kjusu Imperia Universitato.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Brysch-Herzberg, M., Jia, G.-S., Sipiczki, M., Seidel, M., Li, W., Assali, I., & Du, L.-L. (2023). *Schizosaccharomyces lindneri* sp. nov., a fission yeast occurring in honey. *Yeast*, *40*, 237–253. https://doi.org/10.1002/yea.3857